
Multi-Scale Modelling of the Microvasculature in the Human Cerebral Cortex

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Abstract

Cerebrovascular diseases are by far the largest causes of death in the UK, as well as one of the leading causes of adult disability. The brain's healthy function depends on a steady supply of oxygen, delivered through the microvasculature. Cerebrovascular diseases, such as stroke and dementia, can interrupt the transport of blood (and hence oxygen) rapidly, or over a prolonged period of time. An interruption in flow can lead to ischaemia, with prolonged interruptions leading to tissue death and eventual brain damage.

The microvasculature plays a key role in the transport of oxygen and nutrients to brain tissue; however, its role in diseases such as dementia is poorly understood, primarily due to the inability of current clinical imaging techniques to resolve microvessels, and due to the complexity of the underlying microvasculature. Therefore, in order to understand cerebrovascular diseases, it is necessary to be able to resolve and understand the microvasculature. In particular, generating large-scale models of the human microvasculature that can be linked back to contemporary clinical imaging is important in helping plug the current imaging gap that exists.

A novel statistical model is proposed here that generates such large-scale models efficiently. Homogenization theory is used to generate a porous continuum capillary bed (characterised by its permeability) that allows for the efficient scaling up of the microvasculature. A novel order-based density-filling algorithm is then developed which generates morphologically accurate penetrating arterioles and venules, also demonstrating that the topology of the vessels only has a minor influence on CBF compared to diameter. Finally, the capillary bed and penetrating vessels are coupled into a large voxel-sized model of the microvasculature from which pressure and flux variations through the voxel can be analysed. A decoupling of the pressure and flux, as well as a layering of flow, was observed within the voxel, driven by the topology of the penetrating vessels. Micro-infarctions were also simulated, demonstrating the large local effects they have on the pressure and flux, whilst only causing a minor drop in CBF within the voxel.

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Publications

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Chapter 1

Introduction

1.1 Background

With approximately 100 billion neurons in an average human brain, the energy requirements of this organ are extremely high. Despite comprising only 2% of an average human adult's weight, it consumes 20% of our inspired oxygen. The brain, unlike the liver, has no ways of storing energy. Therefore, a constant supply of oxygen and glucose – and hence blood – is essential to maintain the healthy function of the brain. Any reductions in flow can very quickly (tens of seconds to minutes) lead to brain damage and cell death.

Oxygen is transported in blood to the brain through the cardiovascular system, primarily attached to haemoglobin in the red blood cells. Where regions of the body

require oxygen, the oxygen unbinds from the haemoglobin and diffuses across blood vessel walls to be used in metabolism with ATP and glucose to generate the energy required. Carbon dioxide, as a waste product, diffuses into the blood and is carried away to the lungs to be expired.

The blood is transported through the blood vessels, which are essentially hollow tubes. These vessels are grouped functionally into arteries, capillaries, and veins. The arteries transport oxygenated blood to the capillaries where the oxygen diffusion into the tissue mainly occurs, and the veins take deoxygenated blood from the capillaries, along with any waste products, to the lungs for the blood to be re-oxygenated. In particular, the brain is fed by two pairs of arteries, the internal carotid arteries (ICAs) and the vertebral arteries (VAs). These feed into the Circle of Willis, a collateral flow redundancy in the brain, before branching off into various other arteries such as the middle cerebral arteries and posterior cerebral arteries. These feed blood to different regions of the brain.

The major arteries bifurcate into smaller arteries and eventually into pial arteries on the surface of the brain. From these pial arteries bifurcate micro-scale penetrating arterioles that penetrate into the cerebral cortex, supplying the capillary bed, and hence brain tissue, with oxygenated blood. The capillary bed then drains into the penetrating venules, back to the pial veins, and eventually the blood is taken back to the lungs. The penetrating arterioles are able to dilate, in response to increased metabolic demand, to provide an increase in blood flow and oxygen transport to that specific region of the cortex. Additionally, it is also thought that the capillaries are able to contract and dilate due to pericytes that surround the endothelial wall of the capillary vessels, hence also regulating the flow through the microvasculature.

This carefully controlled micro-environment, where most of the oxygen diffusion occurs can, however, malfunction. Any disruptions to blood flow to the brain can result in catastrophic consequences for the patient. Dementia is one such cerebrovascular disease which, since 2015, overtook cardiovascular disease to become the largest cause of death in the UK. The World Health Organization predicts that dementia will become an epidemic due to the lack of any known prevention or cure, and an ageing population. Dementia is characterised by a decline in memory, language, problem-solving, and other cognitive skills that affect a person's day-to-day life, making them reliant on care. Due to the prolonged nature of dementia – characterised by a gradual neurodegeneration over time – the cost to society, health services and social care is high. Dementia is also one of the main causes of disability in old age.

The major cause of dementia is Alzheimer's disease – characterised by formations of amyloid plaques and neurofibrillary tangles in the brain tissue. It is currently thought that a malfunction of the microvasculature results in amyloid-beta being cleared less efficiently from the brain, hence resulting in build-up and formation of plaques, and eventual neurodegeneration. Another major cause of dementia is vascular dementia. The causes of vascular dementia are varied, but all are due to disruption of the microvasculature. Stiffening of vessel walls, increased tortuosity of vessels, and micro-infarctions are all believed to cause a change in local perfusion and oxygen transport. Post-mortem studies of patients with dementia have found small lesions (0.1 – 1 mm in diameter) in the cortex. However, whether these micro-infarctions are a cause of or result of dementia and cognitive decline is unknown, primarily due to the inability of current clinical imaging techniques to resolve such small scales in-vivo in humans.

1.2 Current Gaps and Questions

A wide variety of experiments have been conducted, and models built, to help better understand blood and oxygen transport in the microvasculature. The vast majority of the experiments have been conducted on animals, such as rats, mice, and primates, both in-vivo and ex-vivo. In humans, only ex-vivo measurements of the microvasculature are possible. Additionally, experiments have been conducted occluding penetrating vessels in the cerebral cortex of rats and mice, effectively simulating micro-infarctions in-vivo.

When studying the cerebral microvasculature, mathematical models are essential to understand blood and oxygen transport, particularly at such small scales, due to the inability of imaging them in-vivo in humans. The mathematical models currently available can broadly be split into two branches: whether they are animal or human, and whether the architecture of the blood vessel networks are cast-based or statistical (cast-based meaning they are reconstructed from imaged networks). Using such models, a great deal of information has been gathered about the behaviour of the microvasculature, such as the layering of flow with depth in the cerebral cortex, the complex overlapping territories of the penetrating arterioles and venules, and the robustness of the microvasculature deeper in the cortex. However, there are still gaps and questions that need to be addressed.

Most experiments, in-vivo imaging, and models, are based on animal cerebral microvasculatures, due to the relative ease of imaging animals in-vivo. However, the mapping between animal (in particular rodent) microvasculatures, and the human microvasculature is unknown. As a result, human models are required based on physiological data.

There is an imaging gap with regards to human microvasculatures. Currently there are no ways to image the microvasculature in-vivo in humans. Most clinical imaging techniques have resolutions of around 1 mm or above, meaning that the role of the microvasculature in determining the global parameters, such as CBF, is largely unknown. It is possible, however, to image ex-vivo human brains down to the micro-scale, allowing for a full reconstruction of the microvasculature. This, however, is a time-consuming and expensive technique, possible only for small regions of the vasculature.

Based on the data collected from ex-vivo images it is possible to reconstruct the microvasculature or to use the data to generate statistical models of the microvasculature (generating numerous networks based on physiological data as opposed to one network). However, the scalability of these models is problematic due to the high vascular density of the cerebral microvasculature. To model 1 cm³ of tissue would require approximately 8 million vessels to be modelled. Large-scale models are necessary to help us better understand blood flow and oxygen transport in health and pathology, and link the microvasculature to clinical imaging techniques (such as MRI and CT). As well as this, the models in the literature are heavily dependent on the prescribed boundary conditions.

Experiments have been carried out on rats and mice to simulate micro-infarcts to assess their impact on cognition and local blood and oxygen transport. Such experiments are not possible in humans, hence requiring mathematical models to be built assessing the impact of these micro-infarcts in human brains. Having such large-scale models of micro-infarcts that can be compared to clinical imaging data is useful in linking the microvasculature back to large-scale observable properties of the blood flow.

1.3 Motivation

We need to understand the microvasculature in order to understand cerebrovascular diseases such as dementia. Therefore, the motivation of this thesis is to develop a model of the microvasculature that: is able to model micro-scale blood flows for large regions of the brain; is based on statistical morphological data from which numerous networks can be constructed and modelled; is based on human physiological data; and is generalizable to any available data set such that, in future, when more data on different regions of the brain becomes available, the model can be adapted to model those regions.

In particular, the model will aim to build large volumes of the human microvasculature computationally efficiently, allowing for the scaling up of the microvasculature to large regions that can be compared directly to clinical imaging voxel data. The model will also aim to mitigate the effects of boundary conditions, which have been found to heavily influence flow solutions in models. This is achievable through the use of homogenization theory on the capillary bed (as explained in Chapter 3). This large-scale model will improve our understanding of microvascular networks on a perfusion imaging voxel-scale, which will potentially aid with the analysis of perfusion imaging data.

As well as this, the model proposed and developed in this thesis will be used to investigate the role of micro-infarcts in the human brain, by coupling statistically generated penetrating arterioles to a homogenized capillary bed and simulating occlusions. This model will be very useful in investigating vascular dementia in humans on the microvascular level; analysing the effects of occlusions at the local-scale, whilst also being able to link the global averaged properties (such as CBF) back to clinical imaging data.

Such a physiologically based large-scale model of the human microvasculature could help to clarify the underlying mechanisms behind the control and regulation of perfusion in the capillary bed, as well as to provide an improved understanding of haemodynamic conditions in occluded microvasculatures, potentially helping to understand and delay the onset of vascular dementia.

1.4 Synopsis

The literature review (Chapter 2) provides an overview of the relevant physiology of the brain. I look at current models of flow and viscosity of blood, as well as providing an in-depth analysis of the current mathematical models of the capillary bed, penetrating arterioles and venules, and large-scale models of the microvasculature (which combine both the capillaries and penetrating vessels). Both human and animal models are considered. I then justify the aims of this thesis by looking at the current imaging modalities available, both in-vivo and ex-vivo, and demonstrating that there is an imaging gap with regards to the human microvasculature that can only currently be filled with mathematical models. Finally, two major cerebrovascular pathologies (stroke and dementia) are reviewed with emphasis on the role of the microvasculature. Again, this justifies why it is important to develop mathematical models of the human microvasculature in health and disease.

Chapter 3 presents the theory behind homogenization. This theory is developed for healthy capillary beds and applied to homogenizing the blood flow, the interstitial flow, and oxygen transport in the brain. It is this theory that allows us to scale-up models of the microvasculature into larger volumes. The results of the homogenization of the blood flow are then used on an adapted statistical model of the human capillary bed to determine its permeability. This converged permeability is validated by calculating the CBF.

Chapter 4 deals with developing a model of the blood vessels the next scale up from the capillary bed – the penetrating arterioles and venules. This is necessary in order to develop large models of the microvasculature, as a perfusion imaging sized voxel will have penetrating vessels present. An algorithm is developed which samples statistical information on the human penetrating vessels such as area ratios, bifurcation angles, length-to-diameter ratios, and densities. The novel order-based density-filling algorithm is validated against morphometric data. Murray’s exponent is also calculated to determine whether it is an emergent property in vascular trees. The vascular resistance for the trees is calculated, and subsequently a first order approximation of CBF. This is used to determine the variability of the CBF with the quasi-random topology of the statistically generated penetrating vessels (the varying densities and sizes of the trees generated by the algorithm allow for the quantification of the variability of the approximate CBFs of the trees).

Chapter 5 combines the previous two models, coupling the statistically generated penetrating vessels to the homogenous capillary bed in a coupled ODE/PDE model. As the capillary network has been replaced by a homogenous, porous, isotropic medium, the capillary network is thus modelled by a Darcy flow model with the permeability pre-calculated in Chapter 3. This allows for large regions of the microvasculature to be modelled efficiently (e.g. $2 \times 2 \times 2.5$ mm). The capillary bed is coupled to the penetrating arterioles and venules through their terminal nodes. As the capillary bed permeability is calculated assuming periodic boundary conditions (from homogenization theory), the boundaries perpendicular to the pial surface are also periodic (mitigating the effect of the boundary conditions on the solution). The pial surface and grey/white matter interface have no flow boundary conditions with justification. This model is solved using a linear solver.

An occlusion is simulated in the large-scale model demonstrating the large effect one penetrating vessel has on blood pressure and flux in its direct vicinity. The results of the penetrating vessel occlusion are compared to the results of experiments that have been published in the literature on occluding penetrating vessels in rodent brains.

Finally, in Chapter 6, I summarise and discuss the findings of this thesis, whilst also discussing the limitations of the work presented here and how these limitations affect the results. I conclude by presenting the potential future work that can be undertaken based on the work that has already been completed in this thesis.

Chapter 2

Literature Review

2.1 The Brain

The brain is an extremely complex organ that remains poorly understood. The brain is not only responsible for our basic bodily functions, such as movement, homeostasis, and vision processing, it is also the organ that gives us consciousness, memory, and the ability to be self-aware. This section will give a brief overview of the major components of the brain and its surroundings, in particular with relation to blood flow. The rest of the literature review will focus on the blood flow in the microvasculature which is the specific interest of this thesis.

2.1.1 Structure of the Brain

The brain is protected by several layers: the skull, the pia mater, arachnoid mater, and dura mater (**Fig. 2.1**). Collectively, the three mater layers are known as the

meninges, and their primary function is to protect the brain and central nervous system. In the subarachnoid space, between the arachnoid and pia mater, circulates cerebrospinal fluid (CSF) – (see Section 2.1.4). The brain itself is composed of three regions: the brain stem, the cerebellum, and the cerebrum (**Fig. 2.1**). These regions all work in tandem to allow us to move, think, and sense, but it is the cerebrum –the largest region of the brain – that seems to be responsible for most functions including all voluntary actions, as well as emotions, hearing, vision, language, and memory [128].

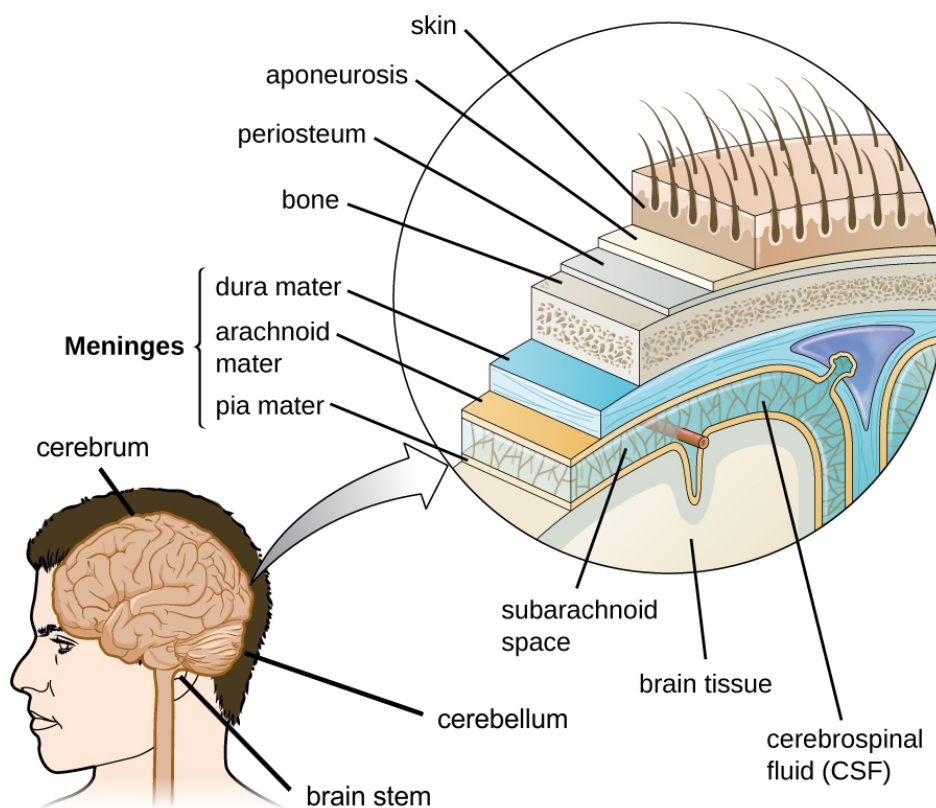


Fig. 2.1 Overview of the location of the three major constituents of the human brain: the cerebrum, cerebellum, and brain stem. The expanded region displays the protective layers of the brain. Adapted from OpenStax [133] under Creative Commons license.

The cerebrum is divided into two hemispheres (the right and left), the cerebral cortex (the outer grey matter) and the underlying white matter (**Fig. 2.2**). The cerebral cortex is only seen in humans and other mammals [203] but is most developed

in humans, where it plays a key role in memory, self-awareness, and thought [119]. The cerebral cortex has a large density of capillaries and so is much greater perfused than the white matter due to the high energy demands of neurons [29]; the white matter is given its colour due to the myelinated sheaths of axons which transmit signals across the brain [128].

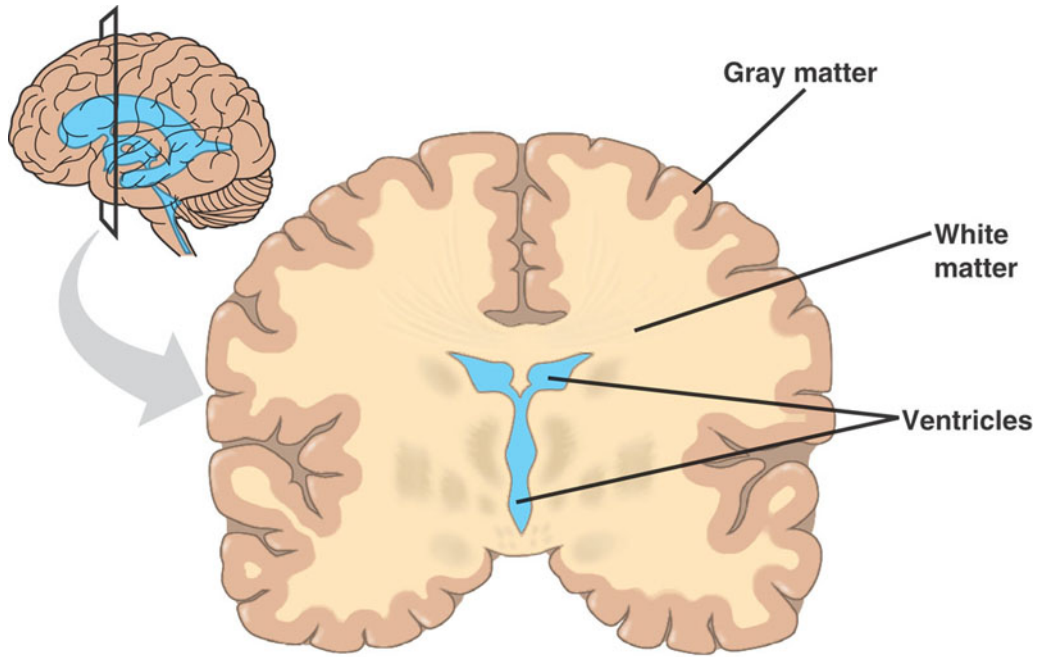


Fig. 2.2 Coronal slice of the brain displaying the outer grey matter, the inner white matter, and ventricles containing the CSF. (Copyright © 2008 Pearson Education Inc., publishing as Pearson Benjamin Cummings).

2.1.2 Cerebral Circulation

On average the brain takes up 2% of the mass of an adult human, whilst consuming 20% of the oxygen supply to the whole body [33]. As a result, the brain needs a constant supply of blood and oxygen to keep functioning normally (between 45 – 65 ml/min/100g of blood [202]), with any drop in flow and resulting starvation of oxygen very quickly leading to tissue death.

The main arteries entering the brain are the vertebral arteries at the back, and the internal carotid arteries at the front. These feed into the Circle of Willis (the ring of arteries in **Fig. 2.3**) which provides collateral flow in case of a reduction of blood flow from a given feeding artery [106]. Bifurcating off the Circle of Willis are the basilar artery, the anterior communicating and anterior cerebral arteries, the posterior communicating and posterior cerebral arteries, as well as the middle cerebral arteries (**Fig. 2.3**). These arteries bifurcate into pial arteries, which are situated in the subarachnoid space on the surface of the brain. The topology of these pial vessels is highly interconnected, containing a large number of loops, which ensure robust delivery of blood to the cerebral cortex [19]. From these pial vessels the penetrating arterioles emerge (Section 2.4) which bifurcate from the pial surface orthogonally down into the grey matter [42]. These arterioles bifurcate until they end as capillaries within the capillary bed (Section 2.3). The venules, in reverse, drain the blood from the capillary bed and back up to the pial surface into veins [42].

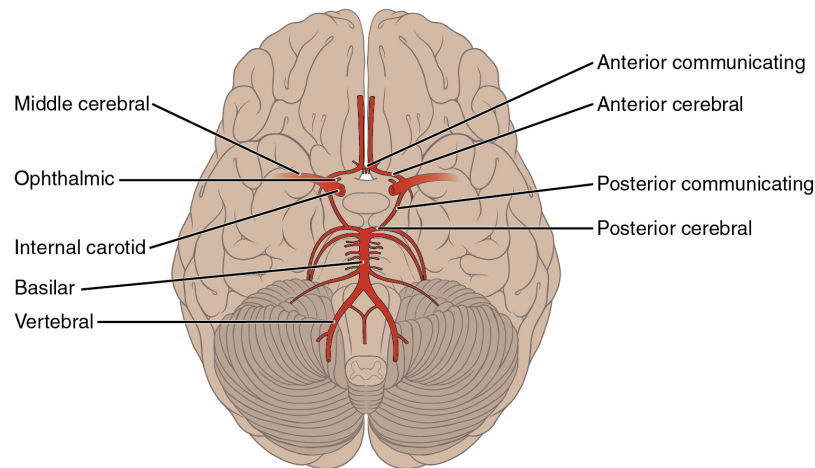


Fig. 2.3 Schematic of the Circle of Willis and its branching arteries in the human brain. Adapted from OpenStax [133] under Creative Commons license.

2.1.3 Blood Brain Barrier

Most solute transport to brain tissue occurs at the penetrating arteriole and capillary scale within the brain vessel hierarchy. There exists, however, a blood brain barrier (BBB) for the vessels that supply the central nervous system (CNS). The BBB is created by a combination of: the tight junctions of endothelial cells that form the walls of the capillaries, discontinuous pericytes that partially surround the endothelium, and astrocyte end-feet (**Fig. 2.4**). The combination of these mechanisms leads to the CNS microvasculature having a hydraulic permeability that is much lower (2 orders of magnitude smaller) than typical values in other capillary vessels [104,105].

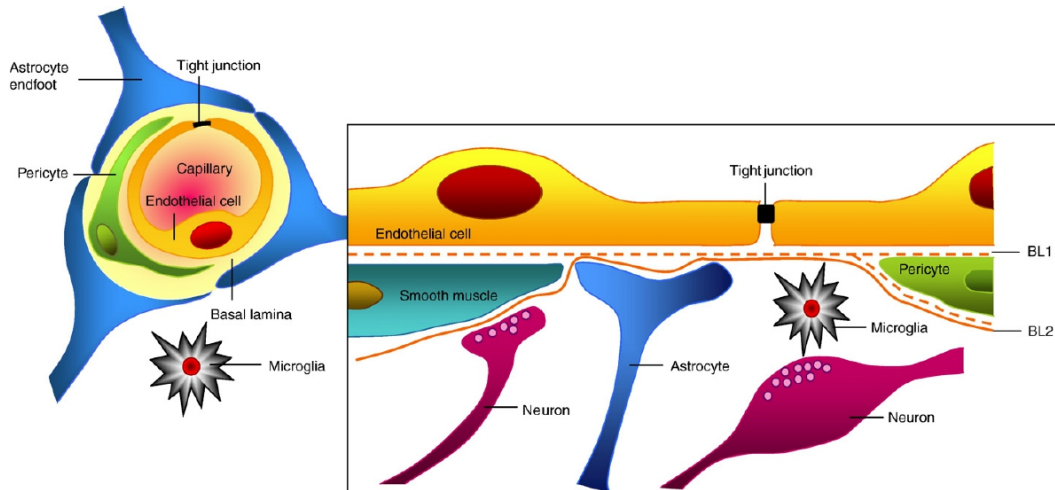


Fig. 2.4 Schematic of the BBB displaying the endothelial tight junctions in the capillary vessel walls, as well as the astrocyte end feed that almost completely envelop the capillary, and the discontinuous pericytes. Reproduced with permission from [2].

The BBB regulates trans-vascular exchange selectively, with a healthy BBB permitting only certain molecules such as essential nutrients, amino acids, and peptides to enter the parenchyma, whilst rejecting neurotoxins and larger macromolecules [2]. Oxygen and carbon dioxide are able to diffuse across the BBB, with these dissolved gases moving down their concentration gradients.

Breakdown of the BBB has been linked to various pathologies including, but not limited to: multiple sclerosis [34], epilepsy [152], Alzheimer’s disease [204], and hypoxia and ischaemia [88]. It is also now believed that the BBB is a dynamic system, able to change its tight junction function [14], and activity of its transporters and enzymes [3], allowing it to compensate in pathological conditions. The BBB thus acts as an effective barrier to maintain homeostasis of the local ionic environment for normal functioning of the neurons and glial cells.

2.1.4 Cerebrospinal Fluid and Intracranial Pressure

Cerebrospinal fluid (CSF) envelops the entirety of the brain, sitting between the arachnoid mater and pia mater (**Fig. 2.1**), as well as occupying the ventricular system where it is produced (**Fig. 2.2**). The makeup of the CSF is very similar to plasma – it is derived from the filtering of blood within the ventricular system – except that CSF has very few proteins and differing electrolyte levels [159].

The CSF has several functions. It acts as a shock absorber for the brain, minimising mechanical impacts to the skull. It circulates around the brain removing metabolic waste and returning it to the blood stream [159]. CSF also has a homeostatic function, acting to regulate the distribution of substances across brain tissue [78]. The CSF is the main fluid in the glymphatic system of the brain, acting as the clearance/drainage fluid descending from the subarachnoid space in periarterial tunnels, and draining out of the perivenous space [83]. As well as this, traditional lymphatic vessels have been found lining the dural sinuses which carry fluid and immune cells away from the CSF [113]. These drainage systems act to remove neurotoxins and other metabolic waste from the brain parenchyma.

The intracranial pressure (ICP) is the pressure under the skull. The Monroe-Kellie doctrine states that, due to the fixed volume of the skull, changes in the volumes

of tissue (e.g. via tumour growth), CSF, and blood result in changes to the ICP. For small volume changes, the CSF is able to compensate by being displaced into the spinal canal. However, as the volume changes increase (as a result of a haemorrhage in the brain for example) the fixed volume of the skull means that there will be an increase in ICP. The value of ICP for a healthy adult in the supine position is considered to be ≤ 20 mmHg [94]. The mean arterial pressure (MAP) of the artery tree (**Fig. 2.3**) must thus be larger than the ICP in order to keep blood flowing to the brain. In pathological states the ICP can rise causing the vessels to contract, leading to impairments in blood circulation. Changes in ICP can be caused by a variety of reasons, such as increased volume due to brain tumours, oedema due to infarction, haematoma, and disorders in CSF circulation [172].

2.1.5 Neurovascular Coupling

Due to the brain lacking metabolic energy stores – in particular glucose and oxygen – there is a very tight coupling between CBF and metabolism, with local demands in increased metabolism leading to an increase in CBF to that region. This increased metabolic demand comes from neuronal activity. The neurovascular unit comprises the blood vessel, smooth muscle, neuron, astrocyte, and microglia (**Fig. 2.4**). The exact signalling pathways, from neuronal spikes to increased CBF to the region, are complex and not covered here. The traditional view of neurovascular coupling can be seen in **Fig. 2.5**. Neural activation leads to release of neurotransmitters, and energy consumption via ATP, glucose and oxygen. The molecular by-products of metabolism then affect the smooth muscle causing vasodilation and an increase in CBF. This view has been around since 1890 [156] and effectively models the neurovascular unit as a feedback loop. However, it is now thought that changes in metabolic consumption and CBF occur in parallel, both mediated by neural signalling mechanisms rather than by sensing oxygen consumption [39,55].

This coupling between the neurons and changes in blood flow and metabolic consumption allows us to infer – by measuring changes in blood flow– the neuronal activity in the brain. Some clinical imaging techniques – such as fMRI – use this coupling to indirectly determine the functionality of our brains. These measurement techniques will be discussed in more detail in Section 2.6.

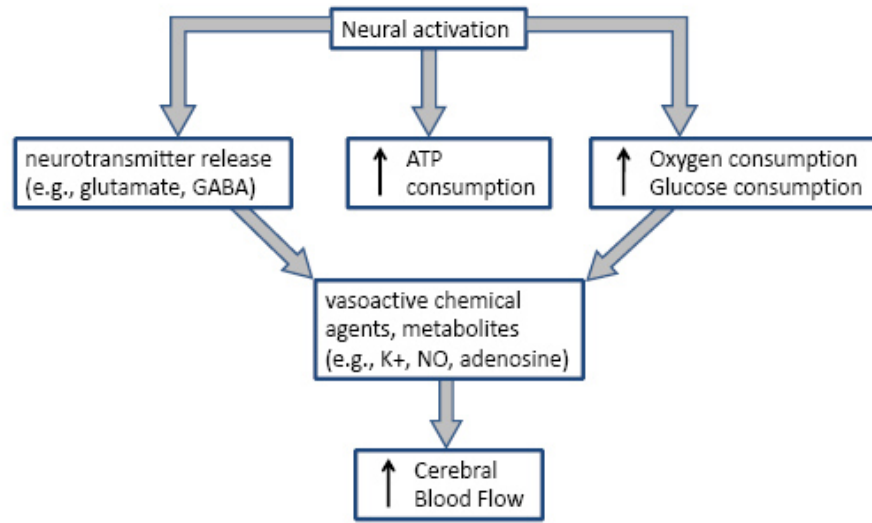


Fig. 2.5 Traditional view of neurovascular signalling pathways. It is now thought that these pathways occur in parallel, with both changes in metabolic consumption and CBF occurring at the same time due to neural signalling mechanisms [39,55]. Reproduced from [136] under Creative Commons license.

2.2 Cerebral Blood Flow and Oxygen Transport

Before looking at the smallest scale of the cerebral microvasculature, I first review what these vessels carry – most importantly blood, oxygen, and glucose. In order to model blood and oxygen transport in these vessels, it must first be understood how blood behaves at these small scales – in particular, the relationship between shear stress and shear strain for this fluid is essential to be able to develop models for blood flow.

2.2.1 Blood

Blood is a fluid connective tissue that circulates through the cardiovascular system. Blood is not a fluid in the traditional sense, but rather a suspension of various, ions proteins, platelets, erythrocytes (red blood cells), and leukocytes (white blood cells) within a fluid – the plasma (**Fig. 2.6**). The normal total circulating blood volume is around 8% of body weight (5.6 L for a 70kg person) [15]. The composition of blood in an average adult human is given in **Table 2.1**.

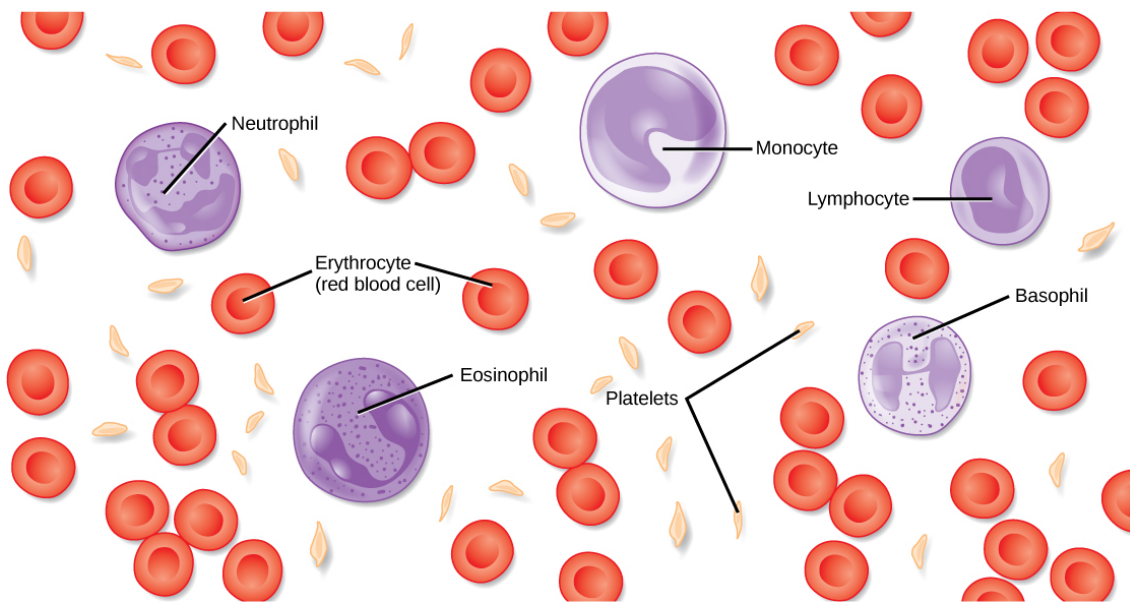


Fig. 2.6 Composition of blood – A suspension of erythrocytes, leukocytes (basophils, eosinophils, neutrophils, monocytes, and lymphocytes), and platelets in plasma. Reproduced from OpenStax [133] under Creative Commons license.

As can be seen, on average 55% of the blood volume is plasma, and just under 45% is made up of red blood cells (RBCs). The haematocrit is defined as the volume fraction of blood that is comprised of red blood cells. The rest of the blood is made up of white blood cells (WBCs) and platelets. The plasma, as it is mostly comprised of water, can be assumed to be a Newtonian fluid. However, the suspension of RBCs within this fluid in large quantities (45% of the volume) is what predominantly gives

blood its non-Newtonian behaviour. The WBCs and platelets only comprise around 1% of the blood volume and so the RBCs dominate this non-Newtonian behaviour.

The mean diameter of RBCs is around $7.5 \mu\text{m}$ [15] which is larger than the smallest capillaries. However, these cells are extremely deformable, with a very flexible outer membrane, which allows them to pass through small vessels. At such small scales the RBCs pass through the vessels in single file, in what is known as plug flow [137]. This is a clear example of how the existence of these cells in the plasma results in non-Newtonian behaviour of blood flow, especially apparent at small scales (the scales in which this thesis is interested). The modelling of this non-Newtonian behaviour is discussed in Section 2.2.3.

Composition of Blood	
Plasma (%)	50-60
Water (% of plasma)	91-92
Protein (% of plasma)	7-8
Solutes (% of plasma)	1-2
Electrolytes e.g. Na^+ (% of plasma)	1
Haematocrit (%)	39-50
Leukocytes (%)	1
Platelets (%)	<1

Table 2.1 Composition of blood in an average adult human. Data taken from [15,155].

Finally, one of the major jobs of blood, in particular the RBCs, is the binding and transportation of oxygen, and removal of carbon dioxide. Typically 98% of oxygen is bound to the erythrocytes and 2% is dissolved in plasma [137]. Each RBC contains

haemoglobin (which gives blood its red colour due to the haem group) which can bind rapidly and reversibly to oxygen forming oxyhaemoglobin. This allows for the quick and efficient transport of oxygen throughout the human body.

2.2.2 Haemodynamics

The starting point for any fluid flow is the Navier-Stokes equation which is defined in (2.1) for a Newtonian fluid (a fluid of constant viscosity):

$$\rho \left(\frac{\partial \mathbf{u}_c}{\partial t} + \mathbf{u}_c \cdot \nabla \mathbf{u}_c \right) = -\nabla p_c + \mu \nabla^2 \mathbf{u}_c \quad (2.1)$$

where $\mathbf{u}_c$ is the velocity of the blood, μ is the dynamic viscosity, p_c is the blood pressure, and ρ is the density of blood. Due to the low velocity of blood flow in the capillaries and penetrating arterioles and venules ($0.1 - 10 \text{ mm s}^{-1}$) [139] the dynamics of the flow are dominated by viscous effects. This can be seen by calculating the Reynolds number (defined as the ratio of inertial forces to viscous forces) for a $6 \text{ }\mu\text{m}$ diameter capillary. For a velocity of 1 mm s^{-1} , a density of 1040 kg m^{-3} [91], and a viscosity of $4 \times 10^{-3} \text{ Pa s}$ at 45% haematocrit [148], the Reynolds number is approximately 1.4×10^{-3} , indicating that viscosity dominates at this scale and the advection term can be neglected. As well as this, steady-state flow can be shown to be valid by calculating the Womersley number (defined as the ratio of transient inertial forces to viscous forces). Assuming a heart rate of 1 Hz , along with the above assumptions for density, viscosity and diameter, the Womersley number is approximately 3×10^{-3} , indicating that viscosity dominates over the transient forces and hence the unsteady term can be neglected. This leads to

$$-\nabla p_c + \mu \nabla^2 \mathbf{u}_c = 0 \quad (2.2)$$

which is Stokes flow for an incompressible fluid. From this equation, it is straightforward to arrive at Poiseuille's equation for flow through a cylindrical pipe –

by assuming negligible circumferential and tangential velocities, uniform pressure over the cross-section of the vessel, and no-slip flow conditions at the vessel wall:

$$Q = \frac{\pi r^4}{8\mu L} \Delta P \quad (2.3)$$

where L is the length of the vessel, r is the tube radius of the vessel, ΔP is the pressure drop across the vessel, and Q is the flow rate through the vessel.

Blood, however, is not a Newtonian fluid due to the dense suspension of RBCs, leukocytes, and platelets in the plasma (see Section 2.2.1). At the microvascular level, the RBCs are also of the same order in size as the diameter of the vessels they are flowing through, and therefore the interaction between these RBCs and endothelial cells lining the vessel walls becomes important when considering the rheology of blood. The modelling of such dense suspensions with cell-wall interactions is very difficult to do [52].

The above equations are not valid for blood due to the starting assumption of a Newtonian fluid. However, we can measure ΔP , L , Q , and r for a given vessel and invert (2.3) to get

$$\mu_{app} = \frac{\pi r^4}{8QL} \Delta P \quad (2.4)$$

where μ_{app} is the apparent dynamic viscosity for the blood flowing in a given circular cylindrical tube with tube radius r [52]. Using this correlation to derive the apparent viscosity makes no assumptions about the nature of the flow (other than it being 1D) – it is just the observed or apparent viscosity of the blood which takes into account the various cell-vessel interactions – as we only measure the input and output conditions of the blood, along with the geometry of the vessel. Therefore, using the above correlation means blood can be modelled using Poiseuille’s equation with a

(constant) apparent viscosity in each vessel. Experimentally derived correlations that determine the apparent viscosity (for a given haematocrit and vessel radius) are discussed below.

2.2.3 Viscosity of Blood

Blood has significant non-Newtonian properties. The RBCs, as has been stated, contribute most of the viscosity of the blood. There are three effects that occur on the small scale (vessels $< 1\text{mm}$ diameter) that cause large changes in the viscosity of the blood due to the presence of the RBCs: the Fåhræus-Lindqvist effect (and its inverse), the Fåhræus effect, and phase separation at bifurcations.

2.2.3.1 Fåhræus-Lindqvist effect

The Fåhræus-Lindqvist effect was identified in 1931 by Fåhræus and Lindqvist when conducting in-vitro flow experiments on glass tubes [47]. They found that vessels below $300\text{ }\mu\text{m}$ in diameter exhibited a drop in viscosity. Since then, others have found that this decrease continues until a diameter of around $10\text{ }\mu\text{m}$ [142]. Below $10\text{ }\mu\text{m}$ there is a marked increase in apparent viscosity up to a diameter of $2.7\text{ }\mu\text{m}$ which is the critical diameter (inverse Fåhræus-Lindqvist effect). Below this diameter the deformable RBCs can no longer pass through the capillaries. Pries et al. have collated and analysed several studies to determine empirical relationships between the relative apparent viscosity of the blood (the ratio of the apparent viscosity to the plasma viscosity) and the tube diameter and discharge haematocrit (defined as the volume flow rate of RBCs as a fraction of the total volume flow rate) (**Fig. 2.7**) [142].

However, in-vivo measurements of apparent viscosity are substantially higher than those predicted by the in-vitro empirical equations [146]. As a result, new empirical equations were developed by Pries et al. that better fit this in-vivo experimental data, resulting in larger values of apparent viscosity, particularly at the

smallest scale diameters ($5 - 20 \mu\text{m}$) (**Fig. 2.8**) [146]. These were developed through experimental observations of blood flow in rat mesentery networks [145,146], effectively determining the apparent viscosities of the networks by measuring pressure drops and flow rates and using (2.4).

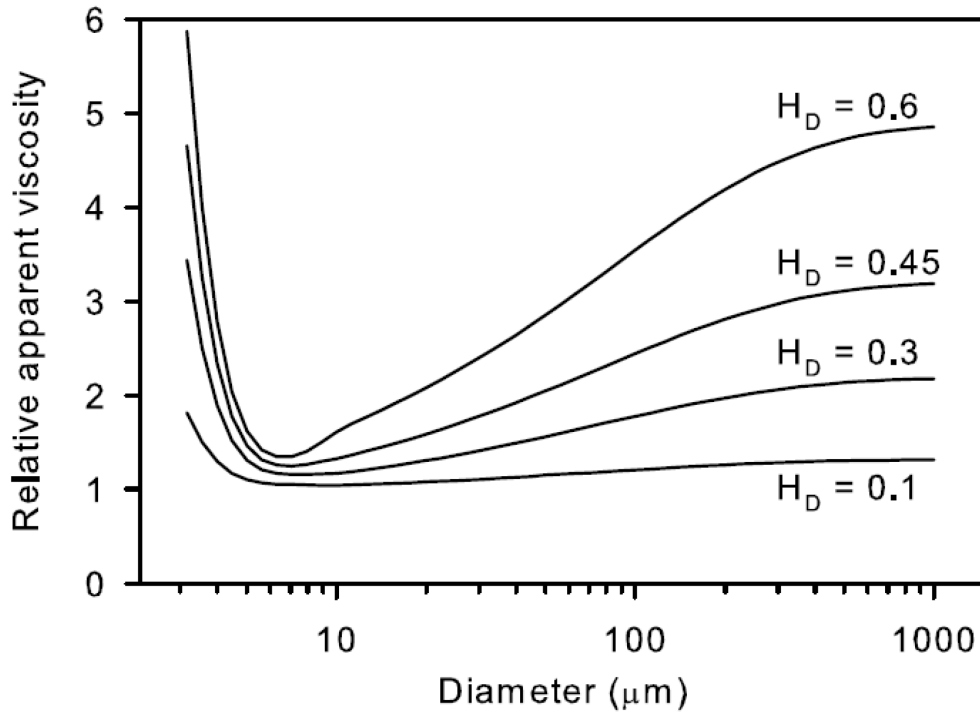


Fig. 2.7 Dependence of relative apparent viscosity of blood in glass tubes on diameter for different levels of discharge haematocrit, according to the empirical equations found in [142]. Reproduced with permission from [164].

Possible reasons for this large difference between in-vitro and in-vivo apparent viscosity include: unaccounted for effects of WBCs, asymmetry of RBC flow, and retardation of the plasma flow at the vessel wall due to the presence of macromolecules [164]. It was found that the main cause of the difference was a $0.5 - 1 \mu\text{m}$ [140,151,185,191] wide layer of macromolecules bound to the endothelial cells lining the vessel walls, known as the endothelial surface layer (ESL).

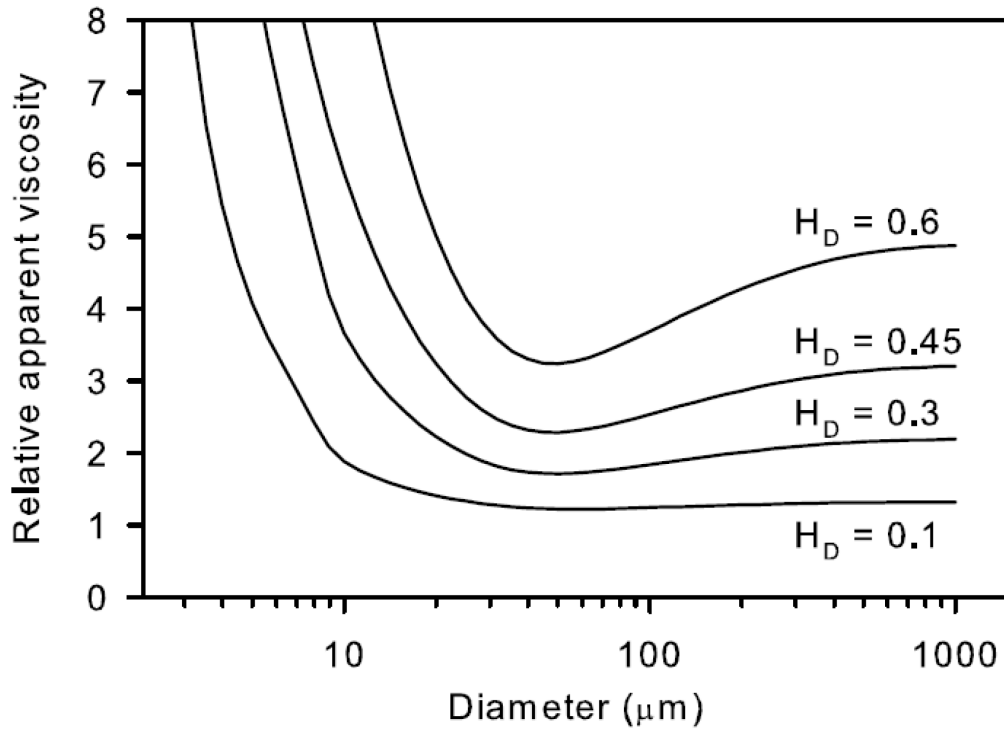


Fig. 2.8 Dependence of relative apparent viscosity of blood in-vitro in rat mesentery on diameter for different levels of discharge haematocrit, according to the empirical equations found in [146]. Reproduced with permission from [164].

As a result, a further empirical model was developed to take into account the effect of this ESL on the apparent viscosity. The ESL is explicitly modelled with a thickness that is determined by the vessel diameter d_i (μm) and discharge haematocrit H_D :

$$\mu_{vitro} = \mu_p \left[1 + (\eta_{0.45} - 1) \frac{(1 - H_D)^{C_D} - 1}{(1 - 0.45)^{C_D} - 1} \right] \quad (2.5)$$

$$\eta_{0.45} = 220e^{-1.3d_i} + 3.2 - 2.44e^{-0.06d_i^{0.645}} \quad (2.6)$$

$$C_D = (0.8 + e^{-0.075d_i}) \left(-1 + \frac{1}{1 + 10^{-11} \cdot d_i^{12}} \right) + \frac{1}{1 + 10^{-11} \cdot d_i^{12}} \quad (2.7)$$

$$\mu_{vivo} = \mu_{vitro} \cdot \left(\frac{d_i}{d_{i,eff}} \right)^4 \quad (2.8)$$

where μ_p is the viscosity of blood plasma (1.2 mPa s), μ_{vitro} is the in-vitro blood viscosity, and $\eta_{0.45}$ is the relative apparent viscosity of blood for a discharge haematocrit of 0.45. The coefficients multiplying d_i all have units of $1/\mu\text{m}$. $d_{i,eff}$ is the effective diameter of the blood vessel that takes into account the thickness of the endothelial surface layer and a haematocrit-dependent effect on the flow resistance, as derived using correlations detailed in Pries and Secomb [143]. This model leads to a closer agreement between observed behaviour and predictions of network haemodynamics than previous empirical relations. It again predicts a larger viscosity than the in-vitro relationships, but has a more complex viscosity-diameter relationship (Fig. 2.9).

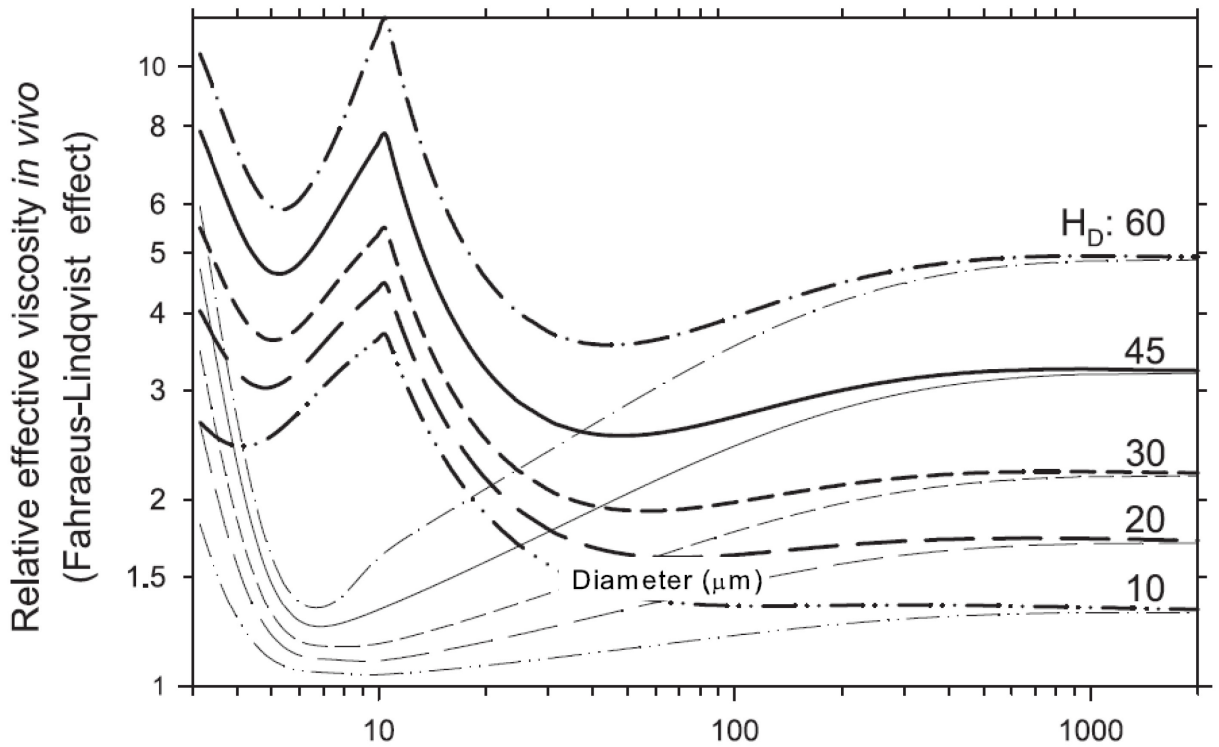
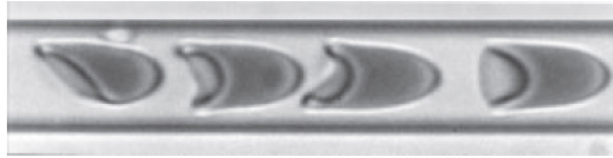


Fig. 2.9 Dependence of effective relative blood viscosity on measured anatomic vessel diameter where H_D is the discharge haematocrit (%). Thick black lines represent the in-vivo model taking into account the ESL (2.5 – 2.8) and the thin grey lines the in-vitro model [142]. Reproduced with permission from [143].

2.2.3.2 Fåhræus effect

The Fåhræus effect was identified in 1929 and states that if blood is allowed to flow from a large reservoir into a small cylindrical tube, the haematocrit in that tube (defined as the tube haematocrit) will be less than that of the reservoir [46]. The reason for this drop in haematocrit is due to the nature of particulate pipe flow where the RBCs tend to flow through the centre of the vessel with the plasma forming a layer on the surface of the vessel wall (**Fig. 2.10**). As flow through the centre of a vessel is fastest, the average RBC velocity is larger than the average plasma velocity leading to a drop in the haematocrit in that vessel.

$$H_D = 0.15, d_i = 7 \mu\text{m}$$



$$H_D = 0.30, d_i = 7 \mu\text{m}$$



$$H_D = 0.45, d_i = 7 \mu\text{m}$$



Fig. 2.10 Flow of RBCs through 7 μm diameter glass tubes at different discharge haematocrits (H_D). The RBC free layer can be seen at the vessel walls. Adapted with permission from [143].

Pries et al. [145] collated data on in-vitro RBC flow and developed an empirical model for the ratio of tube haematocrit to discharge haematocrit for RBC flow in glass tubes (where again d_i has units of μm and its coefficients units of $1/\mu\text{m}$):

$$\left(\frac{H_T}{H_D}\right)_{\text{vitro}} = H_D + (1 - H_D) \cdot (1 + 1.7e^{-0.415d_i} - 0.6e^{-0.011d_i}) \quad (2.9)$$

An in-vivo relationship was also developed using this in-vitro relationship by replacing d_i with $d_{i,ph}$ in (2.9) (with $d_{i,ph}$ being the physical diameter of the vessel after the thickness of the ESL has been accounted for) and then multiplying by a scaling factor [143]:

$$\left(\frac{H_T}{H_D}\right)_{\text{vivo}} = \left(\frac{H_T}{H_D}\right)_{\text{vitro}} \cdot \left(\frac{d_i}{d_{i,ph}}\right)^2 \quad (2.10)$$

The results of both the in-vitro and in-vivo empirical relationships can be seen in **Fig. 2.11**.

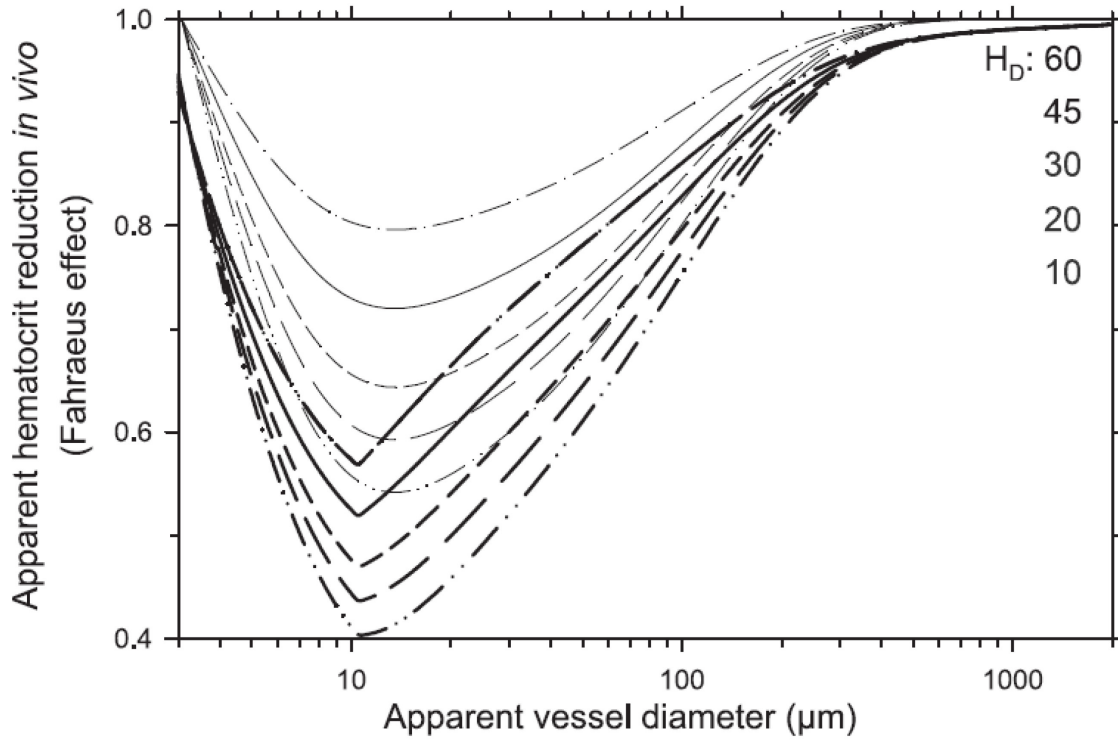


Fig. 2.11 Ratio of the volume concentration of red blood cells in microvessels (H_T) to their concentration in blood discharged from vessels (H_D), against vessel diameter in-vivo. Thick black lines represent the in-vivo model taking into account the ESL (2.10) and the thin grey lines the in-vitro model (2.9). Reproduced with permission from [143].

2.2.3.3 Phase Separation

The final major effect on blood viscosity is phase separation (or plasma skimming). This is an effect due to the network nature of the microvasculature. At bifurcations and trifurcations within a vessel network, RBCs and plasma are observed to be unevenly distributed over the two daughter vessels (**Fig. 2.12**).

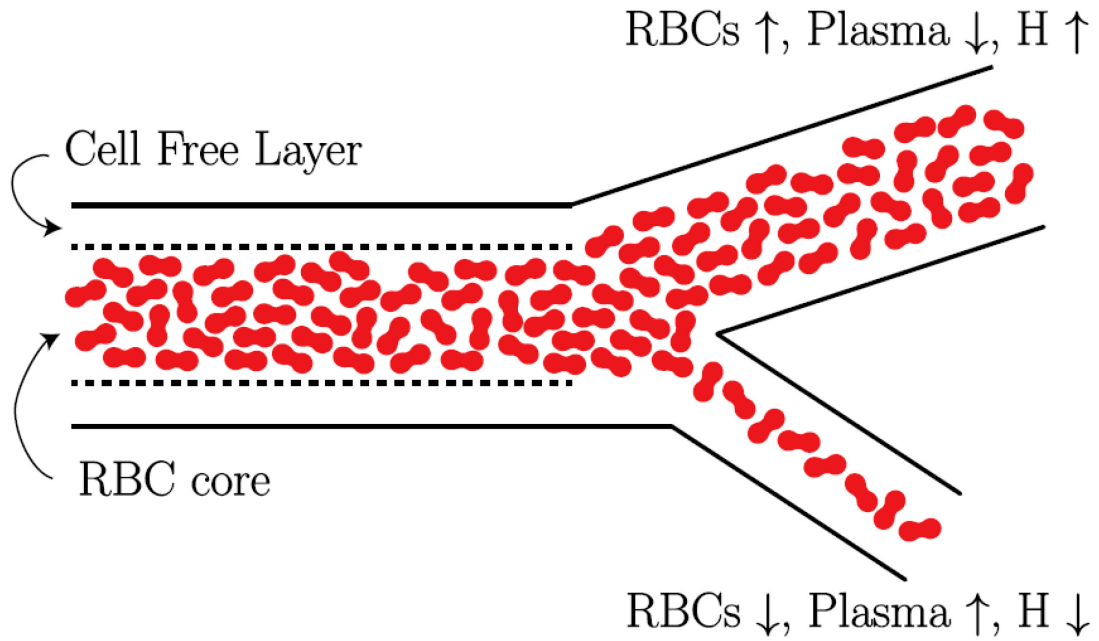


Fig. 2.12 Schematic of phase separation/plasma skimming at a bifurcation. Reproduced with permission from [102].

Pries et al. again developed empirical relationships (based on rat mesentery data) which determined the distribution of blood and cell flows at each bifurcation [145]. This was dependent on the diameters of the parent and two daughter vessels, the discharge haematocrit of the parent vessel, and the fractional blood flow entering that branch. As this model is non-linear it requires an iterative procedure to solve for the haematocrit distribution. Despite this, it is very commonly used when phase separation is accounted for [21,60,111,157]. Other phase separation models have since been developed such as those by Guibert et al. [60], Gould and Linninger [57], and Lee et al. [102].

2.2.4 Oxygen Transport

Blood acts as a vector to supply and to remove solutes across the human body. In particular, oxygen is carried to be metabolised in various cells. The main carrier of oxygen in the blood is the haemoglobin in the RBCs which transports around 98% of the oxygen, with the other 2% being dissolved in the plasma [137]. Once blood reaches an area of lower oxygen concentration, indicated by lower pH due to the release of carbonic acid from cellular respiration [82], the oxyhaemoglobin dissociates releasing oxygen into the plasma. This oxygen diffuses across the capillary endothelial cell layer into the brain cells driven by a concentration gradient.

The binding of the oxygen to haemoglobin can be described as a sigmoidal shape curve and approximated by the empirical Hill equation [165]. There is a very tight coupling between oxygen delivery and oxygen consumption within the brain, due to the very high energy demands of constant neuronal firing and hence the need for ATP consumption. The delivery of oxygen and other solutes is controlled by the cerebral blood flow (CBF). The rate of consumption of oxygen is defined as the CMRO_2 . These are coupled through the oxygen extraction fraction (OEF) which is the amount of oxygen extracted from the bloodstream.

On the most general (continuum) level, the mass transport equation can be used to model oxygen transport through the bloodstream and the cerebral tissue:

$$\frac{\partial c}{\partial t} + \nabla \cdot (\mathbf{u}c - D\nabla c) = g \quad (2.11)$$

where c is the concentration of the solute, $\mathbf{u}$ is the velocity field, D is the diffusion coefficient of the solute, and g the metabolic term (which is non-linear). It is generally considered a good approximation to assume advection dominates in the bloodstream and vice-versa in the tissue.

The solubility of oxygen and dissociation of oxyhaemoglobin are primarily dependent on pressure, temperature and pH [118] which makes the modelling of oxygen transport in a capillary network very complex. One of the earliest discrete oxygen transport models is the Krogh cylinder model [99] which assumes that each vessel supplies oxygen to a surrounding cylinder of tissue of set radius. This can be modified so that consumption of oxygen by the vessel wall is included either by changing the wall permeability, or by incorporating the wall metabolic demand into the diffusion equation [10,120]. For capillaries, due to the very thin walls, the thickness of the capillary wall can be considered part of the interstitium (tissue surrounding the blood vessels). As a result, the concentration gradient is defined from the inside of the capillary wall into a large distance into the tissue [114]. Hsu and Secomb also used Green's functions to model capillaries as line sources within a tissue continuum applied to a section of rat cerebral cortex [72,163]; this was computationally efficient, as well as being more realistic (modelling oxygen in a cerebral network).

This is only a very brief review of oxygen modelling in the cerebral microvasculature as the aim of this thesis is to model blood flow. The general mass transport equation (2.11) will be homogenized in Chapter 3 with stated assumptions, but from then on only blood flow will be simulated.

2.3 The Cerebral Capillary Bed

The human body contains approximately 50,000 miles of capillaries [155]. These are the smallest scale vessels in the microvasculature. It is at this scale that most of the perfusion and oxygen transport occurs, although more recently the idea that most oxygen transport occurs at the capillary scale has been challenged, with pre-capillary arterioles thought to contribute a large part of the oxygen transfer [158]. They are also the most numerous blood vessels in the microvasculature, hence the most

computationally demanding in any models. These vessels are effectively the next scale up from the RBCs. This section of the literature review will review the physiology of the cerebral capillary bed, as well as models developed for analysing perfusion and oxygen transport at this scale. Finally, homogenization theory will be reviewed with a particular application to capillary networks.

2.3.1 Capillary Physiology

Capillaries are the smallest vessels with the thinnest vessel walls. The vessel wall consists of an endothelial cell layer and thin basal membrane (**Fig. 2.13**) and is on average $0.5 \mu\text{m}$ thick, with the average diameter of a capillary around $6.5 \mu\text{m}$ in humans [101]. The endothelial cells have tight junctions with the vessel walls also partially surrounded by pericytes and astrocyte end feet (see **Fig. 2.4**). Together, this forms the blood brain barrier as previously discussed. Capillaries are different from the larger arterioles and venules which also have vessel walls containing collagen (arterioles and venules) as well as smooth muscle and elastin (arterioles) [15].

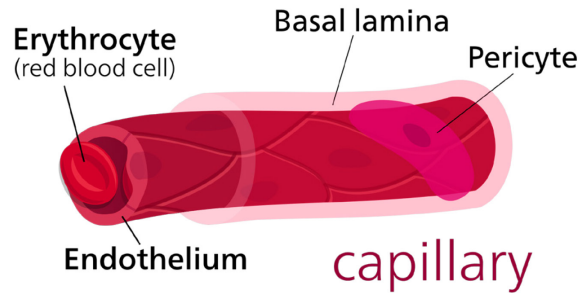


Fig. 2.13 Schematic of a continuous capillary vessel with tight junctions between the endothelial cells at the vessel wall. Reproduced from [90] under Creative Commons license.

The capillary walls also have an endothelial surface layer lined with macromolecules. This layer reduces the lumen size of capillaries as well as slows the plasma on the surface of the vessels resulting in the larger viscosities seen in-vivo (Section 2.2.3). Most transport across the vessel walls occurs through diffusion, with

oxygen and carbon dioxide easily able to diffuse across the endothelial cells. Larger molecules, such as amino acids and peptides, are regulated by the blood brain barrier [2].

2.3.2 Capillary Geometry/Topology

The topological data available on the capillary network can be split into two groups: human and other animal data. It is unsurprising that the latter is more numerous with far more studies conducted on rats, mice, and monkeys than humans. One reason for this is the ability to image flow in capillaries (using techniques such as two photon microscopy) in small mammals. Human topological data at this length scale can currently only be extracted ex-vivo.

The microvascular capillary structure, including its topology, branching angles, and connectivity, is not well understood due to its irregular dense features. One feature that is clearly observable is the heterogeneity of the cerebral capillary network for different regions of the brain [20,26,59,160,161]. Not only are there depth dependent variations of density in the cortex [26,101] but also between primary and non-primary regions of the brain [160]. The overall volume fraction of the microvasculature ranges between 1 and 3% of total brain volume depending on species [20,101,154,183,190].

The cerebral capillary network has been described as “mesh-like” and highly interconnected giving it a robustness (or redundancy) to drops in flow [19,61] (**Fig. 2.14, Fig. 2.17**). This is valid above a certain length-scale (approx. $50\ \mu\text{m}$), at which point the irregular features of the capillary bed appear space-filling and hence homogenous [109]. The diameter of the capillary vessels ranges from around $4\ \mu\text{m}$ up to $10\ \mu\text{m}$, although the upper limit is contested, with no clear indication when the transition between pre-capillary arteriole and capillary occurs [26,190].

There are data available on the topology of rat cortex [19,127], mouse cortex [19,20,183], primate cortex [4,59,154,190], and human cortex [26,42,101]. There is a good recent review of the microvasculature of these various species in Schmid et al. [160], but for the purposes of this literature review I will concentrate on the human microvasculature.

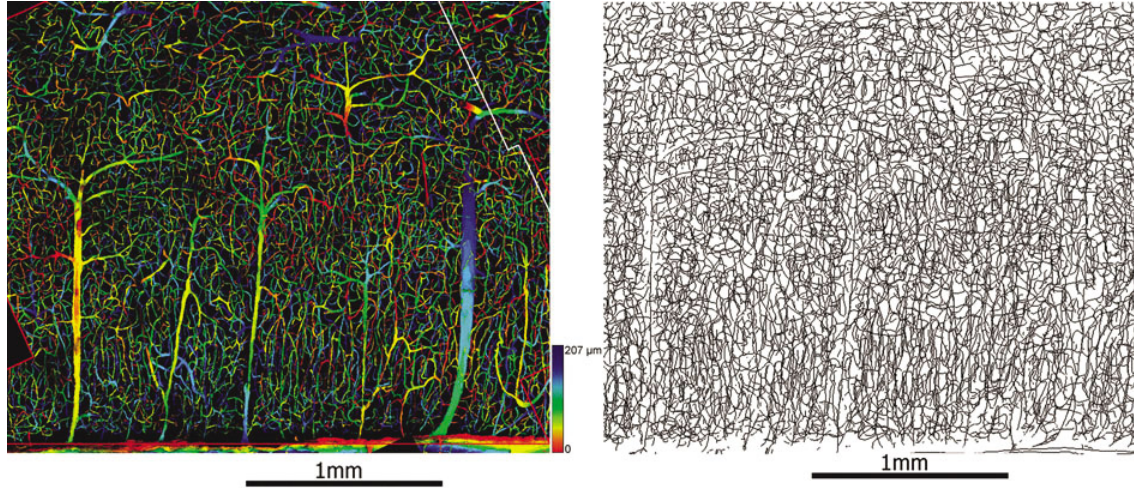


Fig. 2.14 Image of the human cerebral network in the collateral sulcus of the temporal lobe. Both a depth coded projection (left) and corresponding skeleton (right) shown. Mesh-like structure of smaller capillary vessels is clearly shown. Reproduced with permission from [26].

Human cerebral capillary data are currently scarce in comparison with other species data, with the majority coming from 3 imaged regions of the collateral sulcus in the temporal lobe [26,101]. These studies were the first to provide quantitative morphometric data for the human cerebral network. Confocal laser microscopy was used to analyse India-ink injected samples of the brain. These produced detailed maps of the microvasculature with micrometre resolution. From these maps, various statistical parameters were derived such as density of the capillary network, connectivity, length distributions, diameter distributions, vessel orientation, skewness, vascular area, and so forth. Examples of the data generated are shown in **Fig. 2.15** and **Fig. 2.16**. It was found that the capillary density was higher in the middle third

of the cortical depth for this region of the cortex [26]. As well as this, the inter-capillary spacing or mesh-width was found to be on average $50\text{ }\mu\text{m}$, likely due to that being the limit of tissue oxygen diffusion [87].

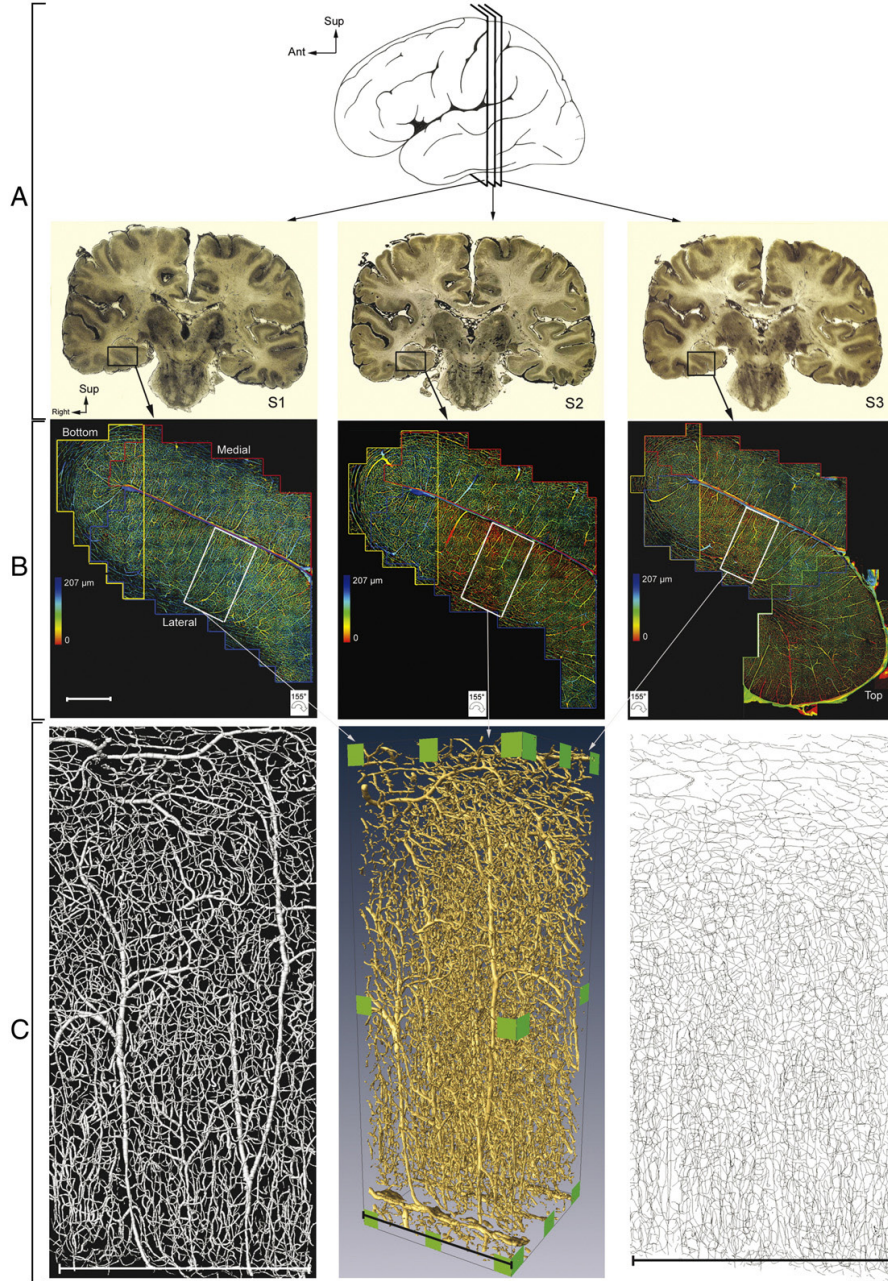


Fig. 2.15 Examples of the casts generated and reconstructed from 3 regions of the human brain. Reproduced with permission from [101].

This topological data of the capillary network are important as it allows for the reconstruction and simulation of flow in these networks [110,111] or the generation

of statistical networks that match these morphological parameters [176]. It is this latter modelling technique that will be extended, with justification, in this thesis.

	Complete network				Capillaries	
	Diam (a)	1/√D (b)	Length (c)	Ln(L) (d)	Diam (e)	Length
Mean	7.82	0.38	52.67	3.54	6.47	52.95
Standard deviation	3.52	0.07	50.38	0.96	1.70	49.75
median	7.19	0.37	36.14	3.59	6.45	36.07
Interquartile range	5.69/9.03	0.33/0.42	15.99/74.3	2.77/4.31	5.20/7.74	16.01/75.7
Skewness	2.96	0.22	2.27	-0.1	0.03	1.97
Kurtosis	20.62	3.23	14.37	2.18	2.21	10.5

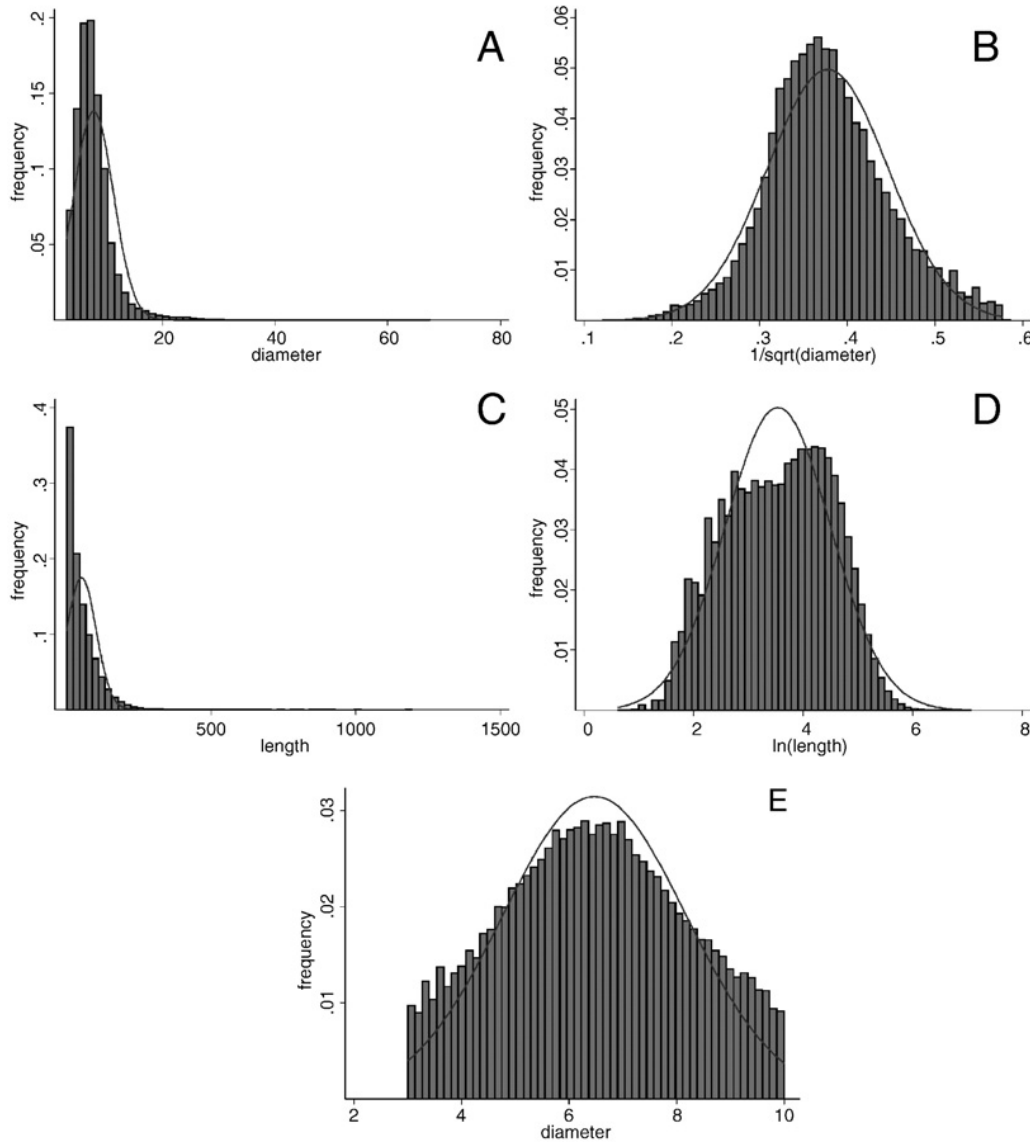


Fig. 2.16 Example of the data generated from the imaged sections of brain as shown in Fig. 2.15. Here diameter and length distributions are quantified for the cerebral network, delineating between the full network and capillaries. The 5 subfigures plot the histograms for the a) diameter, b) $1/\sqrt{\text{diameter}}$, c) length and d) $\ln(\text{length})$

of all the vessels in the vascular network. *e)* represents the histogram for the diameters of the capillary network. The solid black line is the analytical best fit to these distributions. Reproduced with permission from [101].

2.3.3 Models of the Capillary Bed

Due to the inability of current clinical imaging techniques (such as MRI) to distinguish the microvasculature, and subsequently its effects on large-scale perfusion and oxygen transport, mathematical models are essential to help us discern this link. As well as distinguishing between human and non-human models, the modelling techniques can also be broadly split into whether the blood vessel architectures are cast-based models or statistical models. The former reconstructs the vessel network imaged from a given region of the brain (e.g. **Fig. 2.17**). This allows for a specific region of the brain to be analysed in detail. The results, however, are restricted to that given region, and often that given sample due to the large variability observed between samples [68,160,176]. As well as this, these cast based simulations are heavily reliant on the boundary conditions imposed [110]. These boundary conditions are very difficult to elucidate due to the large number of vessels that often are cut when samples are imaged.

The latter approach, the statistical one, depends heavily on knowledge of network characteristics and data. This approach improves as more samples of brain tissue are analysed to provide more statistical information on the network for a region of the brain. The advantage of the statistical approach is its ability to generate a large number of networks that match the morphological data. This allows for the analysis of a large number of networks, and their variability, whilst conforming to the statistical properties derived from the morphological networks. Attempting to image and reconstruct large numbers of casts, in comparison, is extremely costly and time consuming [55].

Nearly all the cerebral capillary models in the literature have appeared in the past 10 years (**Table 2.2**). One of the earliest models of blood-oxygen flow in a capillary is the Krogh cylinder model [99]. This model assumes a standard spatial arrangement of the capillary vessels, each supplying a concentric volume of tissue with oxygen. Whilst useful for tissue with aligned capillaries, such a model is not suitable for mesh-like capillary geometries like that found in the brain.

Author	Species	Capillaries	Penetrating Vessels	Type of Model	Number of Vessels	Model Size - Length x Width x Depth (μm) ³
Zagzoule & Marc-Vergnes, 1986 [201]	Human	Y	Y	Lumped Parameter, 2D	316,858	Whole brain
Secomb et al., 2000 [163]	Rat	Y	N	Cast, 3D	50	140x150x160
Beard, 2001 [17]	Rat	Y	N	Cast, 3D	50	150x160x140
Boas et al., 2008 [21]	Rat	Y	Y	Vascular Anatomical Model, 2D	254	N/A
Fang et al., 2008 [48]	Rat	Y	Y	Cast, 3D	N/A	230x230x450
Reichold et al., 2009 [149]	Rat	Y	Y	Cast, 3D	N/A	N/A (volume 2.8 mm ³)
Guibert et al., 2010 [59]	Marmoset	Y	Y	Cast, 3D	16,000	N/A (volume 18 mm ³)

Lorthois et al., 2011 [110]	Human	Y	Y	Cast, 3D	10,318	N/A (volume 1.6 mm ³)
Su et al., 2011 [176]	Human	Y	N	Statistical, 3D	1073	500x500x500
Safaeian & David, 2013 [157]	Human	Y	N	Statistical, 3D	492	600x380x250
Blinder et al., 2013 [20]	Mouse	Y	Y	Cast, 3D	25,498 (average)	1200x1450x1900
Linninger et al., 2013 [107]	Human	Y	Y	Both, 3D	256,000	3000x3000x3000
Gagnon et al., 2015 [53]	Mouse	Y	Y	Cast, 3D	N/A	600x600x662
Schmid et al., 2017 [161]	Mouse	Y	Y	Cast, 3D	23,496 (average)	1130x1130x1480 (average)
Gould et al., 2017 [58]	Mouse	Y	Y	Cast, 3D	23,496 (average)	1130x1130x1480 (average)

Table 2.2 List of models of the cerebral microvasculature found in the literature. The ‘Type of Model’ refers to the architecture/geometry of the model.

Similarly, casts by Fang et al. (rat brain) [48] and Gagnon et al. 2015 (mouse brain) [53] were reconstructed from 2-photon imaging, with Fang et al. solving a 3D dynamic diffusion-advection equation in the tissue and vasculature. Gagnon et al. used their simulations of the 3D cast models to predict the BOLD signal from first principles, quantifying the contribution of individual vascular compartments to the BOLD signal [54]. Guibert et al. reconstructed marmoset brain vasculature modelling

blood flow [59]. They found that the boundary conditions had a very strong effect on the flow solutions, whereas phase separation had a minor effect on the haemodynamics. More recently, Schmid et al. [161] and Gould et al. [58] used the same casts of mouse cortex vasculature to model blood flow and oxygen transport. Schmid et al. found clear laminar pathways of the RBCs in the flow, shunting between penetrating arterioles and venules (indicating a depth dependence on flow and pressure characteristics). Gould et al. coupled the microvascular cast to a model of blood flow and oxygen transport down to the cellular level (modelling neuronal and glial cells). They found that the wide variation in haemodynamic states in the quasi-random capillary bed was “responsible for relatively uniform cortical tissue perfusion and oxygenation” [58].

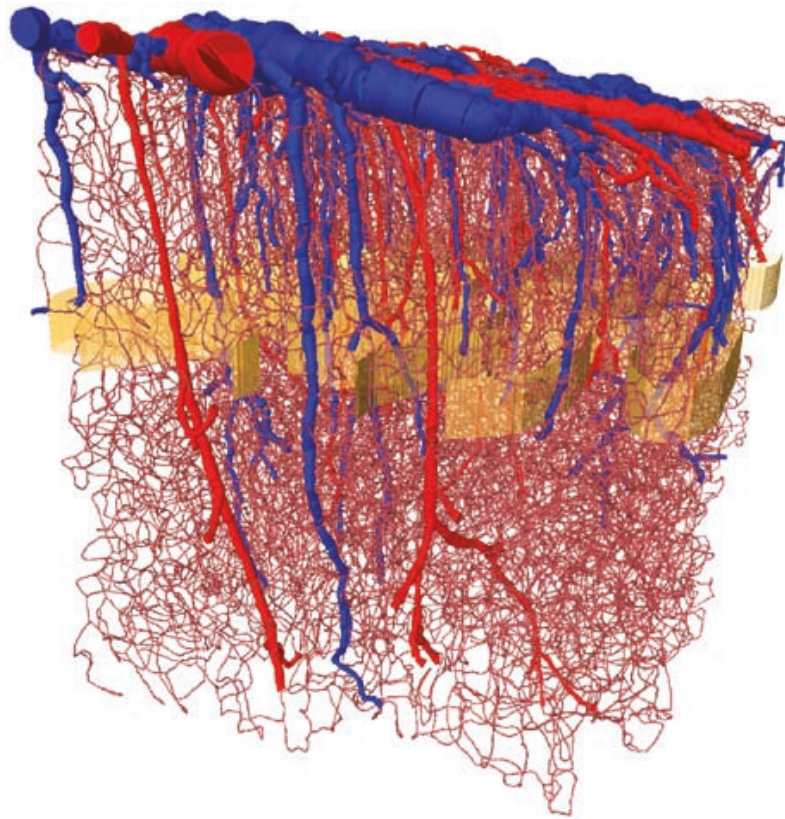


Fig. 2.17 A cast model of a region of the murine cortex displaying the ‘mesh-like’ capillary network, as well as the tree-like penetrating vessels, and surface pial vessels. Reproduced with permission from [20].

All the cast models previously discussed come from the cerebral microvasculature in animals. There is, however, one cast model of the human microvasculature listed in **Table 2.2**. Lorthois et al. [110] reconstructed one of the casts imaged by Cassot et al. [26]. A steady state blood flow model was used, along with a correction for viscosity [144]. Boundary conditions were investigated, determining an upper/lower bound for the baseline perfusion rate of the vasculature. It was found that boundary conditions had a large effect on the perfusion.

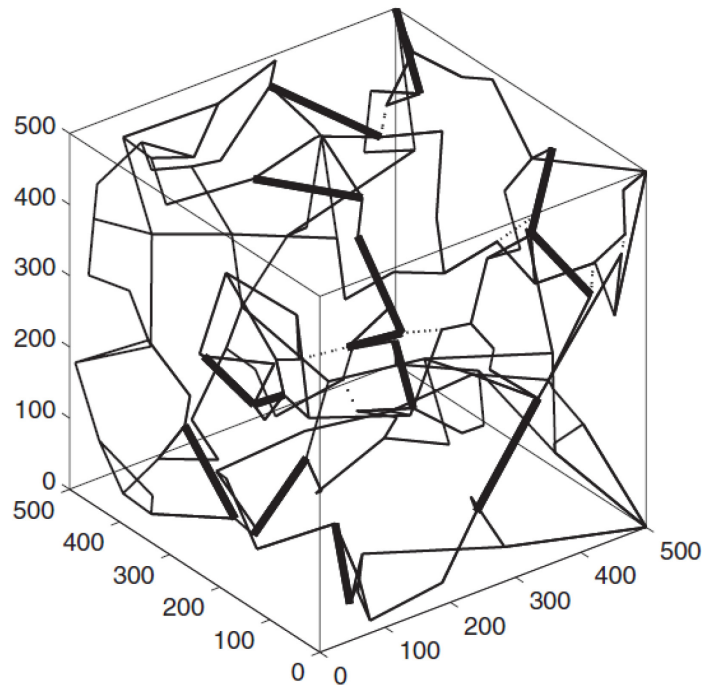


Fig. 2.18 Statistical model of the human cerebral microvasculature (axes units in μm). Adapted with permission from [176].

Due to the difficulty in imaging cerebral vascular networks, it has been suggested that the data obtained from these casts can be used to generate statistical networks. These statistical models conform to morphological statistical parameters, such as those in **Fig. 2.16**, but are not constrained to the original shape or size of the casts. Su et al. showed how a network capillary model could be constructed from the available data (**Fig. 2.18**), using parameters such as lengths, diameters, and

connectivities [176]. This capillary model was validated against morphological data [26,101]. Safaeian and David also modelled the capillary network statistically, using Voronoi tessellation [157]. This model was used to study oxygen transport in capillary networks, finding that large increases in CBF are required for moderate increases in OEF. A similar Voronoi tessellation model was used by Linninger et al. [107] to model large volumes of the human cerebral cortex (27 mm^3) coupled to penetrating arterioles and venules. It should be noted that all of these human models are based on the dataset generated by Cassot et al. and Lauwers et al. [26,101].

2.3.4 Homogenization of the Capillary Bed

There are intrinsic difficulties associated with both the cast and statistical models of the microvasculature. Firstly, the scalability of the models; the human cerebral capillary network has a density of between $7,500 - 10,000 \text{ vessels/mm}^3$ [26,101]. Therefore, to model large volumes of the brain becomes extremely computationally expensive if the capillaries are modelled individually. It may not be fully feasible to resolve large vascular systems. Secondly, the lack of knowledge about boundary conditions means it is difficult to determine perfusion characteristics purely from the geometry of the network.

Continuum-based mathematical modelling techniques, specifically homogenization, aim to deal with these issues. Homogenization is an averaging technique which derives macro-scale models using micro-scale models, assuming that there is scale separation between the two sizes and that the micro-scale model is periodic (see **Fig. 3.2**). Using such methods results in the discrete micro-scale representation being replaced with averaged integral quantities of the capillary bed valid over the larger region of interest, reducing the computational cost substantially [69,168,199]. A key advantage of this continuum approach is that the results are

independent of the choice of boundary condition. The method of homogenization is explained in detail in Chapter 3.

Homogenization has been used in a wide variety of engineering and physics problems: from modelling materials with microstructure [126,153,200], to developing models of water and petroleum reservoirs [9,192]. Its use in biology, however, has been limited. Shipley and Chapman [168] developed these mathematical averaging techniques on a leaky capillary bed to derive an averaged macroscopic Darcy flow model. The permeability tensor of this flow model is what links the capillary scale topology to macro-scale blood flow, and is the property of interest when scaling up discrete models. Characterizing this tensor for a given network allows for the modelling of larger continuum models of the cerebral microvasculature.

Smith et al. [169] have previously used the averaging techniques derived by Shipley and Chapman on a 2D model of non-leaky rat myocardial capillaries, characterising the network with the calculated permeability tensor. Reichold et al. [149] formulated an ‘upscaling’ approach for averaged quantities of an artificial 2D grid. This was an early demonstration of the efficacy of volume averaging techniques on the microvasculature. However, there have been no applications of homogenization theory on physiologically accurate 3D cerebral networks.

2.4 The Penetrating Arterioles and Venules

The next scale up from the capillary bed is the penetrating vessels. These vessels descend from the pial surface, penetrating into the grey matter. They control the supply of blood and oxygen to the capillary bed, as well as contributing a large part of the oxygen transfer to the tissue [59,158]. The structure of these vessels however – in particular their topology – is intrinsically different to the capillary bed, hence warranting their separate treatment.

2.4.1 Arteriole and Venule Physiology

Common to the arterioles, venules and capillaries are the endothelial cell layer and thin basal membrane. Both arterioles and venules also have layers of smooth muscle surrounding them; the venules to a much lesser extent than the arterioles [15]. The arteriole walls also contain elastin, allowing them to expand/contract [137]. These smooth muscles regulate the vascular resistance of the arterioles by changing the vessel diameter [161]. As a result, arteriolar walls are much thicker than venule walls. This stretch/contractile capability also helps arterioles to control blood flow with high pressure and pulsatility. Capillaries and venules don't require thick walls as by that stage in the microvasculature the pulsatility has been severely dampened [18].

Post-capillary venules have almost no smooth muscle since they are functionally more similar to capillaries than veins [15]. Consequently, it is arterioles that serve as the flow regulators for the capillary bed. Contraction of the smooth muscle cells in the wall of the arteriole can change the diameter of the lumen resulting in large changes to vascular resistance (see Poiseuille flow equation (2.3)) that can reduce or increase blood going to the capillary bed. Therefore, pre-capillary and penetrating arterioles are predominantly responsible for the increase in CBF that results from neural activation [49].

2.4.2 Penetrating Vessel Geometry/Topology

Data that specifically deal with the topology of bifurcating penetrating vessels in the human cortex can also be found in the literature. Branching angles, asymmetry, length distributions, diameter distributions, length-to-diameter ratios, total vessel length density, tree densities, area ratios, tortuosity, and Murray's exponent are among the parameters determined from the analysis of the casts that have been imaged [26–28,42,101,112].

Human cerebral penetrating arterioles have diameters that range from 10 – 240 μm and similarly venule diameters range from 10 – 120 μm [42]. It should be noted that generally speaking the arteriolar diameters are smaller than the venular diameters (35 μm vs. 65 μm for group 4 vessels on average – group 4 vessels being those that penetrate down to the white/grey matter interface) [42]. This is the opposite case in mice where average diameters are 11 μm for arterioles and 9 μm for venules [20].

From human data readily available (samples of tissue that total 28.6 mm^3 analysed by Cassot et al. and Lauwers et al. [26,101]) it is found that the number of arterioles and venules have a 2:1 ratio in the vasculature [27] (opposite to the 1:3 ratio for rats [19,127]). This correlates with the fact that the venules are larger than the arterioles in humans, thus draining larger volumes of the tissue. The shapes of the two forms of penetrating vessels also differ, with the venule often penetrating deeper and branching out laterally from the penetrating trunk. The arterioles on the other hand have more of a candelabra (or weeping willow) shape, where they tend to branch up towards the pial surface (**Fig. 2.19**) [42]. The majority of the penetrating vessels terminate roughly halfway into the grey matter. The main trunk of the vessels is often found to maintain its diameter throughout, i.e. there is not much tapering [42]. It is interesting however, that the sinuousness of these vessels tends to increase closer to the white matter, and more generally with age, potentially an indicator for vascular degeneration [42].

Despite the penetrating vessels comprising approximately one fifteenth of the number of vessels in the microvasculature (arterioles 385 / mm^3 ; venules 281 / mm^3) they contribute ~52% of the vascular volume [101]. This is due to their larger diameters (and diameter squared dependence on volume). Little is known about the pial surface distribution of the penetrating vessels [160]. Duvernoy et al. [42] suggested a ring distribution, where each penetrating venule is surrounded by a ring of arterioles.

Blinder et al. [20] suggested a rhombic lattice where one arteriole is surrounded by 6 venules (due to the greater incidence of venules in the rat cortex). However, the general distribution of these vessels is still an area of open research.

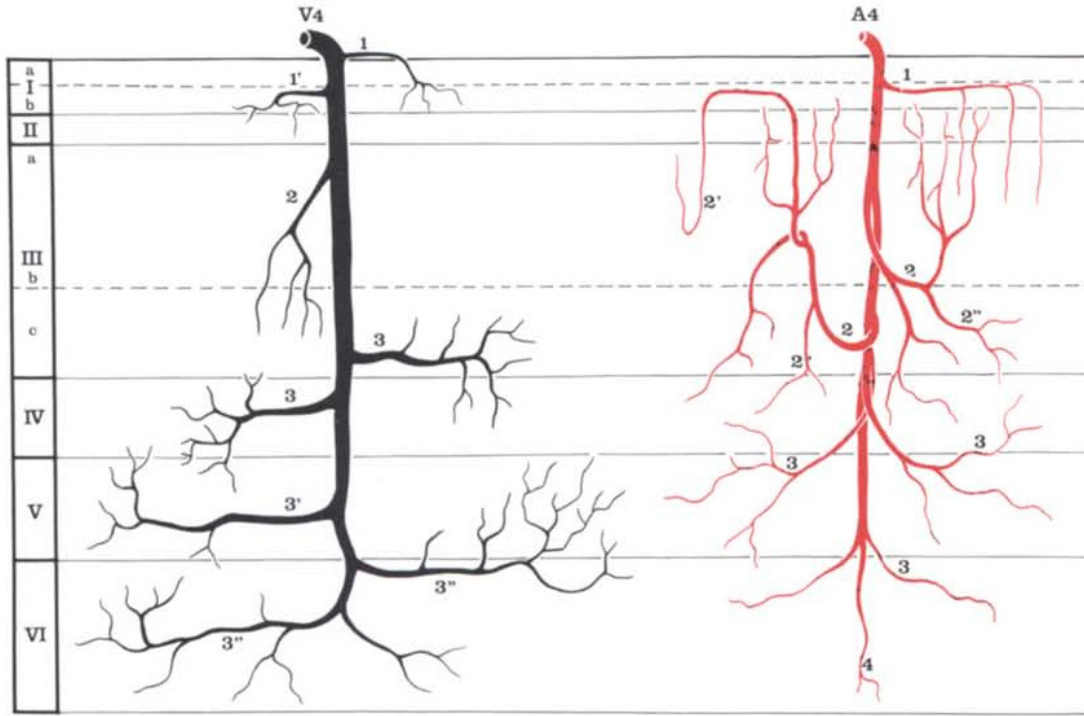


Fig. 2.19 A typical penetrating venule (left) and penetrating arteriole (right) in the human cerebral microvasculature. Notice the more lateral branching of the venule, as opposed to the more ‘bell-like’ arteriole. Reproduced with permission from [42].

The bifurcating nature of the vascular trees lends itself to a hierarchical ordering and analysis. A popular way of characterizing this hierarchy is the diameter-defined Horton-Strahler taxonomy [71,84,173]. Starting at the terminal vessels, given order 0, the parent vessel is given an order one above the largest order of its two daughter vessels if the diameter is larger than a given threshold [84]. Using such a scheme, Cassot et al. found that human penetrating vessels have approximately 4 – 5 orders of branching [26]. Additionally, these trees were found to be quasi-fractal, obeying scaling laws [28].

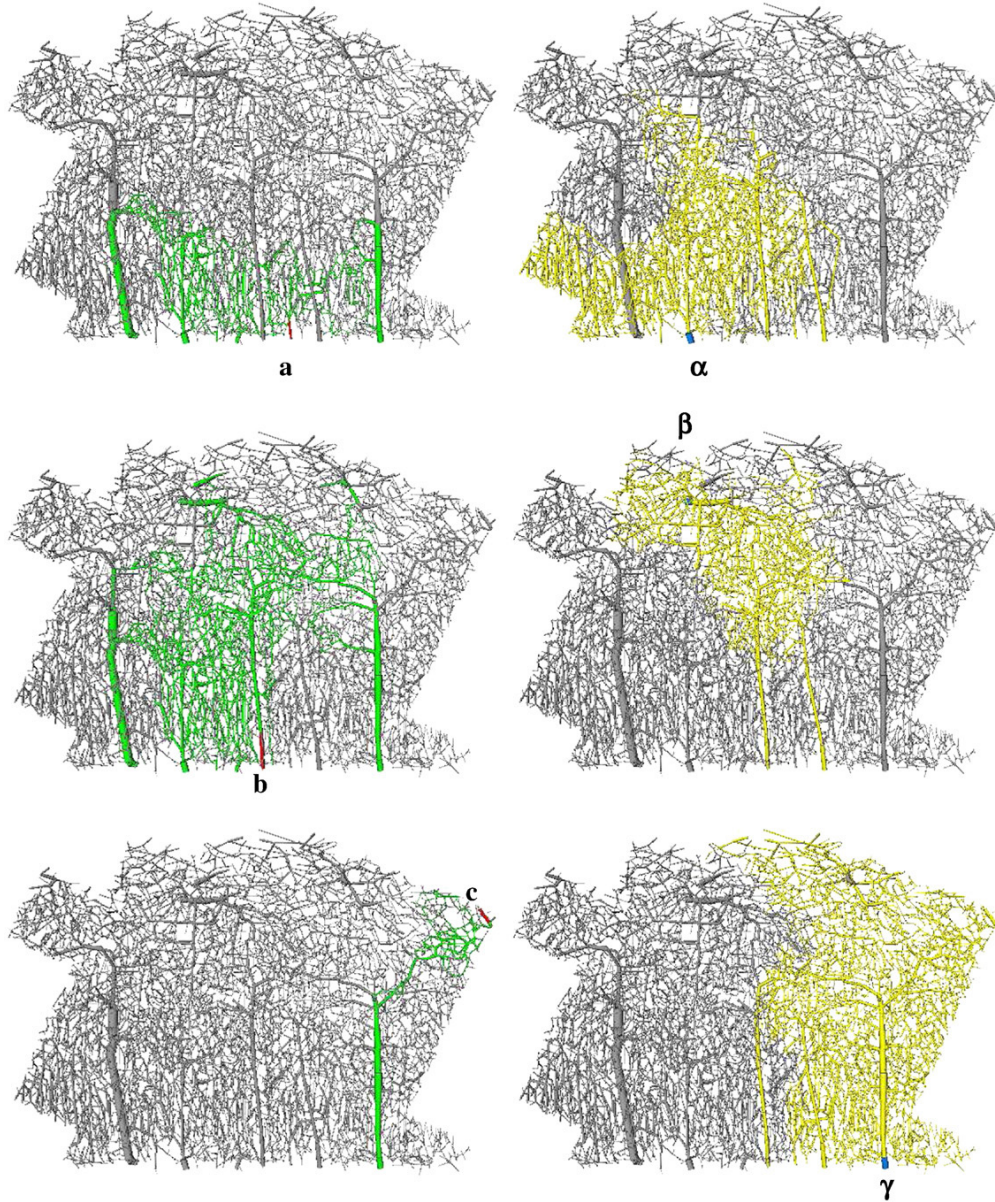


Fig. 2.20 Vascular regions in the human microvasculature fed and drained by the arterioles (a, b, c) and venules (α , β , γ) respectively. These regions do not display clear columnar regions for each penetrating vessel, nor a pairing between the arterioles and venules. The regions fed and drained overlap substantially. Reproduced with permission from [110].

Finally, the volume of tissue fed by these penetrating arterioles is also of interest. Guibert et al. [61] determined this volume using blood flow simulations on a cast of a region of marmoset cortex. They found that a penetrating arteriole fed a radius of $386 \mu\text{m}$ with a 2 mm depth on average. This, however, is an averaged value.

In reality, there is a large amount of interconnectivity in the supply of blood to tissue. They found that there is a degree of robustness in the network – which provides redundancy to protect against occlusion. On average, one penetrating venule collected flow from 14 arterioles and there was approximately a 50% overlap between territories of neighbouring penetrating arterioles and venules [61]. These overlapping territories were also observed by Lorthois et al. (**Fig. 2.20**) [110]. Robustness of the network also increased with depth in the cortex. The flow regions of these vessels were found not to overlap with functional cortical columns, refuting the concept of a neurovascular unit [20]. Despite this, it was still found that the tissue directly surrounding a penetrating vessel, particularly in the superficial layers, depended substantially on the blood supplied by that penetrating vessel [20,129,166].

2.4.3 Penetrating Vessel Occlusions

Studies in patients with dementia identified a large number of cortical micro-infarcts with diameters in the order 0.1 – 1 mm [11,85,98,186]. However, whether these are predictive of or a cause of dementia is as yet unknown due to the inability of conventional clinical imaging to resolve at the length scale of penetrating vessel diameters.

As a result, some research has been conducted with rat and mice models to determine the effect of a penetrating vessel occlusion on the surrounding tissue. Nishimura et al. [129] occluded penetrating vessels in the rat parietal cortex finding clear conical volumes of tissue with reduced RBC flow, with flow restoring 350 μm radius from the occlusion, and deeper into the cortex. This led to the penetrating vessels being labelled as a “bottleneck in the perfusion of the neocortex”. Similarly, Shih et al. [166] also occluded penetrating vessels in rat cortex. The measured necrotic tissue damage was also conical in shape (**Fig. 2.21**) with greater robustness to damage deeper and further from the penetrating vessel (230 – 250 μm radius of damage). They

also found that larger vessels (hence larger flux vessels) led to larger volumes of damage, indicating a coupling between penetrating vessel diameter and volume of tissue fed. This coupling of diameter and tissue fed/drained was also shown by Lorthois et al. in human brain blood flow simulations [110], and Blinder et al. in the mouse cortex [20]. From these simulations, Blinder et al. observed a shunting of flow between arterioles and venules surrounding the penetrating occlusion, stopping the reperfusion of the microvasculature surrounding it.

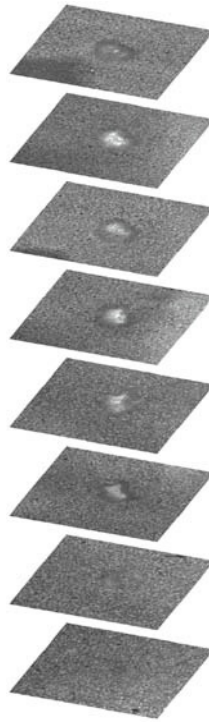


Fig. 2.21 Clear conical region (central white stain) of necrotic damage post-occlusion of a penetrating arteriole in rat cortex. Adapted with permission from [166].

2.4.4 Models of the Penetrating Vessels

To the best of my knowledge, no models of penetrating vessel occlusions in the human microvasculature are available. There are, however, models of the penetrating vessels. One of the earliest attempts to model the tree-like nature of the penetrating vessels was developed by Murray in 1926, hence becoming known as Murray's Law [121]. This law is a very elegant optimization between energy required to pump blood around

the body (volume of the blood) and frictional losses in the blood vessels (assuming Poiseuille flow). From this optimization, it is found that for maximum efficiency in the circulation the parent and daughter vessels are related by the cube of their radius [121,122]:

$$r_0^3 = r_1^3 + r_2^3 \quad (2.12)$$

where r_0 is the radius of the parent vessel and r_1 and r_2 are the radii of the daughter vessels at the bifurcation. More generally, however, Murray's Law is written using an arbitrary power k which can vary in the microvasculature. Analysis of the human cerebral microvasculature found that Murray's law is "broadly acceptable as a macroscopic approximation, but inadequate when employed at bifurcation level" [28]. The k exponent for these cerebral penetrating vessels was found to vary by up to 3 orders of magnitude, with a median of 3.58 and inter-quartile range of 2.28 – 14.5 [28]. This indicates that the assumption of optimality on the microvascular scale may not in fact be valid, with bifurcation design likely being more complex.

Regardless, based on these optimality principles, models of generating arterial trees have been proposed, such as constrained constructive optimization (CCO) [162] and staged growth CCO [86] (see **Fig. 2.22** for an example of a tree generated using CCO). These are heuristic models that aim to use optimality principles, such as Murray's law, to generate arterial trees. Linninger et al. used CCO to construct the 36 penetrating arterioles and 12 penetrating venules in their model of the human microvasculature [107]. Although using such optimality principles to construct the microvasculature is useful, it likely does not fully reproduce the large variations in the microvasculature observed (due to the assumption of a constant k exponent).

The clear majority of models of the penetrating vessels are cast based. Guibert et al., Reichold et al., Schmid et al., Fang et al., Blinder et al., and Gagnon et al. all

reproduced casts of the penetrating vessels in order to model flow through them (**Table 2.2**) [20,48,53,59,149,161]. Guibert et al. found that the pressure boundary conditions imposed at the inlet of the arterioles and outlet of the venules have large effects on the CBF, whilst modelling phase separation has a minimal effect [59]. Reichold et al. observed that the dilation of the penetrating vessels does lead to increased flow in the capillary bed but this increase is limited to a cortical depth that coincides with the depth of the deepest feeding arterioles, indicating a strong topological dependence of flow in the microvasculature dominated by the penetrating vessels [149]. Schmid et al. observed a layering of flow in the microvasculature whereby RBCs tended to move in layers through the capillary bed between one arteriole and one venule [161]. Gould et al. found that the heterogeneity of the structure of the rat microvascular cast was responsible for relatively uniform cortical tissue perfusion and oxygenation [58].

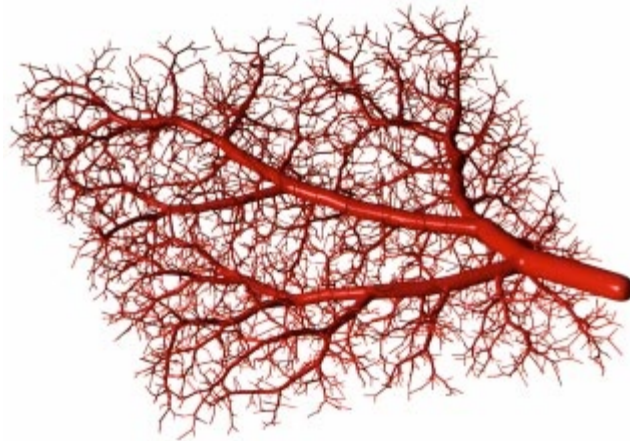


Fig. 2.22 Example of an arteriolar tree generated using staged growth CCO branching principles. Adapted with permission from [86].

Human models of the penetrating vessels are limited. As mentioned previously, Linninger et al. developed a model of the penetrating vessels using CCO [107]. Lorthois et al. on the other hand used a cast based model of the human microvasculature, again observing the strong dependence on boundary conditions, as well as the fairly

large overlapping regions of tissue fed and drained by the arterioles and venules [110]. There are, as far as I am aware, no statistical models of the penetrating vessels in the human cerebral microvasculature.

2.5 Large-Scale Models of the Microvasculature

Large-scale models of the microvasculature are defined here as being models with a length scale in the order of mm. Such models are valuable in that this is the length scale which coincides with the current typical resolution of clinical imaging techniques e.g. fMRI [123] (see Section 2.6). Therefore, having models at this length scale will allow for a clearer comparison between the microvascular architecture and what is clinically observable (such as CBF and transit time distribution (TTD)).

The large-scale models available in the literature have been discussed in the previous sections (Sections 2.3 and 2.4), linked to the discussion of the capillary networks and penetrating vessels, whose combination forms larger microvascular models. The majority of these large-scale models are cast models (only Linninger et al. have a coupled statistical and cast based model [107]). These models are valuable as they allow us to observe how flow behaves on the micro-scale, and hence how the topology of the microvasculature influences parameters such as CBF. However, these models are all heavily dependent on boundary conditions, and are specific to a given region. Therefore, the simulation results are restricted to the experimental conditions from which they were derived.

Lorthois et al. used a cast based model of the human microvasculature, the total volume of which was 1.6 mm^3 with approximately 10,000 vessels (**Table 2.2**). Linninger et al. developed both a statistical and cast based model of the human microvasculature with a total volume of 27 mm^3 and 256,000 vessels (**Table 2.2**). As can be seen, the larger these models get, the rapidly more computationally expensive

it becomes to model blood flow through every vessel. If 1 cm^3 of the microvasculature was to be modelled, it would be necessary to model flow through approximately 8 million blood vessels. This makes achieving whole organ models of the brain very difficult to do.

However, whole organ models of the microvasculature have been achieved for more simply vascularized organs such as the heart [74,117] and the lungs [45]. Such models were developed by assuming a homogenized capillary network (at differing scales) coupled to a cast of the major feeding arteries and veins (**Fig 2.23**). It is therefore possible to develop whole organ models of the human brain, although it will be more difficult due to the high density and variability in the microvasculature. Nevertheless, using homogenization techniques, it should be possible to scale-up the modelling of the microvasculature beyond order mm voxels.



Fig. 2.23 Whole organ model of porcine heart, using a cast for the major arteries and veins, with flow through the microvasculature treated as Darcy flow based on a homogenization of the network at separate scales. Reproduced with permission from [117].

2.6 Imaging the Microvasculature

Despite the importance of the microvasculature in maintaining a healthy supply of blood and oxygen to the brain, little is known about the specific effects that the topology of these vessels has on blood and oxygen transport. This is predominantly due to the low resolution of modern clinical imaging techniques; only managing to discern vessels larger than approximately 0.8 – 0.9 mm in diameter [123]. Therefore, imaging the microvasculature currently requires more invasive techniques that are more readily applied to ex-vivo tissue and in-vivo in animals.

2.6.1 Ex-vivo Measurements

Ex-vivo techniques have been used on primate, rat, and human brain [26,138,154,190]. These measurements have provided us with the majority of our knowledge on microvasculature densities, branching angles, lengths, diameters, etc. Numerous methods have been used, including intravascular dye injections [19,42], staining of vessel components [51,89,179], and vascular corrosion casts [42,150,190]. Their use permits imaging of large regions of the microvasculature at high resolution [198].

For example, Cassot et al. [26] used sample sectioning with confocal laser microscopy on 300 μm thick slices of human cerebral tissue that had India-ink injected through the vasculature (**Fig. 2.24**). Plouraboué et al. [138] used epoxy-resin cast primate microvasculature imaged using high resolution synchrotron tomography (ionizing radiation imaging). Weber et al. [190] used vascular corrosion casts of primate brain imaged using scanning electron microscopy. Blinder et al. [19] used two-photon microscopy (TPM) to image rat brain – a technique more commonly used in-vivo. All these imaging modalities are able to produce full images of the microvasculature down to μm resolution.

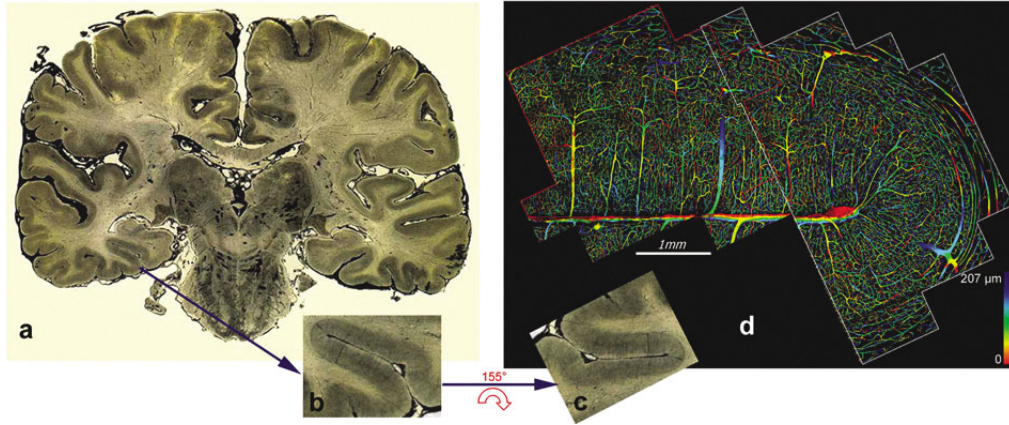


Fig. 2.24 Cast of the human microvasculature generated using India-ink perfused sections of the collateral sulcus in the temporal lobe imaged with confocal laser microscopy. Reproduced with permission from [26].

Although ex-vivo measurements can provide us with detailed maps of the microvasculature, they are not without their problems. Firstly, the imaging depth is limited due to the finite penetration of the imaging methods [68]. As a result, each cast imaged can only be at most a few hundred microns deep making it difficult to quantify the volumetric parameters and 3D nature of the networks. As well as this, the treatment of the vascular networks often results in vessel shrinkage, requiring a correction to be made to better represent the true diameters of the vessels [68]. The diameter uncertainty is particularly problematic due to the fourth power of radius that the flow depends on (Poiseuille's Law), heavily influencing the flow in the simulated networks.

2.6.2 In-vivo Measurements

2.6.2.1 Non-Invasive

Non-invasive techniques are characterised by their relatively low resolution with regards to the microvasculature [37]. The microvessels below approximately 1 mm in diameter remain 'unseen' using these imaging modalities [123]. Diffuse correlation spectroscopy (DCS) can be used non-invasively to measure CBF in humans. Due to

the need to penetrate through the skull, there is no depth resolution, with only superficial CBF measured, with a lateral resolution of 1 – 3 cm [93]. However, blood vessel imaging is not possible due to the poor resolution.

Transcranial Doppler (TCD) is another non-invasive technique often used to measure cerebral blood flow velocity (CBFV) in the major arteries e.g. the posterior cerebral artery [1]. This technique is based on ultrasound penetrating into cerebral tissue. CBF can be estimated from the CBFV assuming a constant area of the vessel. TCD is simple to use and inexpensive, allowing for a continuous monitoring of CBF in the brain. However, for the purposes of the cerebral microvasculature, it is unable to resolve any small scale vessels below the major arteries.

The most commonly known (and popular) non-invasive imaging method is magnetic resonance imaging (MRI). The physics behind MRI is beyond the remit for this brief literature review, but it is based on the principle of nuclear magnetic resonance, where hydrogen atoms release detectable radio frequencies post-relaxation [35]. MRI has many advantages over other imaging modalities, as it does not require harmful ionizing radiation (like that found in X-ray CT scans), and has resolution down to just below 1 mm [123] (depending on the strength of the magnetic field). However, the trade-off is poor temporal resolution, with patients often required to spend tens of minutes up to hours in MRI scanners, as well as the claustrophobic discomfort and noise often associated with it [35].

It should also be noted that MRI does not measure absolute CBF directly, but rather measures the MR signal intensity of concentration fields. This signal can then be interpreted by making assumptions about the underlying vasculature (such as a constant haematocrit), and using some sort of model inversion (such as a deconvolution) to extract a CBF value [25].

2.6.2.2 Invasive

There are a number of techniques available that invasively measure the cerebral microvasculature (and RBC flow) down to a resolution of $1\ \mu\text{m}$ [37,55]. One such technique is multi-photon laser scanning microscopy (MPM) which detects photoluminescence “from endogenous or exogenous chromophores” with images created from the sequential point measurements [55]. This technique is typically applied using a glass-covered cranial window, with depth penetration greater than 1 mm into the cortex [70,97]. However, the time resolution is poor, requiring 10s of minutes to acquire a 3D angiogram over a 1 mm field of view [55]. Two-photon microscopy, which has been extensively used to measure RBC velocities, vessel diameters [38,95,96,178] and the reorganisation of flow post-penetrating vessel occlusion [129], is a subset of MPM.

Optical coherence tomography (OCT) is another invasive in-vivo technique utilising a cranial window. It does not require contrast agents, and works by detecting scattering within tissue, in a similar fashion to ultrasonic pulse-echo imaging (but optical) [170,184,189]. Spatial resolution is between 1 and tens of μm with a typical penetration depth of around 1 mm in rodent brain [170]. However, there is currently a lateral and depth trade-off when imaging. OCT has an advantage over MPM with a much better time-resolution, taking seconds rather than minutes for a comparable volume of tissue [55].

Finally, photoacoustic imaging (PAI) uses excitation light that is absorbed by chromophores, releasing heat. The images are formed by detecting ultrasonic waves generated by thermoelastic tissue expansion [188,196]. Spatial resolution is found to be a few μm with a depth penetration of approximately 1 mm [73].

Comparing the in-vivo invasive and non-invasive techniques, it is clear that there is an imaging gap, where invasive techniques are able to image the entire microvasculature (down to μm resolution), whilst the non-invasive techniques struggle to obtain resolutions much below 1 mm. Therefore, in human brains, ex-vivo imaging is required in order to visualise the microvasculature. Along with in-vivo imaging of primate and rodent microvasculature, data can be generated to build mathematical models of the microvasculature that can help in interpreting non-invasive signal dependence on the microvasculature.

2.7 Cerebrovascular Disease

Cerebrovascular diseases can lead to life-changing conditions, often requiring 24/7 care for the patient, and eventual death. The incidence of these diseases, such as dementia, is increasing due to an aging population and the associated risks of infarcts and haemorrhage increasing with it [124]. Due to the low resolution of the clinical imaging modalities discussed above, the role of the microvasculature in such diseases is poorly understood.

2.7.1 Dementia

In 2015, dementia overtook cardiac disease to become the leading cause of death in England and Wales with 11.5% of deaths recorded that year being attributed to dementia [132]. One in every 14 of the over 65 population has dementia [6], which rises drastically to 1 in 6 of people over the age of 80 [7]. Of the 850,000 people living with dementia in the UK, 40,000 are under the age of 65, indicating the very strong correlation with age of this disease [7,77]. Dementia is characterised by a decline in memory, language, problem-solving, and other cognitive skills that affect a person's day-to-day life, making them reliant on care [6]. Dementia is currently irreversible, with no known cure.

The major causes of dementia are Alzheimer's disease (AD) and Vascular dementia (VaD) with 70 – 80% of all cases attributed to them [56]. In AD, a high density of amyloid plaque and neurofibrillary tangles are found in the brain parenchyma [180]. This is currently attributed to the accumulation of Amyloid-beta peptides, which trigger apoptosis [195]. Since Amyloid-beta is produced naturally in the brain, its accumulation is likely due to the inability of the vasculature to clear it resulting from vascular damage [100]. Therefore, although Alzheimer's disease has traditionally been thought to be due to the formation of amyloid plaques and neurofibrillary tangles, it is now appreciated that there is also a strong vascular component to this pathology, [182].

The path to vascular dementia, however, has no characteristic indicators such as the Amyloid-beta accumulation in AD [31]. The path to VaD is through cerebrovascular disease which eventually leads to brain injury and dementia [31]. VaD can occur through ischaemia within the cortex, white matter or thalamus, as well as in large, small, and micro blood vessels [23]. VaD can also be characterised by its aetiology, such as embolism, hypoperfusion, or amyloid angiopathy [32]. Post-mortem studies of patients with dementia have identified a large number of cortical micro-infarcts, 0.1 – 1 mm in diameter [11,85,98,186] (**Fig. 2.25**). Therefore, some forms of VaD may result from an accumulation of these micro-infarcts in the microvasculature, particularly in the penetrating vessels [129,166]. However, identifying these micro-infarcts, which could potentially be predictors for dementia, is very difficult to do due to the low resolution of current typical clinical imaging.

Although AD (neurodegenerative) and VaD (cerebrovascular disease) were traditionally thought to be different pathologies, increasing evidence suggests an overlap of reciprocal interactions between these two diseases [76]. Vascular factors, in

particular, play a role in both diseases [75]. Additionally, coexistence of VaD and AD is found in brain samples of a large number of patients [23].

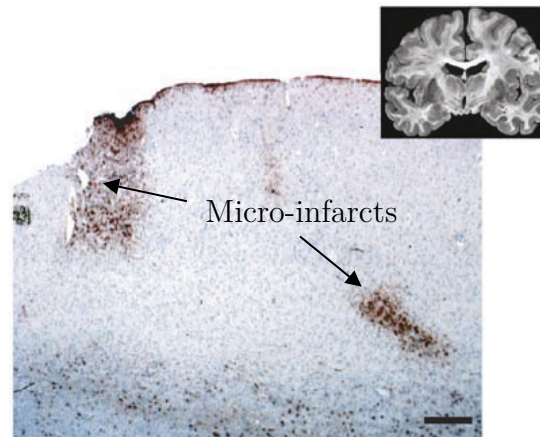


Fig. 2.25 Histology of micro-infarcts detected in the cortex of an elderly human (ex-vivo). Adapted with permission from [166].

2.7.2 Stroke

Stroke is a sudden disruption of blood and oxygen transport to a region of the brain, resulting in sudden cognitive decline. It is the fourth largest cause of death in the UK, and the second largest in the world [174]. Although deaths from strokes have been decreasing in the UK, stroke still results in a high incidence of disabilities, often requiring prolonged care [174].

A stroke can be ischaemic (resulting from a blocked vessel) or haemorrhagic (resulting from a burst vessel). Approximately 85% of strokes are ischaemic [125], with 20% of these fatal. Half of the haemorrhagic strokes are fatal [8]. There also exist transient ischaemic attacks (TIA) which are ‘mini-strokes’ often as a result of a temporary reduction of blood flow to a region of the brain. These are often predictors for more severe stroke later in life [194]. The main types of ischaemic stroke are embolic – a clot blocking a blood vessel – or thrombotic – build-up of atherosclerotic plaque on vessel walls causing narrowing and potential clot formation in the vessel [5] (**Fig. 2.26**).

Haemorrhagic strokes on the other hand are caused by burst vessel walls, often as a result of weakened vessels due to high blood pressure or aneurysms. Once the vessel wall bursts, the bleed into the brain can be classified as intra-cerebral (where a vessel has burst within the brain) or subarachnoid (where a vessel has ruptured on the surface of the brain). The haemorrhage results in raised intracranial pressure.

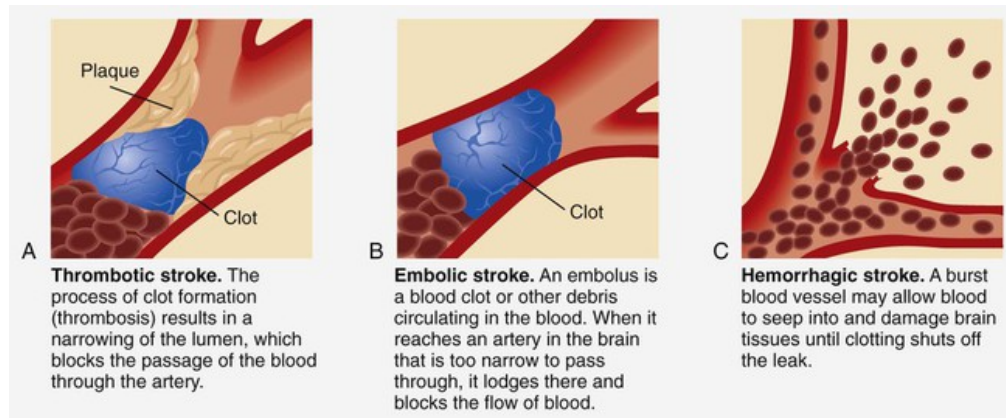
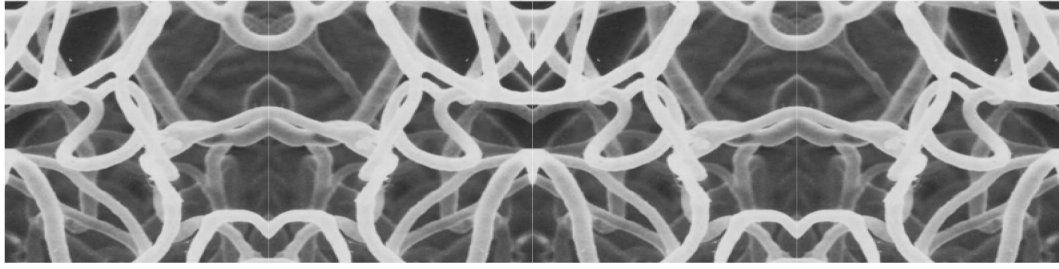


Fig. 2.26 Schematic of the types of stroke: ischaemic (A,B) and haemorrhagic (C). Reproduced from [130].

Identification and treatment of stroke is time-critical, with treatment dependent on whether it is an ischaemic or haemorrhagic stroke. Ischaemic strokes can be treated with blood thinners to dissolve the clot (such as recombinant tissue-plasminogen activator), although this can result in haemorrhage [193]. Aspirin is also prescribed with the onset of stroke to help thinning of blood [92]. Surgery and angioplasty are also methods used to remove blockages in vessels. Haemorrhagic strokes have very different treatments, however, often requiring the blood to be drained (through a bore in the skull) to alleviate ICP. The aneurysm can then be treated by clamping the vessel [66]. Due to the need for quick imaging and determination of stroke, only CT and MRI scans tend to be used due to their relative speed and resolution.

2.8 Summary

In this literature review I have provided a brief overview of the functions of the human brain before analysing in-depth the microvasculature. Various scales were considered, starting with blood rheology, building up to the capillary network, penetrating vessels, and finally large-scale models of the microvasculature. Data available on the anatomy, geometry, and topology of the microvessels were discussed, as well as the available modelling techniques used to model blood flow and oxygen transport in the microvasculature (including homogenization theory applied to biological networks). Next, the various imaging modalities were considered, including ex-vivo and in-vivo, identifying the imaging gap that currently exists with clinical imaging (vessels sub 1 mm in diameter being ‘invisible’ to these modalities). Finally, two major cerebrovascular diseases were discussed, stroke and dementia, along with the difficulties associated with their early diagnosis and treatment, presenting a pressing need to better understand the human microvasculature in both health and pathological conditions. It is hoped that mathematical models, such as the ones presented in this thesis, will go some way in helping us to plug the current clinical imaging gap, and to understand better the effects of the underlying microvasculature on blood and oxygen transport in the brain.



Chapter 3

Multi-scale Homogenization of Blood Flow and Oxygen Transport in 3-dimensional Human Cerebral Capillary Networks

The work presented on blood flow in this chapter can be found published in El-Bouri, W. K. and Payne S. J. (2015), ‘Multi-scale homogenization of blood flow in 3-dimensional human cerebral microvascular networks.’ *J Theor Biol.*, 380: 40–47. doi: 10.1016/j.jtbi.2015.05.011. The work presented on oxygen transport can be found published in El-Bouri, W. K. and Payne, S. J. (2015), ‘Multi-scale homogenization of the mass transport equation in periodic capillary networks.’ *Proceedings of the 4th*

international conference on computational & mathematical biomedical engineering, pp. 178-181. The homogenization theory has also been used as part of a paper in Payne S. J. and El-Bouri, W. K. ‘Assumptions for modelling blood flow and volume in the cerebral vasculature’, submitted for publication. As well as this, the methods used in this chapter are being used with collaborators at the Spanish National Centre for Cardiovascular Research which will lead to a further joint publication.

Abstract

The microvasculature plays a crucial role in the perfusion of blood through cerebral tissue. Current models of the cerebral microvasculature are discrete, and hence only able to model the perfusion over small voxel sizes before becoming computationally prohibitive. In order to gain a more comprehensive understanding of perfusion in the brain and to provide comparisons and validation against imaging data, larger models are required. Therefore, the first step in this project is to scale-up the blood flow and oxygen transfer in the capillary network in order to be able to generate these large microvascular models.

In this chapter, multi-scale homogenization methods were employed to develop continuum models of blood flow and oxygen transport in a capillary network model of the human cortex. Homogenization of the local-scale blood flow equations produced Darcy’s law, with the permeability tensor encapsulating the capillary bed topology. Similarly, homogenization of the mass transport equation led to an averaged form of the mass transport equation. Seven cases were considered for mass transport depending on the dominant transport mechanisms at the macro-scale. A statistically accurate network model of the human cerebral cortex microvasculature was adapted

to impose periodicity (necessary for the homogenization), and the elements of the permeability tensor calculated over a range of voxel sizes.

The permeability tensor was found to converge to an effective permeability as voxel size increased. This converged permeability tensor was isotropic, reflecting the ‘mesh-like’ structure of the cerebral microvasculature, with off-diagonal terms normally distributed about zero. A representative elementary volume of $375\ \mu\text{m}$, with a standard deviation of 4.5 % from the effective permeability, was determined. Using the converged permeability values, the cerebral blood flow was calculated to be around $55\ \text{mL min}^{-1} 100\text{g}^{-1}$, which is in very close agreement with experimental values. These results are the first step in this project that aims to develop large multi-scale statistical models of the human cerebral microvasculature.

As the rest of this thesis will concentrate on blood flow, the oxygen transport equations are left as theory for future research and application.

3.1 Introduction

The brain is particularly vulnerable to severe reductions in blood supply, with damage occurring within tens of seconds. Any mismatch between the metabolic rate in the brain cells and oxygen supply leads to localised damage of tissue, known as ischaemia, and eventual infarction if the supply of blood, and hence oxygen, is not restored. On a large scale, this is often referred to as a stroke.

Increasingly, the underlying microvascular architecture is recognised as being key to the perfusion rate and the delivery of oxygen to surrounding tissue [26,101,149]. Haemodynamically based functional imaging techniques, such as fMRI, make use of the coupling between neuronal activity and local increases in blood flow and metabolism. However, the spatial resolution of such imaging techniques is not high

enough to pick out the microvasculature and its effects on perfusion. As a result, little information is available on the interplay between the microvascular topology and tissue scale perfusion and oxygen transport. Characterising this interplay, and the response of the vascular system to haemodynamic disturbances, is likely to help to improve the treatment of vascular diseases.

In order to aid with understanding blood flow in the brain, a number of models have been built of blood and oxygen transport through the main groups of vessels in the vasculature. These models are most often discrete networks with 1-dimensional blood flow through the vessels. On the local-scale, these models are useful for analysing perfusion and oxygen transport through individual vessels and the surrounding extracellular space. Secomb et al. [163] and Fang et al. [48] have developed models of blood and oxygen transport that can be applied to any 3-dimensional network, and which were applied to transport in the rat cortex.

Similarly, others have reconstructed a rat cortex network from experimental data, using it to investigate the effects of local vascular dilation and occlusion [149]. Lorthois et al. applied the transport models to a reconstructed network from the human cerebral cortex [110]. Su et al. also used the same cerebral cortex data, but rather than reconstruct the observed network, they developed a statistical algorithm to fit morphometric parameters and statistically to model the microvasculature [176]. Such an algorithm is valuable as it bypasses the labour intensive and time-consuming method of experimentally extracting the structure of the microvasculature at each section in the brain. Additionally, such statistical algorithms are easily adaptable for varying statistical parameters at different cerebral locations.

However, all of these models are currently only computationally reasonable for small tissue sections. Building larger models so that accurate comparisons can be made

with imaging data becomes very difficult using current discrete modelling techniques. Boundary conditions of imaged sections are also unknown and have been shown to have a strong effect on flow properties [110]. This makes it very difficult to determine the effect of the microvasculature on tissue scale fluid transport. In order to gain a more comprehensive understanding of the perfusion and pathophysiological conditions associated with the vasculature, a mathematically rigorous multi-scale model will be required, which is not currently achievable.

Continuum-based mathematical modelling techniques, specifically homogenization, aim to deal with these issues. Homogenization is an averaging technique which derives macro-scale models using micro-scale models, assuming that there is scale separation between the two sizes and that the micro-scale model is periodic. Using such methods results in the discrete micro-scale representation being replaced with averaged integral quantities of the capillary bed valid over the larger region of interest, reducing the computational cost very substantially. A key advantage of this continuum approach is that the results are independent of the choice of boundary condition.

Shipley and Chapman used these mathematical averaging techniques on a leaky capillary bed to derive an averaged macroscopic Darcy flow model [168]. The permeability tensor of this flow model is what links the capillary scale topology to macro-scale blood flow, and is the property of interest when scaling up discrete models. Characterizing this tensor allows us to build larger continuum models of the cerebral microvasculature. The idea of scale separation introduces the concept of a representative elementary volume (REV). This is generally defined as the minimum volume within which the property of interest remains relatively constant [16]. Therefore, determining the REV of a network is important to minimise computation

time. Smith et al. have previously used the averaging techniques derived by Shipley and Chapman on a model of non-leaky rat myocardial capillaries [169].

In this chapter I adapt the techniques developed by Shipley and Chapman for capillary networks in tumours to derive macro-scale flow equations for non-leaky capillaries, with no-slip boundary conditions on the capillary walls, as well as to derive macro-scale mass transport equations for a variety of time scales. I then adapt the discrete model developed by Su et al. in order to generate periodic, statistically accurate, 3-dimensional models of the cerebral microvasculature for the first time. The flow equations are used to calculate the permeability tensors of various voxel sizes in order to determine convergence of the permeability. From the converged permeability, an REV is calculated for a given error tolerance. The calculated permeability is validated by calculating CBF and comparing to physiological values.

3.2 Theory

The model of the brain tissue proposed here has two distinct phases: the capillaries and the interstitium (the tissue intercellular fluid surrounding the capillaries). A snapshot of a cerebral capillary network is shown in **Fig. 3.1** where the brain parenchyma has been cleared from the images.

Throughout this chapter, the capillary network is assumed to be periodic in order to apply the homogenization method. This is a fundamental assumption, and its use will be analysed in Section 3.5. However, this periodic assumption allows us to ‘stack’ these micro-scale periodic networks to form larger macro-scale models of the vasculature. The assumption of periodicity is necessary, particularly in multi-dimensional systems, due to the need to remove secular terms. Periodicity is a mathematical tool regularly used in homogenization to simplify the removal of secular terms [12,69,168].

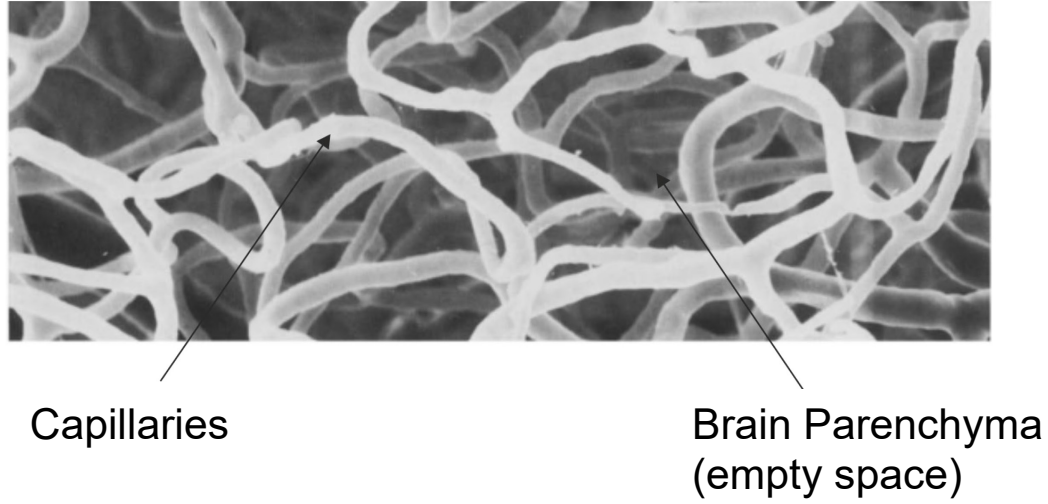


Fig. 3.1 Snapshot of a cerebral capillary network (adapted with permission from [42]). The brain parenchyma here is shown as empty space where tissue cells have been cleared in order to image the vessels.

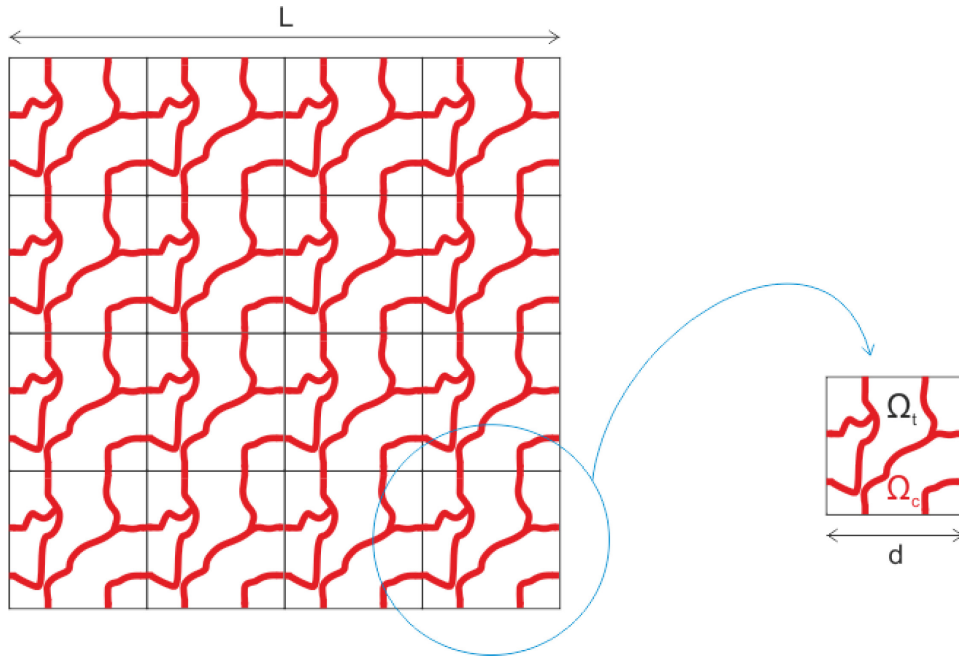


Fig. 3.2 Schematic of the macro-scale network composed of periodic micro-scale cells.

A schematic demonstrating how this periodic assumption allows for scaling up the capillary model is shown in **Fig. 3.2**. Here, d is the micro length scale, L is the macro length scale, and Ω_t and Ω_c are the volumes of the interstitium (brain parenchyma) and capillary networks respectively, with the total volume being $\Omega = \Omega_t + \Omega_c$.

The idea of scale separation introduces the concept of a representative elementary volume (REV). Put simply, the REV is the size of the micro-scale volume that contains enough statistical information about the capillary network in order to be representative of the medium. This REV will be determined for a statistically accurate human cerebral capillary network. First, however, the homogenization theory for the three models of interest will be developed and presented: for blood flow in the capillary network; for fluid flow in the interstitial domain; and for the mass transport across both domains.

3.2.1 Homogenization of Blood Flow in Capillary Networks

The same homogenization methods are used here as in Shipley and Chapman [168] to derive the continuum equations for a normal cerebral vasculature. The brain parenchyma/interstitium, comprised of cells and extracellular space, is assumed to be a porous medium due to the large size of the capillaries in comparison to the inter-cell separation. Blood flow in the capillaries is assumed to be incompressible, of constant haematocrit, and to obey steady-state Navier-Stokes equation (the flow through capillaries is quasi-steady). The validity of assuming constant haematocrit will be examined in Section 3.5. It is also assumed that there is negligible leakage of blood between the capillaries and interstitium, and no-slip, no-leak boundary conditions are imposed for the blood velocity at capillary walls. As a result, the system of equations to homogenize for flow in the capillaries on the micro-scale is:

$$-\nabla p'_c + \mu \nabla^2 \mathbf{u}'_c = \rho(\mathbf{u}'_c \cdot \nabla \mathbf{u}'_c) \quad \text{in } \Omega_c \quad (3.1)$$

$$\nabla \cdot \mathbf{u}'_c = 0 \quad \text{in } \Omega_c \quad (3.2)$$

$$\mathbf{u}'_c \cdot \boldsymbol{\tau} = 0 \quad \text{on } \Gamma_c \quad (3.3)$$

$$\mathbf{u}'_c \cdot \mathbf{n} = 0 \quad \text{on } \Gamma_c \quad (3.4)$$

where subscript c refers to the capillary domain, Ω_c is the capillary volume, Γ_c is the capillary wall surface, ρ is the density of blood, $\mathbf{u}'_c$ is the blood velocity, μ is the blood viscosity, p'_c is the blood pressure, and $\boldsymbol{\tau}$ and $\mathbf{n}$ are the tangential and normal unit vectors respectively.

A small parameter ε , defined as the ratio of the micro- and macro-length scales, is introduced by non-dimensionalizing the set of equations using the following characteristic values:

$$\mathbf{u}'_c = U \mathbf{u}_c, \quad p'_c = \frac{\mu LU}{d^2} p_c + p_0, \quad \mathbf{X}' = d \mathbf{X} \quad (3.5)$$

where U , L , and d are characteristic values of velocity, macro length scale, and micro length scale respectively, $\mathbf{X}'$ is the length variable, and p_0 is a pressure offset. It should be noted here that the prime notation denotes the dimensional variable, and the non-prime notation denotes the dimensionless variable. This is done for visual clarity in the rest of the chapter. The pressure is rescaled using a viscous pressure scale, as viscous forces dominate on the local-scale. To bring the characteristic value of pressure onto the macro-scale it is rescaled by ε^{-2} [167]. The reason for this is that the inter-capillary separation is of $O(\varepsilon)$ which means that there are $O(\varepsilon^2)$ capillaries per unit area. As pressure scales proportionally to the inverse of area then the pressure generated on the macro-scale will be of $O(\varepsilon^{-2})$.

Substituting the non-dimensional variables from (3.5) into (3.1) and multiplying by $d^2/\mu U$:

$$-\nabla p_c + \varepsilon \nabla^2 \mathbf{u}_c = \varepsilon Re_d (\mathbf{u}_c \cdot \nabla \mathbf{u}_c) \quad \text{in } \Omega_c \quad (3.6)$$

where Re_d is the local Reynolds number and is defined as

$$Re_d = \frac{\rho U d}{\mu} \quad (3.7)$$

and ε is the small parameter defined as

$$\varepsilon = \frac{d}{L} \quad (3.8)$$

Similarly, the macro-scale Reynolds number, Re_L is defined as

$$Re_L = \frac{\rho UL}{\mu} \quad (3.9)$$

Therefore, $Re_d = \varepsilon Re_L$, and so the steady state Navier-Stokes equation (3.6) can be written in terms of the macro-scale Reynolds number, as is commonly done

$$-\nabla p_c + \varepsilon \nabla^2 \mathbf{u}_c = \varepsilon^2 Re_L (\mathbf{u}_c \cdot \nabla \mathbf{u}_c) \quad \text{in } \Omega_c \quad (3.10)$$

The appropriate scaling for the dimensionless Reynolds number will be determined later. Finally, non-dimensionalizing the incompressibility equation and boundary conditions (3.2 – 3.4) gives the same form of equations in non-dimensional form.

3.2.1.1 Parameter Values for Blood Flow

The values of the dimensionless numbers will determine what scaling they have and hence where they appear in the expansion of the problem. This determines whether they affect the leading order solution or are part of the correction terms, i.e. are not significant to leading order. Typical parameters for blood flow in capillaries can be found in **Table 3.1**.

The first thing to calculate is ε . The local length scale (d) is around 50 μm , and L is variable depending on what is being defined as the macro length scale. It was decided to set L as 1 mm as this is the same order of magnitude as clinical imaging voxel sizes e.g. MRI. This means ε is approximately 0.05.

The local Reynolds number (Re_d) as defined in (3.7) is found to be 2.6×10^{-3} which gives it a scaling of $O(\varepsilon^2)$. This means that Re_L is $O(\varepsilon)$. However, if L was 1

cm then Re_L would be $O(1)$ and Re_d $O(\varepsilon)$. Due to the uncertainty on how large or small L should be, it was decided to keep Re_L at $O(1)$ in order to retain as much of the physical problem as possible at leading order. Of course, if Re_L turned out to be an order smaller, its contribution would then appear as a correction to the leading order solution. In this particular case, the choice of scaling does not affect the leading order solution, affecting only the correction terms (which are not derived here).

Symbol (Units)	Parameter	Characteristic Value
d (m)	Local length scale	50×10^{-6} [41]
L (m)	Macro length scale	$\approx 1 \times 10^{-3}$
U (m s ⁻¹)	Characteristic blood velocity in capillaries	0.2×10^{-3} [79]
ρ (kg m ⁻³)	Density of blood	1040 [91]
μ (Pa s)	Dynamic viscosity of blood (45% haematocrit)	4×10^{-3} [148]
κ (m ³ s kg ⁻¹)	Hydraulic conductivity	$10^{-12} - 10^{-15}$ (collected in [30])
k (m ²)	Interstitial permeability	$4 \times 10^{-16} - 4 \times 10^{-13}$ (collected in [30])

Table 3.1 Table of typical values of the modelling parameters of interest for the cerebral capillary network and interstitium.

3.2.1.2 Homogenization of the Capillary Networks

Now that the equations have been non-dimensionalized, and the appropriate scalings chosen, it is possible to homogenize the equations. The method is similar to other multiple-scales methods, however with homogenization the goal is to determine an approximate problem rather than an approximate solution. In other words, the original problem will be dependent on both the local- and macro-scale. The aim now

is to find an approximate problem that varies only on the macro-scale but which has coefficients which are determined by solving the local-scale problem.

A fundamental assumption when using homogenization is that the large-scale structure, in this case the capillary bed, is locally periodic. This allows the removal of secular terms in multi-dimensional problems without the need to solve higher order equations explicitly. It is of course possible to homogenize non-locally periodic structures, however finding the solvability condition to eliminate the secular terms becomes very difficult [69]. Periodicity is a mathematical tool regularly used in homogenization to simplify the removal of secular terms. The validity of this assumption for cerebral microvascular networks is discussed later.

Another key concept in homogenization is that of scale separation. As $\varepsilon \ll 1$ the local and macro length scales are well-separated and can be defined as $\mathbf{X}$ and $\mathbf{x} = \varepsilon \mathbf{X}$ respectively. It is emphasised here that $\mathbf{X}$ denotes the micro-scale and $\mathbf{x}$ denotes the macro-scale. Using the assumption of scale separation both $\mathbf{x}$ and $\mathbf{X}$ can be treated as independent variables and so (using the chain rule)

$$\nabla = \nabla_{\mathbf{X}} + \varepsilon \nabla_{\mathbf{x}}, \quad \nabla^2 = \nabla_{\mathbf{X}}^2 + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} + \varepsilon^2 \nabla_{\mathbf{x}}^2 \quad (3.11)$$

Using (3.11) to expand out (3.10) and the non-dimensionalized forms of (3.2 – 3.4)

$$\begin{aligned} \varepsilon^2 Re_L [(\mathbf{u}_c \cdot \nabla_{\mathbf{X}}) \mathbf{u}_c + \varepsilon (\mathbf{u}_c \cdot \nabla_{\mathbf{x}}) \mathbf{u}_c] = & -\nabla_{\mathbf{X}} p_c - \varepsilon \nabla_{\mathbf{x}} p_c + \varepsilon \nabla_{\mathbf{X}}^2 \mathbf{u}_c \\ & + 2\varepsilon^2 \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} \mathbf{u}_c + \varepsilon^3 \nabla_{\mathbf{x}}^2 \mathbf{u}_c \quad \text{in } \Omega_c \end{aligned} \quad (3.12)$$

$$\nabla_{\mathbf{X}} \cdot \mathbf{u}_c + \varepsilon \nabla_{\mathbf{x}} \cdot \mathbf{u}_c = 0 \quad \text{in } \Omega_c \quad (3.13)$$

$$\mathbf{u}_c \cdot \mathbf{n} = 0 \quad \text{on } \Gamma_c \quad (3.14)$$

$$\mathbf{u}_c \cdot \boldsymbol{\tau} = 0 \quad \text{on } \Gamma_c \quad (3.15)$$

Finally, a multiple-scales expansion of the velocity and pressure is proposed of the form

$$\mathbf{u} = \mathbf{u}^{(0)} + \varepsilon \mathbf{u}^{(1)}(\mathbf{x}, \mathbf{X}) + \varepsilon^2 \mathbf{u}^{(2)}(\mathbf{x}, \mathbf{X}) + \dots \quad (3.16)$$

$$p = p^{(0)} + \varepsilon p^{(1)}(\mathbf{x}, \mathbf{X}) + \varepsilon^2 p^{(2)}(\mathbf{x}, \mathbf{X}) + \dots \quad (3.17)$$

In order to maintain periodicity each component of $\mathbf{u}$ and p are periodic in $\mathbf{X}$. The expansions of (3.16 – 3.17) will then be substituted into (3.12 – 3.15) and successive orders of ε equated to determine the leading order homogenized equations for capillary flow and pressure. Substituting the multiple-scales expansion of blood velocity and pressure into (3.12 – 3.15) gives

$$\begin{aligned} & \varepsilon^2 Re_L [((\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) \cdot \nabla_{\mathbf{X}})(\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) + \\ & \quad \varepsilon((\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) \cdot \nabla_{\mathbf{x}})(\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots)] = \\ & -\nabla_{\mathbf{X}} (p_c^{(0)} + \varepsilon p_c^{(1)} + \dots) - \varepsilon \nabla_{\mathbf{x}} (p_c^{(0)} + \varepsilon p_c^{(1)} + \dots) + \varepsilon \nabla_{\mathbf{X}}^2 (\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) \\ & + 2\varepsilon^2 \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} (\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) + \varepsilon^3 \nabla_{\mathbf{x}}^2 (\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) \quad \text{in } \Omega_c \end{aligned} \quad (3.18)$$

$$\nabla_{\mathbf{X}} \cdot (\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) + \varepsilon \nabla_{\mathbf{x}} \cdot (\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) = 0 \quad \text{in } \Omega_c \quad (3.19)$$

$$(\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) \cdot \boldsymbol{\tau} = 0 \quad (3.20)$$

$$(\mathbf{u}_c^{(0)} + \varepsilon \mathbf{u}_c^{(1)} + \dots) \cdot \mathbf{n} = 0 \quad (3.21)$$

Equating powers of $O(\varepsilon^0)$ in (3.18 – 3.21) gives

$$\nabla_{\mathbf{X}} p_c^{(0)} = 0 \quad (3.22)$$

$$\nabla_{\mathbf{X}} \cdot \mathbf{u}_c^{(0)} = 0 \quad (3.23)$$

$$\mathbf{u}_c^{(0)} \cdot \boldsymbol{\tau} = 0 \text{ and } \mathbf{u}_c^{(0)} \cdot \mathbf{n} = 0 \quad \text{on } \Gamma \quad (3.24)$$

and Equating powers of $O(\varepsilon^1)$

$$\nabla_{\mathbf{X}} p_c^{(1)} + \nabla_{\mathbf{x}} p_c^{(0)} = \nabla_{\mathbf{X}}^2 \mathbf{u}_c^{(0)} \quad (3.25)$$

$$\nabla_{\mathbf{X}} \cdot \mathbf{u}_c^{(1)} + \nabla_{\mathbf{x}} \cdot \mathbf{u}_c^{(0)} = 0 \quad (3.26)$$

$$\mathbf{u}_c^{(1)} \cdot \boldsymbol{\tau} = 0 \text{ and } \mathbf{u}_c^{(1)} \cdot \mathbf{n} = 0 \quad \text{on } \Gamma \quad (3.27)$$

From (3.22) it is evident that $p_c^{(0)}$ is constant at the local-scale, hence $p_c^{(0)} = p_c^{(0)}(\mathbf{x})$. In order to determine the leading order problem it is necessary to solve for $\mathbf{u}_c^{(0)}$ and $p_c^{(1)}$. From (3.25) it can be seen that $\mathbf{u}_c^{(0)}$ and $p_c^{(1)}$ are both linear functions of $\nabla_{\mathbf{x}} p_c^{(0)}$ and so solutions are proposed of the form:

$$\mathbf{u}_c^{(0)} = -\mathbf{w}_c^j(\mathbf{X}) \frac{dp_c^{(0)}}{dx^j} \quad (3.28)$$

$$p_c^{(1)} = -P_c^j(\mathbf{X}) \frac{dp_c^{(0)}}{dx^j} + \bar{p}_c^{(1)} \quad (3.29)$$

The form used in (3.28) and (3.29) is motivated due to the need to separate terms in $\mathbf{x}$ and $\mathbf{X}$. Einstein notation has been used here for clarity where j can take the values 1, 2, or 3 and refers to the Cartesian co-ordinate directions. The notation used is a simple substitution for what would otherwise be a dot product of the two j components. $\mathbf{w}_c^j(\mathbf{X})$ and $P_c^j(\mathbf{X})$ account for the local variations in $\mathbf{u}_c^{(0)}$ and $p_c^{(1)}$ and are known as the cell variables. It is from these local variables that the homogenized macro-scale parameters of the blood flow in the capillary network can be determined. These variables are determined by inserting them into (3.23), (3.24), and (3.25) to obtain the cell problem:

$$\nabla_{\mathbf{X}} \cdot \mathbf{w}_c^j(\mathbf{X}) = 0 \quad \text{in } \Omega_c \quad (3.30)$$

$$\nabla_{\mathbf{X}} P_c^j(\mathbf{X}) = \nabla_{\mathbf{X}}^2 \mathbf{w}_c^j(\mathbf{X}) + \mathbf{e}_j \quad \text{in } \Omega_c \quad (3.31)$$

$$\mathbf{w}_c^j(\mathbf{X}) \cdot \boldsymbol{\tau} = 0 \text{ and } \mathbf{w}_c^j(\mathbf{X}) \cdot \mathbf{n} = 0 \quad \text{on } \Gamma \quad (3.32)$$

where $\mathbf{e}_j$ is the unit vector in the j -direction. This is the local periodic cell problem which must be solved numerically in order, as shall be seen, to derive the parameters for the macro-scale problem. Note that (3.31) is a forced Stokes flow problem. From this is derived the Poiseuille equation (making assumptions on the radial and swirl

components of the velocity). Therefore, despite having left in the convective acceleration term in (3.1), to leading order the cell problem is Stokes flow.

From (3.31) and (3.32) it can be seen that the cell problem is underdetermined and hence the local pressure term P_c^j is only defined up to a constant value. A uniqueness condition is thus imposed which states that the volume average of the local pressure is zero:

$$\langle P_c^j \rangle_c = \frac{1}{|\Omega|} \int_{\Omega_c} P_c^j dV = 0 \quad (3.33)$$

Taking a volume average over $\mathbf{u}_c^{(0)}$ (3.28) results in:

$$\langle \mathbf{u}_c^{(0)} \rangle_{\Omega_c} = -\mathbf{K} \nabla_{\mathbf{x}} p_c^{(0)} \quad (3.34)$$

where

$$K_{ij} = \frac{1}{|\Omega|} \int_{\Omega_c} w_{ci}^j dV \quad (3.35)$$

This is Darcy's Law with $\mathbf{K}$ defining the permeability tensor. Therefore, to leading order, the homogenization of the incompressible, steady state Navier-Stokes equations gives Darcy's Law. This result can also be arrived at using other mathematical methods such as mixture theory [147]. The permeability tensor $\mathbf{K}$ is the important result here as this encapsulates the geometry of the problem and how the geometry affects the flow for given pressure gradients. It is an averaged coefficient tensor, calculated by solving the micro cell problem. Determining the converged values of the elements of the tensor allows us to build 'scaled-up' models of blood flow in the cerebral microvasculature.

It has been shown that, to leading order, the volume averaged velocity is equivalent to a surface mean velocity (Darcy Flux) [12,40] and so (3.34) can be

rewritten as:

$$\langle \mathbf{u}_c^{(0)} \rangle_\Gamma = -\mathbf{K} \nabla p_c^{(0)} \quad (3.36)$$

where $\langle \mathbf{u}_c^{(0)} \rangle_\Gamma$ is the surface mean flux taken at the outflow boundaries of the periodic cell problem. This is used to determine the permeability tensor for a periodic cerebral capillary network later in this chapter.

Finally, consider the expanded incompressibility equation (3.26) and volume average over the fluid domain (the integral is over the local domain and hence can be brought in front of the macro-scale divergence operator)

$$\langle \nabla_{\mathbf{x}} \cdot \mathbf{u}_c^{(0)} \rangle_c = \nabla_{\mathbf{x}} \cdot \langle \mathbf{u}_c^{(0)} \rangle_c = -\frac{1}{|\Omega|} \int_{\Omega_c} \nabla_{\mathbf{x}} \cdot \mathbf{u}_c^{(1)} dV \quad (3.37)$$

Using the divergence theorem on the right hand side

$$-\frac{1}{|\Omega|} \int_{\Omega_c} \nabla_{\mathbf{x}} \cdot \mathbf{u}_c^{(1)} dV = -\frac{1}{|\Omega|} \int_\Gamma \mathbf{u}_c^{(1)} \cdot \mathbf{n} dS \quad (3.38)$$

which, along with the boundary condition (3.27) and periodicity on the boundary cell walls, is zero. This leads to the macro-scale volume conservation equation

$$\nabla_{\mathbf{x}} \cdot \langle \mathbf{u}_c^{(0)} \rangle_c = 0 \quad (3.39)$$

Substituting (3.34) into (3.39) leads to the elliptic, macro-scale homogenized equation in terms of blood pressure

$$\nabla_{\mathbf{x}} \cdot (\mathbf{K} \nabla p_c^{(0)}) = 0 \quad (3.40)$$

It is this equation that is solved on the macro-scale to determine blood pressure within the capillary network, and along with (3.36), the volume averaged velocity of the blood. This is done for a large-scale network in Chapter 5. However, before that, the permeability tensor must be found which will encapsulate the effect of the

geometry of the capillary network on macro-scale pressure and velocity. This will be determined in this chapter on a statistically accurate model of a capillary network. Prior to that however, I will first also obtain the homogenized forms of the interstitial domain equations, and the mass transport equations in the next two sections.

3.2.2 Homogenization of Interstitial Domain Flow

3.2.2.1 Model Formulation

As the interstitial region is porous, Darcy's law will be used to model flow in that region, along with incompressibility. Subscript t refers to the interstitial domain.

$$\mathbf{u}'_t = -\frac{k}{\mu} \nabla p'_t \quad \text{in } \Omega_t \quad (3.41)$$

$$\nabla \cdot \mathbf{u}'_t = 0 \quad \text{in } \Omega_t \quad (3.42)$$

where Ω_t is the interstitial volume and k the interstitial permeability (assumed to be constant and uniform).

The boundary condition for this problem is the same as that for the no-leak capillary domain (3.4) as no blood can cross the capillaries for a healthy network.

$$\mathbf{u}'_t \cdot \mathbf{n} = 0 \quad \text{on } \Gamma_t \quad (3.43)$$

Finally, non-dimensionalizing (3.41) using variables defined similarly as in (3.5) gives

$$\mathbf{u}_t = -\kappa \nabla p_t \quad \text{where } \kappa = \frac{kL}{d^3} \quad (3.44)$$

Alternatively, κ can be written as

$$\kappa = \frac{\bar{\kappa}}{\epsilon} \quad \text{where } \bar{\kappa} = \frac{k}{d^2} \quad (3.45)$$

Non-dimensionalizing (3.42) and (3.43) results in the same non-dimensional form of those equations.

It is possible (and preferable) to write the interstitial problem in terms of p_t only – by inserting (3.41) into (3.42) and (3.43) – and so this problem becomes

$$\nabla^2 p_t = 0 \text{ in } \Omega_t \quad (3.46)$$

$$\nabla p_t \cdot \mathbf{n} = 0 \text{ on } \Gamma_t \quad (3.47)$$

3.2.2.2 Parameter Values

The second dimensionless number in the fluid flow model is the hydraulic conductivity. As was seen in (3.45) κ can also be written as $\bar{\kappa}/\varepsilon$ where $\bar{\kappa} = k/d^2$. Using the values of k found in **Table 3.1** it is found that $\bar{\kappa}$ is in the range 3.2×10^{-5} to 3.2×10^{-3} . This means $\bar{\kappa}$ is $\sim \varepsilon^4$ to ε^3 . However, $\bar{\kappa}$ is very sensitive to the choice of d and so it is important to retain in the asymptotic analysis in order to retain as much of the physical problem as possible to leading order. Therefore, although it is likely to be much smaller, the scaling of $\bar{\kappa}$ is set to be $O(1)$ and so κ is $O(\frac{1}{\varepsilon})$. This retains its leading order contribution. However, as the capillary and interstitial domains are uncoupled (there is no leakage of blood between them), the hydraulic conductivity and permeability do not play a role in the homogenization. An advantage of retaining this physical phenomenon to leading order is that it allows for limits to the scalings to be tested. For example, if $\bar{\kappa}$ is an order smaller, this would lead to the interstitial velocity being an order smaller (3.69) (whilst the capillary velocity would remain the same).

3.2.2.3 Homogenization of the Interstitial Domain

The homogenization method for the interstitial domain is very similar to that presented for the capillary domain. The same scale separation is assumed (3.11) and a multiple-scales expansion assumed for the interstitial velocity and pressure (3.16, 3.17). These are substituted into (3.43, 3.46, 3.47) to get

$$\begin{aligned} \nabla_{\mathbf{X}}^2(p_t^{(0)} + \varepsilon p_t^{(1)} + \dots) + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}}(p_t^{(0)} + \varepsilon p_t^{(1)} + \dots) + \\ \varepsilon^2 \nabla_{\mathbf{x}}^2(p_t^{(0)} + \varepsilon p_t^{(1)} + \dots) = 0 \quad \text{in } \Omega_t \end{aligned} \quad (3.48)$$

$$\nabla_{\mathbf{X}}(p_t^{(0)} + \varepsilon p_t^{(1)} + \dots) \cdot \mathbf{n} + \varepsilon \nabla_{\mathbf{x}}(p_t^{(0)} + \varepsilon p_t^{(1)} + \dots) \cdot \mathbf{n} = 0 \quad \text{on } \Gamma \quad (3.49)$$

$$(\mathbf{u}_t^{(0)} + \varepsilon \mathbf{u}_t^{(1)} + \dots) \cdot \mathbf{n} = 0 \quad (3.50)$$

Following the homogenization method already detailed, we start by equating powers of $O(\varepsilon^0)$ in (3.48) and (3.49)

$$\nabla_{\mathbf{X}}^2 p_t^{(0)} = 0 \quad \text{in } \Omega_t \quad (3.51)$$

$$\nabla_{\mathbf{X}} p_t^{(0)} \cdot \mathbf{n} = 0 \quad \text{on } \Gamma \quad (3.52)$$

$p_t^{(0)}$ is found to be locally constant and so $p_t^{(0)} = p_t^{(0)}(\mathbf{x})$ (this can be proved by considering $p_t^{(0)} \nabla_{\mathbf{X}}^2 p_t^{(0)} = 0$ and integrating over Ω_t , then using the divergence theorem with the Neumann boundary condition (3.52) – see Appendix A).

At $O(\varepsilon^1)$:

$$\nabla_{\mathbf{X}}^2 p_t^{(1)} + 2 \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} p_t^{(0)} = 0 \quad \text{in } \Omega_t \quad (3.53)$$

$$\nabla_{\mathbf{X}} p_t^{(1)} \cdot \mathbf{n} = -\nabla_{\mathbf{x}} p_t^{(0)} \cdot \mathbf{n} \quad \text{on } \Gamma \quad (3.54)$$

The second term in (3.53) is zero as $p_t^{(0)}$ is locally constant. However, $p_t^{(1)}$ is not locally constant due to the non-homogenous flux condition in (3.54) (the proof of $p_t^{(0)}$ being constant uses the homogenous flux condition (3.52); when the flux condition is not homogenous, invariance with the local-scale cannot be proven). However, from (3.54) it is evident that $p_t^{(1)}$ is a linear function of $\nabla_{\mathbf{x}} p_t^{(0)}$ and so a solution is proposed of the form

$$p_t^{(1)} = -P_t^j(\mathbf{X}) \frac{dp_t^{(0)}}{dx^j} + \bar{p}_t^{(1)} \quad (3.55)$$

where P_t^j accounts for the local variation in $p_t^{(1)}$. Inserting (3.55) into the $O(\varepsilon^1)$ equations the cell problem is arrived at

$$\nabla_{\mathbf{X}}^2 P_t^j(\mathbf{X}) = 0 \quad \text{in } \Omega_t \quad (3.56)$$

$$\nabla_{\mathbf{X}} P_t^j(\mathbf{X}) \cdot \mathbf{n} = \mathbf{e}_j \cdot \mathbf{n} \quad (3.57)$$

where again $\mathbf{e}_j$ is the unit vector in the j -direction. Similar to P_c^j , P_t^j is only defined up to a constant value and so the same uniqueness condition is imposed for P_t^j as in (3.33). This condition ensures that the $O(\varepsilon)$ correction to pressure ($p_t^{(1)}$) is a function of the macro-scale variable only, i.e. $\langle p_t^{(1)} \rangle_t = \phi_t \overline{p_t^{(1)}}(\mathbf{x})$, where ϕ_t is the volume fraction of the interstitium. However, in this analysis only the leading order equation is what is important and so the calculation of $p_t^{(1)}$ is not necessary.

Finally, at $O(\varepsilon^2)$:

$$\nabla_{\mathbf{X}}^2 p_t^{(2)} + 2\nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} p_t^{(1)} + \nabla_{\mathbf{x}}^2 p_t^{(0)} = 0 \quad \text{in } \Omega_t \quad (3.58)$$

$$\nabla_{\mathbf{X}} p_t^{(2)} \cdot \mathbf{n} = -\nabla_{\mathbf{x}} p_t^{(1)} \cdot \mathbf{n} \quad \text{on } \Gamma \quad (3.59)$$

This is a common boundary value problem with the solvability condition being the integration of (3.58) over Ω_t .

$$\frac{1}{|\Omega|} \int_{\Omega_t} (\nabla_{\mathbf{X}}^2 p_t^{(2)} + 2\nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} p_t^{(1)} + \nabla_{\mathbf{x}}^2 p_t^{(0)}) \, dV = 0 \quad (3.60)$$

As the integration is over the local-scale the third term in (3.60) simply becomes

$$\frac{|\Omega_t|}{|\Omega|} \nabla_{\mathbf{x}}^2 p_t^{(0)} = \phi_t \nabla_{\mathbf{x}}^2 p_t^{(0)} \quad (3.61)$$

Substituting $p_t^{(1)}$ from (3.55) into the second term and using the divergence theorem and periodicity (such that the surface integral is only over the capillary walls)

$$\begin{aligned} \frac{2}{|\Omega|} \nabla_{\mathbf{x}} \cdot \left(\int_{\Omega_t} \nabla_{\mathbf{x}} p_t^{(1)} dV \right) &= \frac{2}{|\Omega|} \nabla_{\mathbf{x}} \cdot \left(\int_{\Omega_t} \nabla_{\mathbf{x}} P_t^j dV \right) \nabla_{\mathbf{x}} p_t^{(0)} = \\ &= \frac{2}{|\Omega|} \nabla_{\mathbf{x}} \cdot \left(\int_{\Gamma} P_t^j n_i dS \right) \nabla_{\mathbf{x}} p_t^{(0)} \end{aligned} \quad (3.62)$$

where n_i is the Einstein notation for the unit normal vector. Using the divergence theorem, periodicity, and the flux boundary condition (3.59) on the first term

$$\frac{1}{|\Omega|} \int_{\Omega_t} \nabla_{\mathbf{x}} \cdot (\nabla_{\mathbf{x}} p_t^{(2)}) dV = \frac{1}{|\Omega|} \int_{\Gamma} \nabla_{\mathbf{x}} p_t^{(2)} \cdot \mathbf{n} dS = -\frac{1}{|\Omega|} \int_{\Gamma} \nabla_{\mathbf{x}} p_t^{(1)} \cdot \mathbf{n} dS \quad (3.63)$$

Substituting for $p_t^{(1)}$ the term reduces to

$$-\frac{1}{|\Omega|} \nabla_{\mathbf{x}} \cdot \left(\int_{\Gamma} P_t^j n_i dS \right) \nabla_{\mathbf{x}} p_t^{(0)} \quad (3.64)$$

Therefore combining (3.61), (3.62), and (3.64) the final continuum equation is found to be

$$\nabla_{\mathbf{x}} \cdot (\mathbf{E} \nabla_{\mathbf{x}} p_t^{(0)}) = 0 \quad (3.65)$$

where

$$\mathbf{E} = \left(\phi_t \delta_{ij} + \frac{1}{|\Omega|} \int_{\Gamma} P_t^j n_i dS \right) \quad (3.66)$$

and δ_{ij} is the Kronecker delta. In practice the second term of $\mathbf{E}$ is a correction term and so $\mathbf{E}$ can be reduced to $\mathbf{E} = \phi_t \mathbf{I}$ where $\mathbf{I}$ is the identity tensor [167].

Finally, to determine the leading order interstitial velocity, compare powers of $O(\varepsilon^1)$ in the expanded version of (3.44)

$$\mathbf{u}_t^{(0)} = -\bar{\kappa} \nabla_{\mathbf{x}} p_t^{(1)} - \bar{\kappa} \nabla_{\mathbf{x}} p_t^{(0)} \quad (3.67)$$

Substituting for $p_t^{(1)}$ from (3.55)

$$\mathbf{u}_t^{(0)} = -\bar{\kappa} \nabla_{\mathbf{x}} P_t^j \nabla_{\mathbf{x}} p_t^{(0)} - \bar{\kappa} \nabla_{\mathbf{x}} p_t^{(0)} = -\bar{\kappa} (\nabla_{\mathbf{x}} P_t^j + \delta_{ij}) \nabla_{\mathbf{x}} p_t^{(0)} \quad (3.68)$$

Taking the average over the interstitial domain it is found that

$$\left\langle \mathbf{u}_t^{(0)} \right\rangle_t = -\bar{\kappa} \mathbf{E} \cdot \nabla_x p_t^{(0)} \quad (3.69)$$

which is Darcy's law for the averaged interstitial fluid velocity. Therefore, the parameter of interest that must be determined on the micro-scale for the interstitial domain is $\mathbf{E}$ which is simply the volume fraction of the tissue within the model. This will be determined later in this chapter. The final section of theory I will present is the homogenization of the mass transport equation, specifically for oxygen transport.

3.2.3 Homogenization of the Mass Transport Equation

The motivation of this section is to develop a homogenized equation for solute transport in capillary beds in the brain. The homogenized form of the blood transport in the capillary and interstitium was derived in the previous sections for a random periodic network structure. The resulting model was uncoupled (due to the lack of blood leakage into the interstitium) but with volume averaged Darcy flow in the capillaries and interstitium. This model will be extended in this chapter to include solute flow through the vasculature and into the interstitium, with metabolic activity in the tissue. This work is based on techniques developed by Shipley and Chapman [168] and Auriault et al. [12].

3.2.3.1 Model Formulation

The advection-diffusion-reaction equation is the standard mass transport equation describing the concentration of a chemical species in time and space. In general, it is defined in multi-dimensional space as

$$\frac{\partial c'}{\partial t'} + \nabla \cdot (\mathbf{u}' c' - D' \nabla c') = g \quad (3.70)$$

where c' (mol m⁻³) is the concentration of the solute, D' (m² s⁻¹) is the diffusion coefficient of the solute, and g is a reaction term.

In the case of blood flow through capillaries it can be assumed that the flow is incompressible and that the diffusion coefficient is constant. The reaction rate, or the metabolic term, is crudely considered initially to vary linearly with concentration. Thus, (3.70) becomes

$$\begin{aligned} \frac{\partial c'}{\partial t'} + (\mathbf{u}' \cdot \nabla) c' &= D' \nabla^2 c' - \Lambda c' \\ \Lambda &= \begin{cases} 0, & \text{in } \Omega_c \\ \lambda, & \text{in } \Omega_t \end{cases} \end{aligned} \quad (3.71)$$

3.2.3.2 Boundary Conditions

The first boundary condition can be stated immediately as a mass flux balance of the solute across the capillary wall. Due to there being no leakage of blood into the interstitium, the only transport mechanism is diffusion. Therefore, the flux must be continuous on Γ (where Γ is the boundary between the capillary and the interstitium).

$$(D'_t \nabla c'_t) \cdot \mathbf{n} = (D'_c \nabla c'_c) \cdot \mathbf{n} \quad \text{on } \Gamma \quad (3.72)$$

where D'_t is the diffusion coefficient of the solute in the interstitium and D'_c the diffusion coefficient in the capillary network.

The second boundary condition is slightly trickier to prescribe. It must be a boundary condition of the species concentration on the capillary-interstitium boundary, i.e. the capillary wall. The simplest is to assume a continuity of concentration across the wall

$$c'_c = c'_t \quad \text{on } \Gamma \quad (3.73)$$

Another option is to assume a concentration jump across the membrane wall [168]. It is also possible to assume that metabolism occurs in the capillary walls although this will not be considered in this work.

For oxygen, it has previously been shown that the most appropriate boundary condition is to treat the capillary wall as part of the tissue as it is so thin [114]. Therefore (3.73) will be chosen as the second boundary condition in this analysis. It should be noted that it is not difficult to extend this analysis to consider cases with different boundary conditions. However, for reasons of space only this particular boundary condition will be analysed here.

3.2.3.3 Nondimensionalization

Characteristic values for each variable are used in order to non-dimensionalize the equations.

$$c' = Cc, \quad \mathbf{u}' = U\mathbf{u}, \quad D' = D_{ch}D, \quad \mathbf{X}' = d\mathbf{X}, \quad t' = t_c t \quad (3.74)$$

D_{ch} is a characteristic diffusion coefficient, d (as before) is the characteristic local length scale (in this case the average capillary separation), C is the characteristic concentration of the solute, and t_c is the characteristic time. The degree of freedom obtained by not defining the time scale at this point will allow us to determine the appropriate scaling of the three mass transport terms later. Again, it should be noted here that the prime notation denotes the dimensional variable, and the non-prime notation denotes the dimensionless variable.

Inserting the equations from (3.74) into (3.71)

$$P_d \frac{\partial c}{\partial t} + Pe_d (\mathbf{u} \cdot \nabla) c = D \nabla^2 c - \mathcal{R}c$$

$$\mathcal{R} = \begin{cases} 0, & \text{in } \Omega_c \\ Da_d, & \text{in } \Omega_t \end{cases} \quad (3.75)$$

with the three dimensionless numbers defined as

$$P_d = \frac{d^2}{D_{ch} t_c}, \quad Pe_d = \frac{Ud}{D_{ch}}, \quad Da_d = \frac{\lambda d^2}{D_{ch}} \quad (3.76)$$

The Péclet number (Pe_d) is defined as the ratio of advection to diffusion, the Damköhler number (Da_d) is defined as the ratio of metabolism/reaction to diffusion, and P_d is some non-dimensional time. Subscript d refers to the micro-scale. As a result, large values of Pe_d and Da_d imply dominant advection and reaction terms on the micro-scale respectively.

It will also be useful to define the Péclet and Damköhler numbers in terms of characteristic times. In order to do so, we define the advection, diffusion, and reaction time scales as follows

$$t_d^{adv} = \frac{d}{U}, \quad t_d^{dif} = \frac{d^2}{D_{ch}}, \quad t_d^{reac} = \frac{1}{\lambda} \quad (3.77)$$

As a result, the dimensionless numbers can be re-written as

$$P_d = \frac{t_d^{dif}}{t_c}, \quad Pe_d = \frac{t_d^{dif}}{t_d^{adv}}, \quad Da_d = \frac{t_d^{dif}}{t_d^{reac}} \quad (3.78)$$

Evidently by relating t_c to t_d^{adv} , t_d^{dif} , and t_d^{reac} it will be possible, depending on what are the dominant processes in the system, to obtain the appropriate scaling for the dimensionless numbers. Before proceeding with the analysis of various cases we will also define the global time scales and dimensionless numbers as

$$\begin{aligned} t_L^{adv} &= \frac{L}{U}, & t_L^{dif} &= \frac{L^2}{D_{ch}}, & t_L^{reac} &= \frac{1}{\lambda} \\ P_L &= \frac{t_L^{dif}}{t_c}, & Pe_L &= \frac{t_L^{dif}}{t_L^{adv}}, & Da_L &= \frac{t_L^{dif}}{t_L^{reac}} \\ P_L &= \frac{L^2}{D_{ch}t_c}, & Pe_L &= \frac{UL}{D_{ch}}, & Da_L &= \frac{\lambda L^2}{D_{ch}} \end{aligned} \quad (3.79)$$

3.2.3.4 Homogenization

The mass transport equation is homogenized in a similar fashion to the fluid transport equations in the prior sections. Again, the macro length variable is defined as $\mathbf{x} = \varepsilon \mathbf{X}$, where $\mathbf{X}$ is the local length variable. Due to the assumption of scale separation

both $\mathbf{x}$ and $\mathbf{X}$ are treated as independent variables such that (3.11) still holds. Substituting (3.11) into (3.75) gives:

$$P_d \frac{\partial c}{\partial t} + Pe_d (\nabla_{\mathbf{X}} \cdot (c\mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c\mathbf{u})) = D \nabla_{\mathbf{X}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c + \varepsilon^2 D \nabla_{\mathbf{x}}^2 c - Da_d c \quad (3.80)$$

where the advection terms have been rewritten in conservation form and it is assumed that the reaction only occurs in the interstitium. Similarly, for the boundary conditions

$$(D_t \nabla_{\mathbf{X}} c_t + \varepsilon D_t \nabla_{\mathbf{x}} c_t) \cdot \mathbf{n} = (D_c \nabla_{\mathbf{X}} c_c + \varepsilon D_c \nabla_{\mathbf{x}} c_c) \cdot \mathbf{n} \quad \text{on } \Gamma \quad (3.81)$$

$$c_c = c_t \quad \text{on } \Gamma \quad (3.82)$$

As before, a multiple-scales expansion is performed with

$$\mathbf{u} = \mathbf{u}^{(0)} + \varepsilon \mathbf{u}^{(1)}(\mathbf{x}, \mathbf{X}, t) + \varepsilon^2 \mathbf{u}^{(2)}(\mathbf{x}, \mathbf{X}, t) + \dots \quad (3.83)$$

$$c = c^{(0)} + \varepsilon c^{(1)}(\mathbf{x}, \mathbf{X}, t) + \varepsilon^2 c^{(2)}(\mathbf{x}, \mathbf{X}, t) + \dots \quad (3.84)$$

To maintain periodicity each component of $\mathbf{u}$ and c is periodic in $\mathbf{X}$. The velocity terms should already be known from the homogenization of the blood flow on the capillary scale.

It is now possible to homogenize the mass transport equation depending on what are the dominant mechanisms at the macro-scale. There are 7 cases to be homogenized, assuming a maximum of an order ε difference between any two mass transport mechanisms. **Table 3.2** summarises the appropriate scalings of P_d , Pe_d , and Da_d for the dominant mechanisms.

Out of these 7 cases, only one is appropriate for oxygen transport in the brain. As this is the most interesting solute transport in this thesis, I will only analyse the case that most resembles oxygen transport on the macro-scale. The other 6 cases,

which can represent a variety of other solutes depending on their respective dimensionless numbers, are presented in Appendix B.

	P_d	Pe_d	Da_d
Advection only	$O(\varepsilon)$	$O(1)$	$O(\varepsilon^2)$
Metabolism only	$O(\varepsilon)$	$O(\varepsilon)$	$O(\varepsilon)$
Advection-Metabolism	$O(\varepsilon)$	$O(1)$	$O(\varepsilon)$
Diffusion only	$O(\varepsilon^2)$	$O(\varepsilon^2)$	$O(\varepsilon^3)$
Advection-Diffusion	$O(\varepsilon^2)$	$O(\varepsilon)$	$O(\varepsilon^3)$
Diffusion-Metabolism	$O(\varepsilon^2)$	$O(\varepsilon^2)$	$O(\varepsilon^2)$
Advection-Diffusion-Metabolism	$O(\varepsilon^2)$	$O(\varepsilon)$	$O(\varepsilon^2)$

Table 3.2 Appropriate scalings of local dimensionless numbers depending on which transport mechanisms are dominant on the global-scale

3.2.3.5 Oxygen Transport on the Macro-Scale

Characteristic values for oxygen transport in the cerebral capillary networks are presented in **Table. 3.3**.

Symbol (Units)	Parameter	Characteristic Value
d (m)	Local length scale	50×10^{-6} [103]
L (m)	Macro length scale	$\approx 1 \times 10^{-3}$
U (m s ⁻¹)	Characteristic blood velocity in capillaries	0.2×10^{-3} [79]
D_{ch} (m ² s ⁻¹)	Characteristic Diffusion	1.8×10^{-9} [22]
λ (s ⁻¹)	Linear metabolic rate	3.2 [64]

Table 3.3 Table of typical values of the modelling parameters of interest for oxygen transport in the brain.

Here, λ is a very crude estimate taken from linearizing the metabolic rate of oxygen (CMRO_2) in brain tissue [64]. Using these values, $Pe_L = 111$ and $Da_L = 1778$. This indicates that advection dominates over diffusion and metabolism dominates over diffusion as well. More specifically, the macro-advection time scale is $O(\varepsilon)$ smaller than the macro-diffusion time scale and the macro-reaction time scale is between $O(\varepsilon)$ and $O(\varepsilon^2)$ smaller than the macro-diffusion time scale. If I choose the $O(\varepsilon^2)$ scaling I find that the system is not homogenizable [12], therefore it is assumed that the reaction time scale is $O(\varepsilon)$ smaller than the diffusion.

Subsequently, $t_c = t_L^{adv} = t_L^{reac} = \varepsilon t_L^{dif}$. Using the equations in (3.79) I find that $P_L = O(\frac{1}{\varepsilon})$, $Pe_L = O(\frac{1}{\varepsilon})$, and $Da_L = O(\frac{1}{\varepsilon})$. On the local-scale that means that $P_d = O(\varepsilon)$, $Pe_d = O(1)$, and $Da_d = O(\varepsilon)$. Hence, (3.80) becomes

$$\begin{aligned} \varepsilon \overline{P_d} \frac{\partial c}{\partial t} + Pe_d (\nabla_{\mathbf{X}} \cdot (c\mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c\mathbf{u})) &= D \nabla_{\mathbf{X}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c + \\ &\varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon \overline{Da_d} c \end{aligned} \quad (3.85)$$

where $\overline{P_d}$ and $\overline{Da_d}$ are both of $O(1)$. Substituting the multiple-scales expansion of $\mathbf{u}$ and c ((3.83) and (3.84)) into (3.85) and into the boundary conditions (3.81, 3.82) yields

$$\begin{aligned} \varepsilon \overline{P_d} \frac{\partial}{\partial t} (c^{(0)} + \varepsilon c^{(1)} + \dots) + Pe_d \{ \nabla_{\mathbf{X}} \cdot ((c^{(0)} + \varepsilon c^{(1)} + \dots)(\mathbf{u}^{(0)} + \varepsilon \mathbf{u}^{(1)} + \dots)) + \\ \varepsilon \nabla_{\mathbf{x}} \cdot ((c^{(0)} + \varepsilon c^{(1)} + \dots)(\mathbf{u}^{(0)} + \varepsilon \mathbf{u}^{(1)} + \dots)) \} &= D \nabla_{\mathbf{X}}^2 (c^{(0)} + \varepsilon c^{(1)} + \dots) + \\ 2\varepsilon D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} (c^{(0)} + \varepsilon c^{(1)} + \dots) + \varepsilon^2 D \nabla_{\mathbf{x}}^2 (c^{(0)} + \varepsilon c^{(1)} + \dots) - \\ \varepsilon \overline{Da_d} (c^{(0)} + \varepsilon c^{(1)} + \dots) \end{aligned} \quad (3.86)$$

$$\begin{aligned} (D_t \nabla_{\mathbf{X}} (c_t^{(0)} + \varepsilon c_t^{(1)} + \dots) + \varepsilon D_t \nabla_{\mathbf{x}} (c_t^{(0)} + \varepsilon c_t^{(1)} + \dots)) \cdot \mathbf{n} &= \\ (D_c \nabla_{\mathbf{X}} (c_c^{(0)} + \varepsilon c_c^{(1)} + \dots) + \varepsilon D_c \nabla_{\mathbf{x}} (c_c^{(0)} + \varepsilon c_c^{(1)} + \dots)) \cdot \mathbf{n} &\text{ on } \Gamma \end{aligned} \quad (3.87)$$

$$(c_c^{(0)} + \varepsilon c_c^{(1)} + \dots) = (c_t^{(0)} + \varepsilon c_t^{(1)} + \dots) \quad \text{on } \Gamma \quad (3.88)$$

Equating $O(\varepsilon^0)$ terms:

$$D\nabla_{\mathbf{X}}^2 c^{(0)} - Pe_d \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(0)}) = 0 \quad (3.89)$$

Defining the operator

$$\mathcal{L}_0 \star := D\nabla_{\mathbf{X}}^2 \star - Pe_d \nabla_{\mathbf{X}} \cdot (\star \mathbf{u}^{(0)}) \quad (3.90)$$

equation (3.89) becomes

$$\mathcal{L}_0 c^{(0)} = 0 \quad (3.91)$$

with the $O(\varepsilon^0)$ boundary conditions being

$$(D_t \nabla_{\mathbf{X}} c_t^{(0)}) \cdot \mathbf{n} = (D_c \nabla_{\mathbf{X}} c_c^{(0)}) \cdot \mathbf{n} \quad \text{on } \Gamma \quad (3.92)$$

$$c_c^{(0)} = c_t^{(0)} \quad \text{on } \Gamma \quad (3.93)$$

Similar to the work in Shipley and Chapman, this system only has constant solutions in $\mathbf{X}$, i.e. $c^{(0)} = \bar{c}(\mathbf{x}, t)$. The proof can be found in Appendix A. Next, at $O(\varepsilon^1)$:

$$\begin{aligned} \overline{P_d} \frac{\partial c^{(0)}}{\partial t} + Pe_d \{ \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(1)}) + \nabla_{\mathbf{X}} \cdot (c^{(1)} \mathbf{u}^{(0)}) + \nabla_{\mathbf{x}} \cdot (c^{(0)} \mathbf{u}^{(0)}) \} = \\ D\nabla_{\mathbf{X}}^2 c^{(1)} + 2D\nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(0)} - \overline{Da_d} c^{(0)} \end{aligned} \quad (3.94)$$

with $O(\varepsilon^1)$ boundary conditions:

$$(D_t \nabla_{\mathbf{X}} c_t^{(1)} + D_t \nabla_{\mathbf{x}} c_t^{(0)}) \cdot \mathbf{n} = (D_c \nabla_{\mathbf{X}} c_c^{(1)} + D_c \nabla_{\mathbf{x}} c_c^{(0)}) \cdot \mathbf{n} \quad \text{on } \Gamma \quad (3.95)$$

$$c_c^{(1)} = c_t^{(1)} \quad \text{on } \Gamma \quad (3.96)$$

From (3.94) the $c^{(1)}$ diffusion term grouped with $Pe_d \nabla_{\mathbf{X}} \cdot (c^{(1)} \mathbf{u}^{(0)})$ gives $\mathcal{L}_0 c^{(1)}$. As $c^{(0)}$ is only a function of the macro-scale variable spatially ($\mathbf{x}$), the cross-diffusion term is 0. Finally, if the remaining two advection terms are expanded and the $O(\varepsilon^1)$ incompressibility equation used from the blood flow homogenization (3.26) then (3.94) can be simplified to

$$\mathcal{L}_0 c^{(1)} = \overline{P_d} \frac{\partial c^{(0)}}{\partial t} + Pe_d (\mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)}) + \overline{Da_d} c^{(0)} = g_1(\mathbf{x}, \mathbf{X}, t) \quad (3.97)$$

The concentration is coupled over the interstitium and capillary domains (due to the continuity of concentration on the capillary wall boundary condition (3.73)) so the Fredholm alternative is used to determine the appropriate solvability condition for (3.97). The solvability condition, as demonstrated by [167], is the volume average of $g_1(\mathbf{x}, \mathbf{X}, t)$ over the local domain, i.e.

$$\frac{1}{|\Omega|} \int_{\Omega} \mathcal{L}_0 c^{(1)} dV = \frac{1}{|\Omega|} \int_{\Omega} g_1(\mathbf{x}, \mathbf{X}, t) dV \quad (3.98)$$

Due to the locally constant flux boundary condition, the first integral is zero. Implementing the solvability condition for the second integral gives:

$$\frac{1}{|\Omega|} \int_{\Omega} \overline{P_d} \frac{\partial c^{(0)}}{\partial t} dV + \frac{1}{|\Omega|} \int_{\Omega_c} Pe_d(\mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)}) dV + \frac{1}{|\Omega|} \int_{\Omega_t} \overline{Da_d} c^{(0)} dV = 0 \quad (3.99)$$

The unsteady state term is integrated over the whole domain, yet the advection term is only integrated over the capillary domain (as there is no blood leakage into the interstitium), and the reaction term is only integrated over the interstitial domain (as metabolism has been assumed to occur in the interstitium only). As a result, the homogenized equation is

$$\overline{P_d} \frac{\partial c^{(0)}}{\partial t} + Pe_d(\tilde{\mathbf{u}}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)}) + \overline{Da_d} \phi_t c^{(0)} = 0 \quad (3.100)$$

where again ϕ_t is the volume fraction of the interstitium and $\tilde{\mathbf{u}}^{(0)}$ is defined as

$$\tilde{\mathbf{u}}^{(0)} = \langle \mathbf{u}_c^{(0)} \rangle_c = \frac{1}{|\Omega|} \int_{\Omega_c} \mathbf{u}_c^{(0)} dV \quad (3.101)$$

Finally, re-dimensionalizing the variables gives us the homogenized equation for advection and reaction dominated flow on the global-scale to leading order – and the appropriate mass transport equation for oxygen transport at this scale.

$$\frac{\partial c'}{\partial t'} + \widetilde{\mathbf{u}}' \cdot \nabla c' = -\lambda \phi_t c' + O(\varepsilon) \quad (3.102)$$

The $O(\varepsilon)$ term is the error of the leading order equation. This term can be found by continuing the multiple-scales expansion and solving for $c^{(1)}$. This will bring diffusion into the equation as a correction term.

3.2.4 Theory Summary

There are three main parts to the theory in this chapter: homogenization of blood flow in healthy capillary networks; homogenization of fluid flow in the interstitium (or tissue) surrounding the capillary networks; and homogenization of the mass transport equation for solutes in both the capillary networks and the interstitium.

3.2.4.1 Homogenization of Blood Flow in the Capillary Networks

Starting with the Navier-Stokes equation to model blood flow in the capillary networks, and the assumption that the networks are healthy (no blood leakage into the interstitial domain), the blood flow was homogenized from the micro- to the macro-scale. It was found that the equivalent representation of a periodic random capillary network on the macro-scale was Darcy flow. The parameter that links the micro-scale network to the macro-scale is the permeability $\mathbf{K}$. This permeability must be determined from a representative elementary volume of the network before large-scale simulations can be run. This will be the next step.

3.2.4.2 Homogenization of Fluid Flow in the Interstitial Domain

As well as homogenizing the blood flow in the capillary networks, the fluid flow in the interstitial tissue is also homogenized. Assuming Darcy flow to begin with, it is found that on the macro-scale the flow through the interstitium is also a volume averaged Darcy flow with the parameter of interest $\mathbf{E}$ being derived from the micro-scale. This parameter was found to be the volume fraction of the tissue within the micro-scale

model multiplied by the identity tensor. This parameter will also be determined from a representative elementary volume of the capillary network. It should be noted, that in the case of an unhealthy vascular network, where there is leakage from the capillary network into the interstitium, the two macro-scale equations for both domains will be coupled, as has already been shown by Shipley and Chapman [168].

3.2.4.3 Homogenization of the Mass Transport Equation

The final part of the theory is the homogenization of the mass transport equation, specifically applied to oxygen transport. It was assumed the concentration of solute was coupled in the capillary and interstitial domain so that the whole domain could be represented by one equation. The homogenization of the micro-scale mass transport equation resulted in a macro-scale mass transport equation, with the parameters of interest being the volume fraction of the tissue (for the metabolic term) and the volume averaged blood velocity (for the advection term).

Hereon in I will mainly be concerned with developing models of blood flow in the cerebral vasculature. As a result, the theory for the oxygen transport equations is left here for others to take forward.

3.3 Materials and Methods

The method for calculating the two tensors of interest ($\mathbf{K}$, $\mathbf{E}$) will now be presented. The homogenized permeability tensor $\mathbf{K}$ is particularly important as this encapsulates the geometry of the periodic substructure. The permeability tensor is the permeability of the capillary network for blood (similar to hydraulic conductivity) and as such has units $\text{m}^3 \text{s kg}^{-1}$. It is an averaged coefficient tensor, calculated by solving the micro cell problem. Determining the converged values of the elements of the tensor allows us to build ‘scaled-up’ models of blood flow in the cerebral microvasculature, as previously stated.

3.3.1 Calculation of the Permeability Tensor

In order to determine the averaged permeability tensor, a periodic boundary value problem must be solved over the periodic micro cell of the capillary network. Assuming the radial and swirl components of the blood velocity at the capillary scale to be zero, and fully developed flow, then the blood follows Poiseuille flow. Integrating the velocity profile of each capillary over the cross-section of capillary m leads to Poiseuille's equation:

$$\mathbf{q}_m^j = \frac{\pi r_m^4}{8\mu_m} \left(\frac{\Delta P_m^j}{L_m} \right), \quad j = 1, 2, 3 \quad (3.103)$$

where $\mathbf{q}_m^j$ is the flux through capillary m , r_m and L_m are the radius and length of the capillary respectively, and ΔP_m^j is the local pressure drop along the capillary length. The viscosity law of Pries et al. is used to account for non-Newtonian rheology for a constant discharge haematocrit of 0.45 [146].

Since the left hand side of (3.36) is a surface mean, it can be calculated by summing the capillary flow rates cutting through a surface of the voxel and dividing by the area of that surface. Therefore, the elements of the permeability tensor can be calculated as such:

$$K_{ij} = \frac{\Sigma \mathbf{q}_{m,surf}^j}{\Gamma^j (\Delta p^i / L)}, \quad i, j = 1, 2, 3 \quad (3.104)$$

where $\mathbf{q}_{m,surf}^j$ is a surface outflow node in the j th direction, Γ^j is the cube outflow surface area, and $\Delta p^i / L$ is the pressure gradient imposed across the periodic cube of length L . A diagram of the periodic surfaces of the cube is shown in **Fig. 3.3**. As a result, three independent pressure gradients are required in order to determine $\mathbf{K}$ for the 3-dimensional structure of the cerebral microvasculature. In our case i, j refer to Cartesian co-ordinates. As an example, if we wish to calculate K_{11} then a pressure

gradient is imposed in the x -direction, the total flow rate through the Γ_1 surface is calculated, and divided by the surface area Γ_1 and the pressure gradient in the x -direction. To calculate the cross flows in the y and z directions, the same procedure is followed but over the Γ_2 and Γ_3 surfaces respectively, yielding K_{12} and K_{13} . Imposing pressure gradients in the y and z directions produces the second and third rows of the tensor respectively, and hence the full 3×3 permeability tensor.

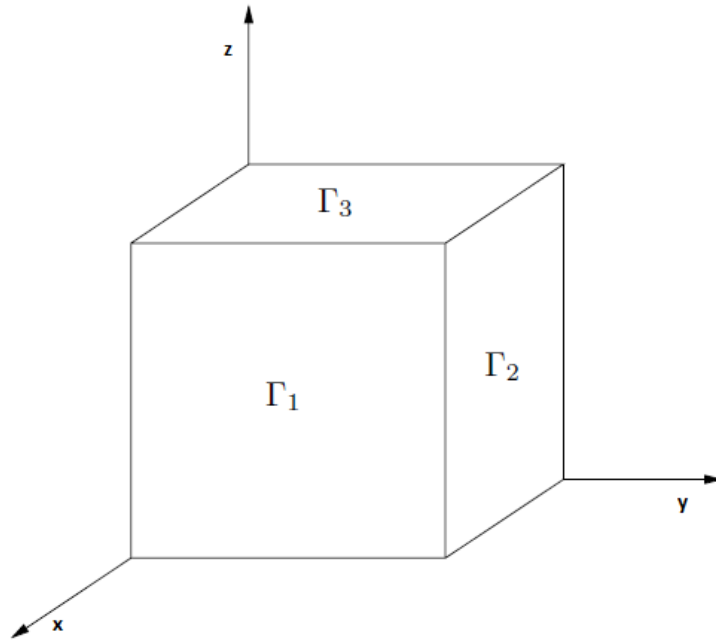


Fig. 3.3 Diagram of the three periodic faces of the capillary cube over which the permeability will be calculated. Γ_1 , Γ_2 , and Γ_3 are the outflow surface areas in the x , y , and z directions respectively.

3.3.2 Calculation of the $\mathbf{E}$ tensor

The calculation of the $\mathbf{E}$ tensor for the interstitial fluid flow model is in principle very simple. As $\mathbf{E} \approx \phi_t \mathbf{I}$, the only parameter that needs to be calculated is the volume fraction of the interstitial domain, which is just one minus the volume fraction of the capillary domain. As the radii and lengths of all the capillaries are known in the statistical model, it is trivial to calculate their volume fraction, and hence the interstitial volume fraction.

3.3.3 Application to Synthetic Cerebral Vascular Networks

The network generation algorithm developed by Su et al. [176] is used here to generate statistically accurate models of the cerebral microvasculature. The homogenization theory already detailed is then applied to this model to calculate the permeability tensor. The model is based on the morphometric data obtained by Cassot et al. [26] and Lauwers et al. [101]. In those studies, quantitative morphometric information was extracted from the collateral sulcus in the right temporal lobe using Indian ink and confocal laser microscopy to analyse sample sections. The vessel density, length distribution of vessels, distribution of the radii of the vessels, and average connectivity were some of the parameters found which allow for the generation of statistically accurate models of the cerebral vasculature. The minimum set of statistical metrics required for the accurate characterization of the capillary bed is still a topic of debate. However, the networks generated in this thesis are statistically accurate based on the statistics that are currently available and presented.

The algorithm, proposed by Su et al., uses a modified spanning tree method (MSTM), where two spanning trees are generated from randomly seeded nodes on a given voxel which then merge to form a random capillary network [176]. The diameters of the capillaries were assumed to be normally distributed with a mean of $6.23 \mu\text{m}$ and a standard deviation of $1.3 \mu\text{m}$, in accordance with experimental data. In order to fit the length distribution of the vessels found in Cassot et al., filters are then applied, which remove and add vessels depending on their length. Details of these filters can be found in Su et al. [176].

The assumption of periodicity made during the homogenization process means it is necessary to modify this model. It is currently not spatially periodic, nor does it have periodicity of blood flow through opposing surfaces of the voxel. Therefore, the model is modified here to introduce spatial and flow periodicity. In order to impose

spatial periodicity, the surfaces of the voxel must be symmetric. This is achieved by randomly seeding three non-opposite faces with nodes and copying their co-ordinates over to their opposing faces. The voxel is split into 27 sub-cubes with the internal nodes seeded randomly but uniformly across these 27 regions to ensure a space-filling capillary network in the voxel. Each surface node has one capillary connected to it from the internal network. Capillaries attached to opposing surface nodes are summed to form one long capillary. Along with the filters used by Su et al., this allows for the generated models to statistically fit the experimental data despite the constraint of spatial periodicity. A typical spatially periodic voxel is shown in **Fig. 3.4**.

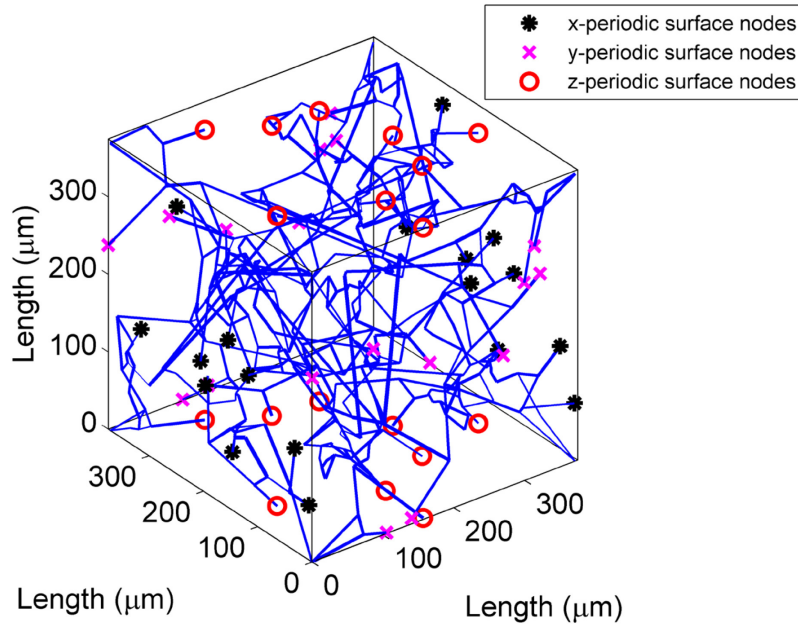


Fig. 3.4 Typical cerebral capillary network, generated in a 375 μm cube with the additional constraint of spatial periodicity.

Various voxel sizes are generated in order to determine how the permeability of the cerebral network varies with size, and whether there is a representative elementary volume. In order to ‘scale-up’ to larger voxel sizes a base voxel must be chosen. As there can only be an integer number of nodes, the size of the periodic base voxel is limited by being able accurately to fit the experimental data whilst having

one surface node on each face. It was found that a $125\ \mu\text{m}$ cube with one surface node on each face is able to match the length distribution of the capillary network, as well as the segment density (in the range $7219 - 8817\ /\text{mm}^3$ for the samples analysed by Cassot et al. [26]). Therefore, one surface node on a $125\ \mu\text{m}$ cube is deemed to be an acceptable base voxel. Larger cubes can be built by stacking $125\ \mu\text{m}$ cubes together.

Finally, the flow over opposing faces of the voxel must also be periodic, i.e. the total influx over one face must be equal and opposite to the outflux over the opposing face. This is achieved by using wrap-around (periodic) boundary conditions on the four faces over which the pressure gradient is not applied.

The capillary networks are simulated 500 times for every cube size so that the average length distribution of the capillaries matches that observed by Cassot et al. [26]. The 10 cube sizes used ranged in length from $125 - 625\ \mu\text{m}$ (see **Table 3.4**). Cube sizes not multiples of $125\ \mu\text{m}$ had the number of surface nodes rounded down to the nearest whole integer. The sizes chosen were such that the rounding error of the surface nodes was kept to a minimum, whilst also giving a good spread of values in the range of sizes being simulated. Cube sizes larger than $625\ \mu\text{m}$ were found to be computationally expensive to simulate, therefore $625\ \mu\text{m}$ was chosen as the upper bound of the sizes.

3.3.4 Calculation of the Representative Elementary Volume

The idea of scale separation introduces the concept of a representative elementary volume (REV). Within the range of the REV the macroscopic permeability coefficient (and $\mathbf{E}$ tensor) do not change significantly. The REV contains a large enough number of capillary scale heterogeneities that the permeability coefficient calculated from it can be considered to be the effective permeability coefficient on the macro-scale [153].

As the periodic voxel simulation size increases, $\varepsilon \rightarrow 0$, and hence the permeability coefficient tensor asymptotically tends to the effective macro-scale permeability. It is therefore expected that as voxel size increases, the calculated permeability (and $\mathbf{E}$ tensor) will tend to a converged asymptotic macro permeability, similar to that observed by Smith et al. for their simulations of capillary networks in the rat myocardium [169].

In order to determine the converged permeability of each element in the tensor, the root mean square error between the variation of permeability with cube size and a curve of best fit of the form:

$$K_{mean} = \Delta K e^{-\frac{L}{L_{ch}}} + K_{eff} \quad (3.105)$$

was minimised, where K_{mean} is the mean permeability at every cube size, L the cube length, L_{ch} a characteristic length, ΔK a stretch coefficient, and K_{eff} the effective, or macro, permeability. The average permeability values at each cube size for each element in the tensor is plotted, and the line of best fit provides a functional equation of how the permeability varies with cube size. This form of equation was chosen to fit the data as it was a simple fit with few parameters. ΔK , L_{ch} , and K_{eff} were found using the `fminsearch` function on MATLAB. It is then possible to calculate the REV for an acceptable error margin by comparing the calculated permeability of a given cube size with the converged permeability, K_{eff} . Determining the minimum REV is important in order to minimise computation time when scaling up models.

3.4 Results

3.4.1 Periodic Network Generation

The cube sizes used, along with the average segment density, are listed in **Table 3.4**. As can be seen, all the mean segment values lie within the range 7219 - 8817 /mm³ found experimentally [26].

Cube Size (μm)	Mean Segment Density (/mm ³)	Mean Segment Length (μm)	No. Surface Nodes (/face)
125	8621 $\pm$ 939	57.5 $\pm$ 5.8	1
180	7744 $\pm$ 697	57.2 $\pm$ 3.2	2
250	7991 $\pm$ 479	57.2 $\pm$ 1.9	4
300	7685 $\pm$ 327	53.5 $\pm$ 1.3	5
375	7763 $\pm$ 229	56.4 $\pm$ 0.9	9
425	8048 $\pm$ 209	54.2 $\pm$ 0.7	11
450	8162 $\pm$ 184	54.6 $\pm$ 0.6	12
500	7934 $\pm$ 164	56.1 $\pm$ 0.6	16
550	8272 $\pm$ 143	54.9 $\pm$ 0.5	19
625	8115 $\pm$ 119	55.0 $\pm$ 0.4	25

Table 3.4 Table of the mean segment densities, mean segment length, and surface nodes of the 10 periodic cube sizes used to model the cerebral capillary network.

The distribution of the number of segments for each cube are normally distributed over the samples (all p-values < 0.05), except for the 125 μm cube. Therefore, the segment density at each cube size can be characterised by a mean and standard deviation. The average length distribution of the simulated capillary networks provides a good match to the experimental results of Cassot et al., despite the additional constraint of periodicity (see **Fig. 3.5** and **Table 3.4**). The mean capillary length density – the product of the mean segment density and the mean

length – ranged from 411 – 496 mm/mm³, which again is within the morphometric range of 411.60 – 613.17 mm/mm³ [101].

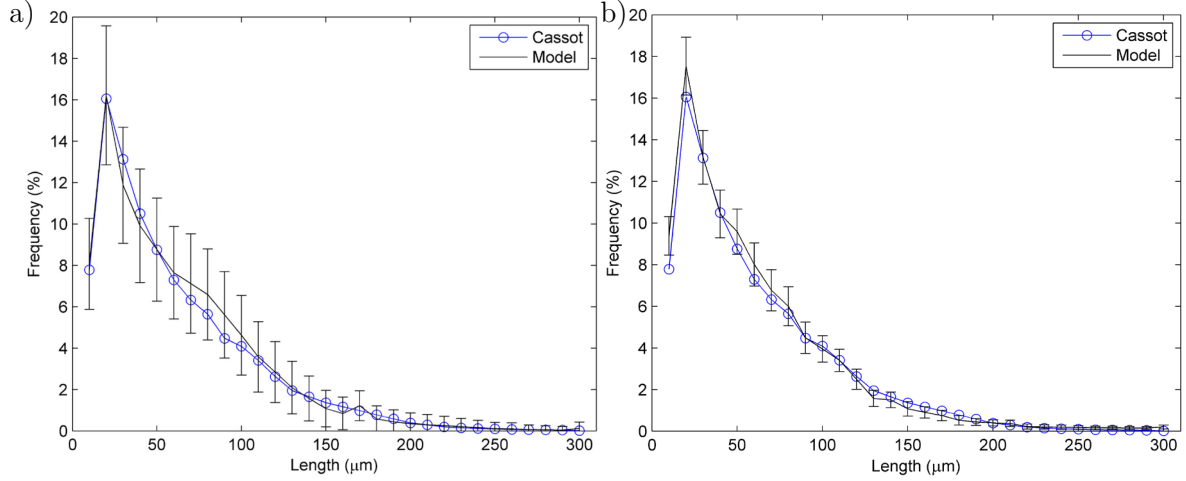


Fig. 3.5 Vessel length distribution of a periodic a) 250 μm voxel and a periodic b) 450 μm voxel compared with experimental data converted from Cassot et al. [26]

3.4.2 Permeability of Capillary Networks

All elements of the permeability tensor were normally distributed at each cube size bar those for the 125 μm cube (Kolmogorov-Smirnov test with significance level 5%). This was due to the very small number of segments in this voxel size (average of 16) which effectively meant there was a discrete distribution of permeability values. Thus, the results of the 125 μm cube are omitted from the rest of the work. The diagonal terms of the permeability tensor all displayed a decreasing permeability as the cube size increased (**Fig. 3.6**). The permeability appeared to converge for all three diagonal terms (**Fig. 3.6**). The exponential line of best fit (3.105) was applied over cube sizes $\geq 180 \mu\text{m}$. The converged permeability values, K_{eff} were found for K_{11} , K_{22} , and K_{33} to be $4.26 \times 10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$, $4.31 \times 10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$, and $4.27 \times 10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$ respectively (the values of ΔK , L_{ch} , and K_{eff} are given in **Table 3.5**). The difference in the three diagonal values was $< 1.2 \%$ indicating isotropy of the tensor. This mirrors the underlying “mesh or net-like structure” [26] of the cerebral microvasculature,

which appears random and isotropic.

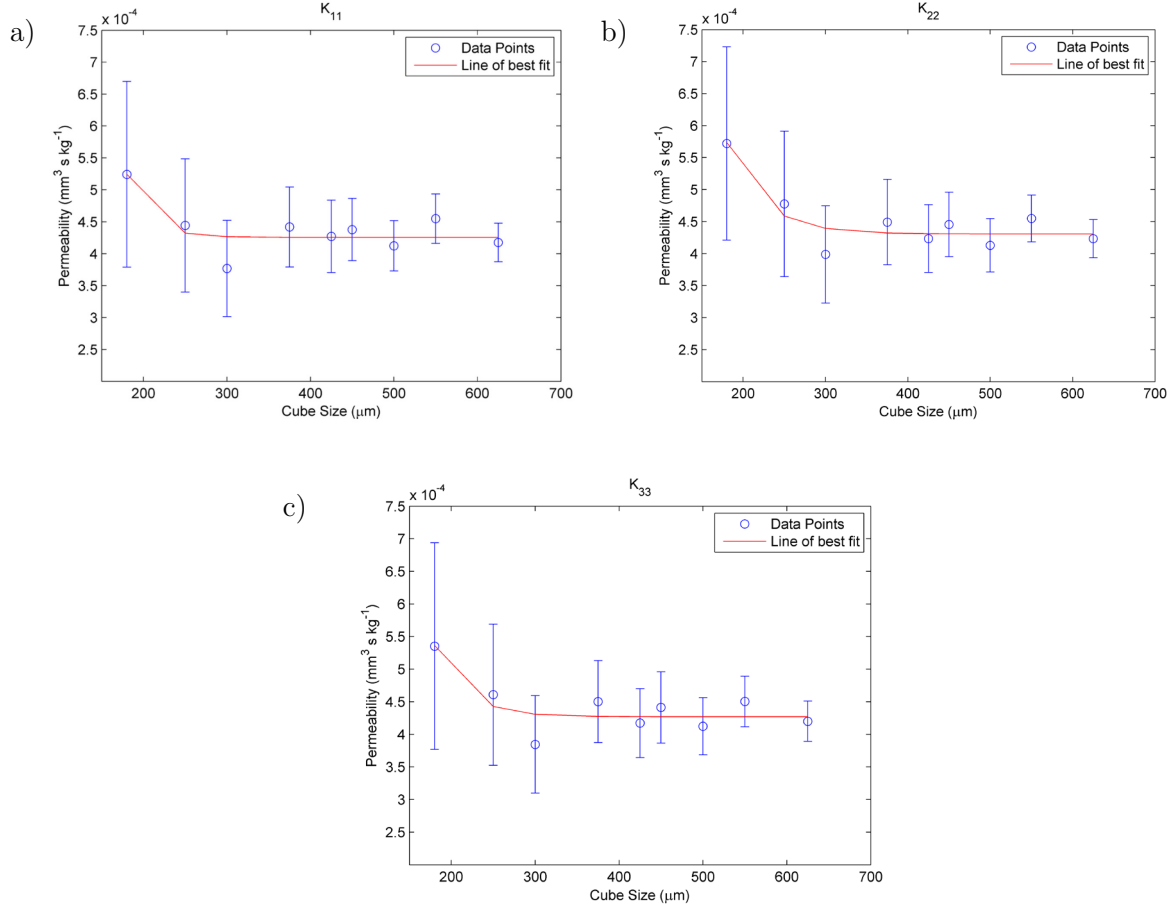


Fig. 3.6 Error-bar plots of the a) K_{11} , b) K_{22} , and c) K_{33} terms of the permeability tensor. Lines of best fit (red) pass through the error-bars of all cube sizes.

As an example, simulations over the 180 μ m, 375 μ m, and 625 μ m voxel sizes gave mean K_{33} values of $(5.35 \pm 1.58) \times 10^{-4}$ mm³ s kg⁻¹, $(4.50 \pm 0.63) \times 10^{-4}$ mm³ s kg⁻¹, and $(4.20 \pm 0.31) \times 10^{-4}$ mm³ s kg⁻¹. The magnitudes of the errors in these values from the converged permeability (K_{eff}) range from around 25 % to 1.6 %. This is in contrast to the error in the mean calculated by Smith et al. which has a maximum value of around 70 % for a 100 x 100 x 21.6 μ m³ voxel [169]. The lack of a sharp increase in permeability at smaller cube sizes is likely to be due to the more random, isotropic nature of the cerebral microvasculature, as opposed to the more aligned myocardial capillaries modelled by Smith et al. It is also likely that the much larger

density of the longitudinally aligned myocardial capillaries will have partially accounted for this difference. The diagonal terms of the permeability matrix were found to be an order of magnitude smaller than those measured by Smith et al. for capillaries aligned parallel to the epicardial surface.

The off-diagonal K terms were found to be on average two orders of magnitude smaller than the diagonal terms, indicating that the majority of blood flow occurs in the direction of the pressure gradient. The off-diagonal pairs ($[K_{12}, K_{21}]$, $[K_{13}, K_{31}]$, $[K_{23}, K_{32}]$) had very similar lines of best fit which all appeared to converge towards a mean of zero (**Fig. 3.7**). A t-test was performed to determine whether the off-diagonal terms were distributed normally about a mean of zero. The null hypothesis could be accepted for all 6 terms, indicating that the mean was zero. Any deviations from zero were purely due to random variations in construction of the networks.

	K_{eff} (mm ³ s kg ⁻¹) x10 ⁻⁴	ΔK (mm ³ s kg ⁻¹) x10 ⁻⁴	L_{ch} (μ m)
K_{11}	4.26	1.03	25.9
K_{12}	-0.019	3.96	3.96
K_{13}	-0.0067	0.76	19.7
K_{21}	-0.026	4.13	3.76
K_{22}	4.31	0.096	42.8
K_{23}	0.037	-0.0014	134
K_{31}	0.00025	0.71	18.6
K_{32}	0.050	-0.0015	133
K_{33}	4.27	0.158	36.2

Table 3.5 Parameters of the lines of best fit for the nine permeability tensor elements.

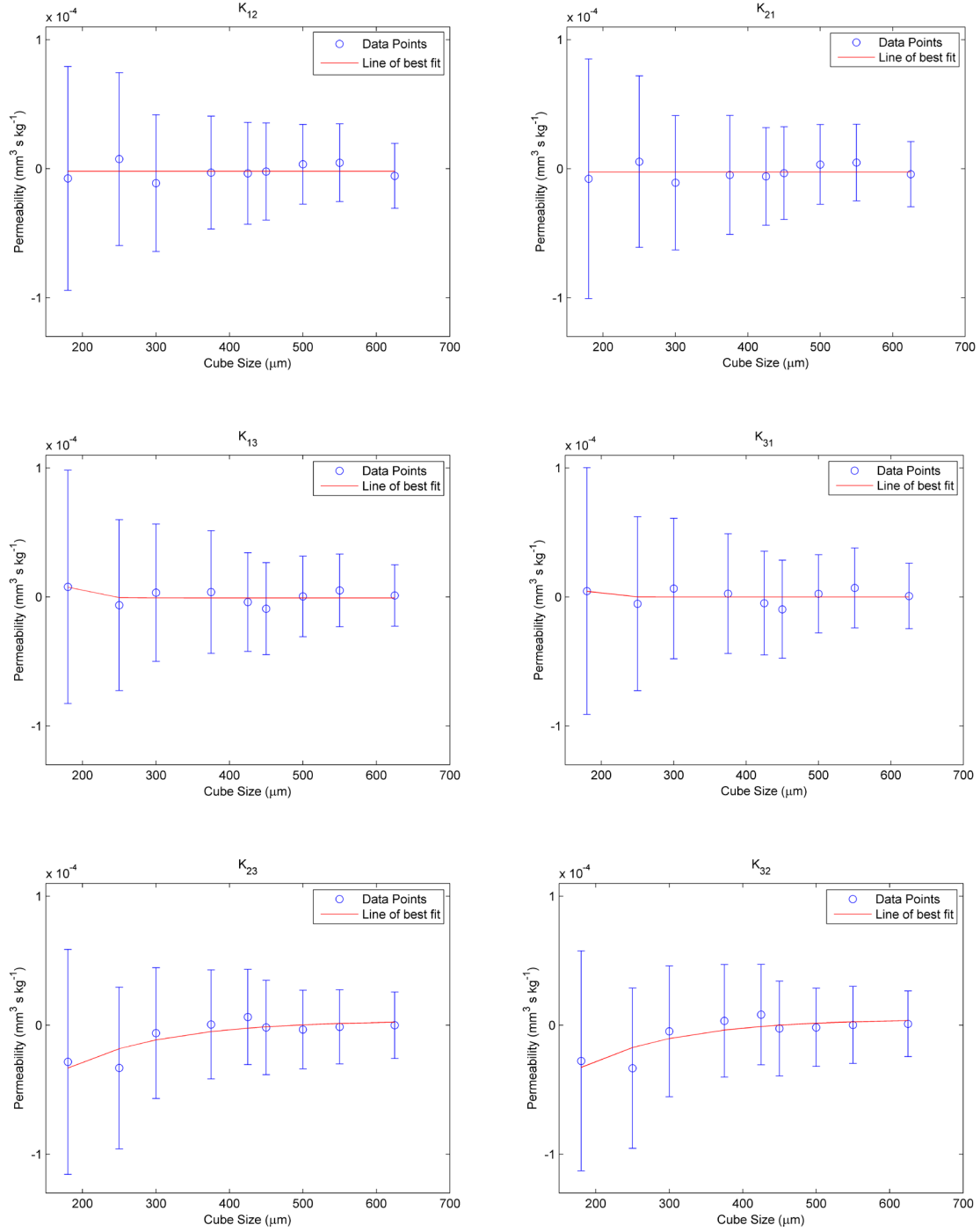


Fig. 3.7 Error-bar plots of the off-diagonal terms of the permeability tensor. Lines of best fit (red) pass through the error-bars of all cube sizes.

The off-diagonal pairs were not perfectly symmetric, with slightly differing parameters and converged permeabilities for the lines of best fit (Table 3.5). However, it is expected that as the number of simulations for each cube size increases,

the off-diagonal pairs will become symmetric. Smith et al. found that the off-diagonal pairs for the myocardial permeability tensor had a mean permeability of order 10^{-8} $\text{mm}^3 \text{ s kg}^{-1}$. This contrasts with the larger off-diagonal mean found in this study, of order 10^{-6} $\text{mm}^3 \text{ s kg}^{-1}$. This is likely due to the ordered nature of the simulated myocardial capillary network which had orthogonal connections between principal direction and cross-flow capillaries. In the cerebral microvasculature, the capillaries can be aligned in any direction in 3-dimensions, thus increasing the amount of cross-flow.

The standard deviation of the diagonal terms dropped linearly with increasing cube size on a log-log plot (**Fig. 3.8**). The standard deviation of the off-diagonal terms similarly displayed a linear drop on a log-log plot. Therefore, the standard deviations drop according to a power law as expected. The coefficients of this power law fit (of the form $K_{ij} = aV^k$) for the 9 elements of the permeability tensor can be found in **Table 3.6**. In comparison to the cross-flow standard deviation, the diagonal standard deviations have a steeper drop. The cross-flow standard deviation is almost symmetric, and it is expected that as the number of simulations is increased it will become symmetric.

	K_{11}	K_{12}	K_{13}	K_{21}	K_{22}	K_{23}	K_{31}	K_{32}	K_{33}
a	0.0468	0.0493	0.0273	0.0459	1.1868	0.0321	0.0068	0.0135	0.0612
k	-1.143	-1.191	-1.089	-1.184	-1.651	-1.107	-0.859	-0.969	-1.171

Table 3.6 Table of power law fit coefficients for the standard deviation of the 9 elements of the permeability tensor ($K_{ij} = aV^k$)

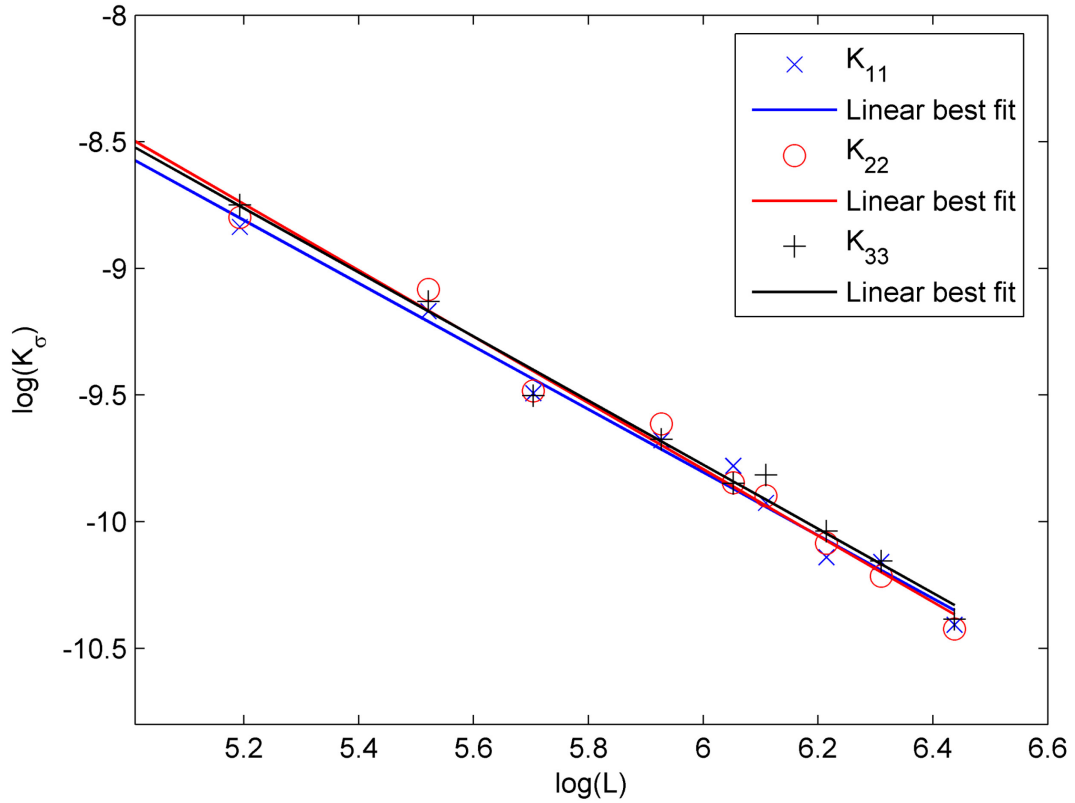


Fig. 3.8 Log-log plot of the standard deviation of the diagonal permeability terms over cube size.

Finally, it can be seen that the permeability matrix is isotropic. At the $625\ \mu\text{m}$ cube size the difference between the 3 diagonal terms is $< 3\%$. This is unsurprising as the statistical capillary model presented here is isotropic by construction (due to the random network generation). It is not certain whether anatomical cerebral capillary networks are isotropic, although qualitatively the cerebral capillary bed appears to have a ‘mesh-like’ robust structure, potentially indicating regions of isotropy in the cerebral microvasculature. This is in contrast to other organs such as the heart which has capillaries aligned along a principal direction in the myocardium. This leads to highly anisotropic permeability tensors as demonstrated by [169] where there was a factor of 10 difference between the principal and non-principal capillary directions.

3.4.3 $\mathbf{E}$ tensor of the Capillary Networks

The interstitial permeability is calculated by multiplying the volume fraction of the interstitium by the identity tensor. This comes from assuming the capillary diameters are much smaller than the characteristic length of the local-scale, hence the integral in (3.66) is just a small order correction to $\mathbf{E}$ [167]. Therefore only E_{11} is plotted as E_{22} and E_{33} are the same (**Fig. 3.9**).

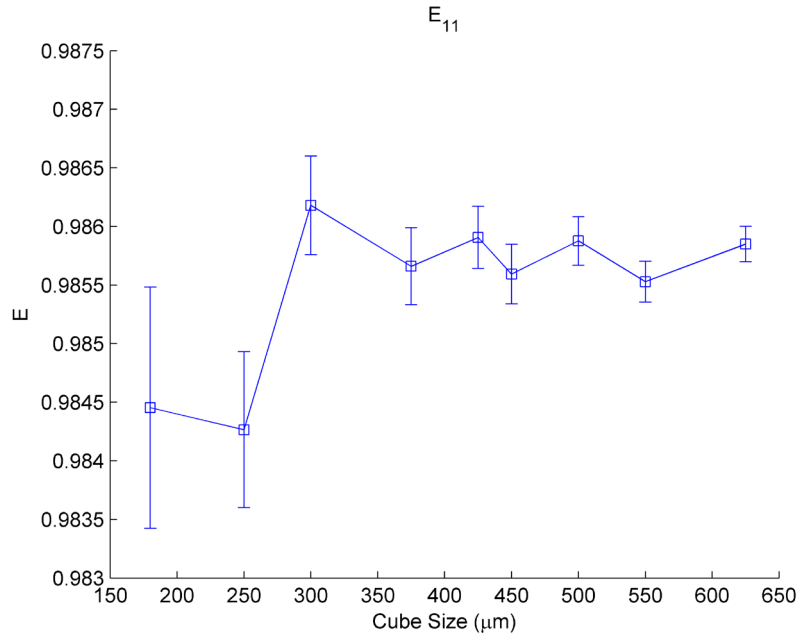


Fig. 3.9 Plot of variation of E_{11} with cube simulation size displaying very little change with cube size.

The difference between the 625 μm value and the 180 μm value is around 0.15%. This minimal difference indicates convergence of the interstitial permeability. Therefore, when determining the representative elementary volume (REV) of the cerebral capillary network, the $\mathbf{K}$ tensor should be used.

3.4.4 Calculation of the Representative Elementary Volume

The choice of an REV is determined by what is deemed an acceptable error from the converged permeability K_{eff} of the diagonal terms (since the off-diagonal terms are

close to 0). For a 375 μm cube the average error was 4.5 %. This drops to 3.5 % at 500 μm and 1.7 % at 625 μm . Larger cube sizes however require a greater computational effort. Therefore, on balance it was decided that the 375 μm cube, with an error of 4.5 % was an acceptable REV for future study of this network.

3.4.5 Perfusion Estimation

The permeability calculated above can be validated, in a similar fashion to Smith et al., using experimental cerebral blood flow (CBF) measurements [169]. Assuming that the pressure gradient is linear across the capillary bed, the perfusion ($\mu\text{m}^3 \text{s}^{-1}$) into a region of the brain can be estimated by integrating the Darcy velocity over a cross-sectional area A (μm^2). If the Darcy velocity is parallel to the principal axis over which the pressure gradient is imposed, then:

$$Perfusion = K_{ii} \times \frac{\Delta p}{l} \times 0.133 \times 10^{-3} \times A \quad (3.106)$$

where the factor 0.133×10^{-3} converts from mmHg to $\text{kg } \mu\text{m}^{-1} \text{s}^{-2}$, K_{ii} is in $\mu\text{m}^3 \text{s kg}^{-1}$, and Δp is a uniform pressure drop of 18 mmHg [110] over a pre-capillary arteriolar post-capillary venular path length of $l \approx 340 \mu\text{m}$ [158]. Perfusion can also be related to the CBF using the following equation:

$$Perfusion = \frac{CBF}{60} \times \frac{\rho}{100} \times A \times l \quad (3.107)$$

where $\rho = 0.96 \text{ g cm}^{-3}$ and is the cerebral density [36,115], the factor of 60 converts from minutes to seconds, and the factor of 100 converts from g to 100g. Substituting (3.107) into (3.106) using the converged permeability values of K_{11} , K_{22} , and K_{33} yielded a CBF in the range $55.1 - 55.8 \text{ mL min}^{-1} 100\text{g}^{-1}$. These results are within the range, and very close to the mean, of the CBF values observed experimentally of $54.7 \pm 6.1 \text{ mL min}^{-1} 100\text{g}^{-1}$ [131].

3.5 Discussion

Generating models of large sections of the brain is extremely difficult with current discrete modelling techniques. In addition to this, the choice of boundary conditions for such models influences the flow and hence permeability of the network. As a result, homogenization was employed here to allow us to scale-up discrete vascular models to continuum models whilst simultaneously removing the boundary condition problem (the macro-scale solution is independent of the choice of boundary conditions).

In this chapter, the blood flow and oxygen transport through an arbitrary capillary network was homogenized. The approach used the assumption of a periodic local-scale to achieve the macro-scale continuum equations. The flow homogenized equations were expressed in terms of the permeability tensors for the capillary and interstitial phases. These tensors encapsulate the vascular structure and hence determine how macro-scale blood flow depends on local-scale topology.

The mass transport equation was also homogenized, with the time scale (t_c) not assigned immediately. The degree of freedom provided by not assigning the time scale immediately allows the dimensionless numbers to be scaled appropriately based on what are the dominant mechanisms. Seven cases were considered (six in Appendix B), depending on which combinations of advection, diffusion, and metabolism were dominant on the macro-scale. Data for oxygen transport in blood were used to determine which mechanisms dominate on the macro-scale for oxygen transport in the microvasculature; it was found that advection and reaction dominate over diffusion. The oxygen transport equations are left as theory and the simulations thereon in only dealt with blood flow.

The homogenization theory developed was then applied to a statistically accurate 3-dimensional model of the human cerebral microvascular network in a

portion of the temporal lobe. It was found that the permeability of the capillaries in the three principal directions was highly isotropic, with less than 1.2 % variation in their converged values. This was due to the “mesh-like” structure of the capillary network, and confirms that when modelling the cerebral microvasculature a 2-dimensional simplification of the model is not a valid assumption, unlike when modelling coronary capillary networks [169]. The off-diagonal permeability was found to be on average two orders of magnitude smaller than the permeability of the principal terms – and appeared to be converging to zero. This indicates that there was very little crossflow in the capillary network, despite its strongly interconnected nature. The standard deviation of the permeability tensor elements dropped exponentially. The exponential drop of the standard deviation with cube size is useful in characterising the capillary network.

The converged values of the diagonal permeability terms were found using an exponential line of best fit. The line of best fit passed through every cube size’s standard deviation error-bar. The converged values are of use when determining what size REV should be used. The choice is of course application dependent. When choosing, there is a trade-off between the computational cost of generating the periodic sub-structure, and the maximal tolerated deviation, or error, from the converged permeability. The REV should also be of a sensible size relative to any clinical data that are being used to validate the model. If attempting to compare the model with perfusion imaging data, which usually have voxel sizes of the order of mm, then the REV should be smaller than the in-plane resolution of the imaging data. For a 375 μm cube the average error is found to be 4.5 % in this study. This drops to 3.5 % at 500 μm and 1.7 % at 625 μm . Larger cube sizes however require greater computational effort. Therefore, on balance it was decided that the 375 μm cube, with an error of 4.5 %, was an acceptable REV for future study. The REV we have calculated is well

below typical MRI resolution, and hence can be used with MRI perfusion data for validation. The interstitial $\mathbf{E}$ tensor was also calculated, simply as the volume fraction of the interstitial domain (around 98% given a 2% capillary density). This was found to vary very little ($< 0.2\%$) across the cube sizes and hence to calculate the REV of the capillary network the permeability ($\mathbf{K}$) should be used.

Finally, the converged values of permeability were validated by calculating CBF in the brain. The converged values gave a CBF between $55.1 - 55.8 \text{ mL min}^{-1} 100\text{g}^{-1}$ which is in very good agreement with healthy human CBF of $54.7 \pm 6.1 \text{ mL min}^{-1} 100\text{g}^{-1}$ [131].

Reichold et al., Secomb et al., and Lorthois et al. all constructed accurate cerebral capillary networks directly from experimental data [110,149,163]. Such networks are difficult to scale-up discretely due to the rapidly increasing computational cost as voxel size increases. The choice of boundary conditions also has a very significant effect on the flow solution. Reichold et al. ‘up-scaled’ a 2-dimensional idealised model of the cerebral capillary bed, where all capillaries have the same diameter. The model built upon here, in contrast, conforms statistically to morphometric data, is 3-dimensional, and the homogenized flow solutions are independent of the choice of boundary condition – something that has previously been found to strongly influence the blood flow simulations [110]. Additionally, the model here has been used to characterise the effect of the microvasculature on tissue scale blood flow to allow for the construction of larger scale cerebral blood flow models. Such information has been previously unavailable.

In a similar study, Smith et al. used the same homogenization methods applied here on synthetic rat coronary capillary networks [169]. The network was assumed to be 2-dimensional with orthogonal connections between capillaries. They found that as

the voxel size of the capillary network increases, the permeability drops and eventually converges. This is also found here for the principal terms, although the magnitude of the drop is found to be smaller than that for a coronary network. This was due to the much more complex, less-dense, random nature of the cerebral microvasculature reducing the variability of flow with varying cube size. Smith et al. also found that the permeability of the network is of order $10^{-3} \text{ mm}^3 \text{ s kg}^{-1}$ in the longitudinal direction and of order $10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$ in the cross capillary direction. We found that the permeability for the cerebral microvasculature is of order $10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$ in all three orthogonal directions, in agreement with the cross-capillary direction (which had a similar order magnitude density as our capillary network). As the larger permeability found in Smith et al. is attributed as compensating for the compression of the capillaries, and hence restricted flow in systole, then it would appear that a permeability of order $10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$ is enough both in the heart and brain to maintain healthy function.

One limitation of the model is the assumption of periodicity of the microvascular network. This assumption was made to simplify the derivation of the homogenized equations; however, it is not yet known whether such an assumption is valid for the cerebral microvasculature. It can be argued that for a sufficiently random microstructure, the approximation of periodicity is a good assumption [13,69]. In the absence of experimental data determining the randomness or periodicity of the capillary networks, periodicity is used to simplify the derivation.

It should be noted that the network generation algorithm used here is based on one set of experimental morphometric data [26] (due to the lack of other accurate data on the cerebral microvasculature). Therefore, the permeability tensor calculated in this chapter characterises only a small region of the brain. The network topology and density may vary substantially within the brain, being closely linked with

functional activity. However, as the algorithm is statistically based, it would easily be possible to modify it for different sets of morphometric data, and to use the same homogenization procedure laid out in this chapter to characterise the permeability of different regions. As more information about the topology and geometry of cerebral microvascular networks becomes available, the more it will be possible effectively and accurately to characterise other regions of the brain.

The blood flow model here assumed a constant haematocrit throughout the capillary network, hence the viscosity in each capillary was only dependent on vessel diameter. It is known, however, that at bifurcations and trifurcations in the network, plasma skimming (or phase separation) takes place, where red blood cells are distributed unevenly across the daughter vessels. This leads to changes in haematocrit throughout the capillary network. This effect has been quantified [141], but was omitted in this chapter. Guibert et al. applied this variable haematocrit model to a model of primate cerebral vasculature, but found that the effects were not substantial [59]. A variable haematocrit model should be incorporated in future work although it is likely to have only but second order importance on blood flow, but is likely more important when considering oxygen transport.

The current discrete model adapted here [176] is able to model voxel sizes of up to $625\text{ }\mu\text{m}$ before becoming computationally too expensive on a standard PC. In order to make comparisons with clinical data, voxel sizes of the order mm will need to be generated. Imaging voxels are usually of the order mm^3 in volume. Such a volume will contain, as well as capillaries, larger structures such as arterioles and venules. If any accurate comparison is to be made between clinical or experimental perfusion data and the model, arterioles and venules must be incorporated into the model in the future, whilst maintaining periodicity. This will be the aim of the next chapter.

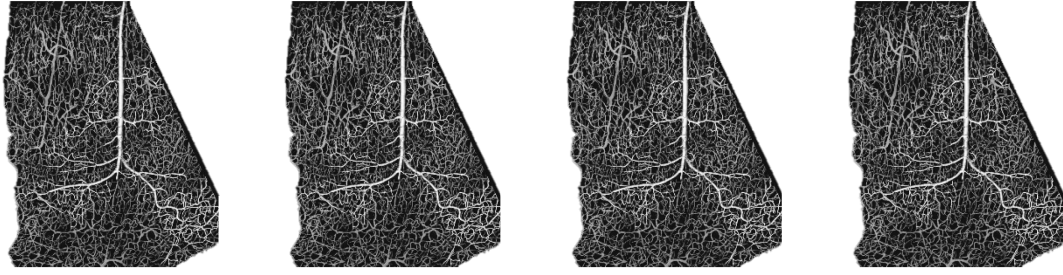
The method presented here provides a novel technique to ‘scale-up’ such discrete networks and to calculate the permeability tensor $\mathbf{K}$. This tensor provides fundamental information about the network flow conductivity and can be used to parameterise large models of the microvasculature. Modelling larger volumes of the brain is important for understanding whole-organ cerebral flow. The permeability tensor calculated here represents capillary bed flow conductivity under normal conditions. The framework developed however, could also be used to quantify the effects of microvascular changes in the brain. Of particular interest is ischaemic stroke, where a statistically accurate network model is already in place [134]. The current stroke model is discrete and only able to model cube sizes of $250\ \mu\text{m}$. Applying the techniques laid out in this chapter will allow us better to determine the effects of ischaemic stroke on the permeability of the capillary beds, as well as to build larger scale models to compare with imaging voxel data.

Chapter Summary

This chapter has developed and presented the theory behind homogenization of blood and oxygen transport in the human cerebral capillary networks. Additionally, it has characterised the tissue scale permeability of the cerebral microvasculature. Mathematical averaging techniques were combined with a statistical, periodic, anatomical model of the cerebral capillary bed to determine the permeability tensor. Calculation of the permeability tensor enabled characterisation of the microvasculature flow properties to be performed at a specific region in the brain. This can then be used to build larger models of perfusion which can in turn be compared to voxel data for validation. The techniques laid out in this chapter could be used to scale-up other microvascular topologies and densities. This will allow larger regions of the brain to be modelled, helping us further to understand the link between the microstructure and global perfusion. The permeability tensor has been shown to

converge to some effective permeability (K_{eff}) at larger voxel sizes, as well as to be isotropic, reflecting the mesh-like structure of the capillary network on the micro-scale. The off-diagonals were normally distributed about zero. These results parameterise the microvasculature for a region of the brain, and allow larger scale continuum models to be built.

In the next chapter I will develop an algorithm to generate statistically accurate arterioles and venules that can then be coupled to this homogenized representation of the capillary network. This is the next step in modelling larger regions of the human cerebral microvasculature.



Chapter 4

A Statistical Model of the Penetrating Arterioles and Venules in the Human Cerebral Cortex

The work presented in this chapter can be found published in El-Bouri, W. K. and Payne, S. J. (2016), ‘A statistical model of the penetrating arterioles and venules in the human cerebral cortex.’ *Microcirculation*, 23: 580–590. doi: 10.1111/micc.12318

Abstract

Models of the cerebral microvasculature are required at many different scales in order to understand the effects of microvascular topology on CBF and to develop large models of the microvasculature. There are, however, no data-driven models at the

penetrating arteriolar/venular scale. In this chapter I develop a data-driven algorithm based on available morphological data to generate statistically accurate penetrating vessels.

A novel order-based density-filling algorithm is developed, based on statistical data including bifurcating angles, length-to-diameter ratios, and area-ratios. Three thousand simulations are presented, and the results validated against morphological data. These are combined with the statistical properties of the previous capillary network (Chapter 3) in order to calculate full vascular network parameters.

Statistically accurate penetrating trees were successfully generated. All properties provided a good fit to experimental data. The k exponent of Murray's Law had a median of 2.5 and an interquartile range of 1.75-3.7. CBF showed a standard deviation ranging from $\pm 18\%$ to $\pm 36\%$ of the mean, depending on the penetrating vessel diameter.

Small CBF variations indicate that the topology of the penetrating vessels plays only a small part in the large regional variations of CBF seen in the brain. The radius of the penetrating vessels had a much larger effect on the CBF, suggesting that the variation of the CBF in the brain is minimally affected by the topology of the vessels, and is more likely to be affected by the varying vessel radii. These results open up the possibility of efficient oxygen and blood flow simulations at perfusion imaging voxel-scales which can be directly validated against perfusion imaging data.

4.1 Introduction

The microvascular architecture is recognized as being key to the perfusion rate and the delivery of oxygen to the surrounding tissue [59,149,166]. Changes in the cerebral

microvasculature topology have been linked to Alzheimer’s disease and vascular dementia [81,116], vascular occlusions [129,166], and brain tumours [24,80].

However, little is known about the link between the microvascular topology and global-scale blood and oxygen perfusion in the cortex. Imaging techniques, such as perfusion MRI, have spatial resolutions that are much larger than the microvasculature and hence are unable easily to discern the link between the microvasculature and global perfusion. Functional magnetic resonance imaging (fMRI) uses the blood oxygen level dependent (BOLD) technique as a surrogate signal to indicate neuronal activity. Its usefulness, however, “depends on an understanding of neurovascular coupling and the underlying vascular architecture” [27]. For example, Gagnon et al. recently attempted to quantify this link using two-photon microscopy on mice [54]. However, in order to better understand the interplay between the micro- and macro-scale, mathematical models are needed that are based on experimental data of the cerebral microvasculature. This will allow us to generate and to analyse many different models and configurations of the cerebral vasculature. These statistical models, informed by one-off casts and animal experiments, can then be used in conjunction with these methods to help us better to understand blood flow in the microvasculature, and to simulate blood flow in a way that is not restricted to particular experimental conditions.

A wealth of data has recently become available on the architecture of the microvasculature in the human cortex (see Appendix C). A series of studies by Duvernoy, Cassot, Lauwers, and Lorthois have produced statistical data on the structure and connectivity of the cerebral microvasculature, from the “tree-like” penetrating arterioles and venules that branch off the surface pial vessels, down to the “mesh-like” capillary networks that fill the grey matter [26–28,101,112]. Distinctions are made between the bifurcating arterioles and venules, and the highly connected

capillary network. Bifurcating angles, area ratios, length-to-diameter ratios (LDR), vessel densities, and lengths are among the variables quantified in the data sets analysed, affording the opportunity to build statistically accurate microvascular models of the cortex.

A statistically accurate capillary model based on this data has already been established by Su et al. [176]. This model, however, modelled capillaries discretely, and so generating voxel size models of the capillary network (order of mm^3) proved computationally very expensive. As a result, in Chapter 3, I used multi-scale methods to homogenize the network and to convert it into a continuum based problem where the permeability of the capillary network is used to generate large voxel size models. A standard MRI voxel will, however, contain both capillaries and penetrating vessels. Therefore, a statistical model of the penetrating arterioles and venules is required in order to couple it with the capillary network in a given voxel. This would then give a representative model of the microvasculature in a typical perfusion imaging voxel, allowing us to explore the link between the microvasculature and perfusion measurements. This will also allow us to look at the relationship between flow and metabolism in more detail.

In previous studies, Lorthois et al. [110] used image segmentation to reconstruct the arteriole, venule, and capillary networks of a given region of the cortex. Guibert et al. [59] also used image segmentation to reconstruct a region of the microvasculature for the primate cortex. Although these reconstructed networks are useful, they are specific to only the region of the microvasculature that has been simulated and hence are neither generalizable nor scalable. Karch et al. [86] used constrained constructive optimization combined with staged growth to generate arterial model trees. This framework works to minimise the intravascular volume of the tree whilst “optimizing the geometric location and topological site of each new

connection” [86]. This method was also used by Linninger et al. [107] to generate penetrating arterioles and venules. Although choosing to minimise intravascular volume is reasonable, it is probably not the only principle at work, and recent analysis of human arterioles and venules has shown that the k value in Murray’s law can vary by 3 orders of magnitude [28]. Additionally, none of these models use the wealth of statistical data now available to construct the penetrating vessels, relying instead on a volume minimisation assumption; they are thus not data-driven models.

Supplementary data in [27] give statistical means, medians, and standard deviations of the area ratios and angles at bifurcations in the arterioles and venules analysed (Appendix C). These are listed by order of the vessel starting at order 4 – being the largest diameter vessels – to order 0 being the terminal vessels. As well as these, vessel densities are given, along with LDR distributions by order [112]. These distributions can be used to generate statistically accurate penetrating arterioles and venules in the human cortex. Using this data to construct these vessels therefore removes the need for any assumptions to be made regarding the minimisation of tree volume or form of the tree.

This work thus introduces a data-driven statistical 3-dimensional model of the penetrating arterioles and venules based on available morphological data, removing the need for any assumptions as used in previous models. A novel order-based density-filling algorithm is developed which generates statistically accurate arteriolar and venular penetrating trees. Three thousand simulations are run and the variables of interest are validated against penetrating vessel morphological parameters found in the literature. The penetrating vessels are then combined with the capillary network already available [176] and the total network parameters are validated against morphological values. The k exponent of Murray’s Law is calculated and compared to the theoretical value. The vascular resistance of the penetrating trees is calculated to

give a first order approximation to the CBF, and to assess the variability of the CBF with the variations in topology modelled by the algorithm.

4.2 Materials and Methods

4.2.1 Morphological Parameters

The data collation of the morphological parameters used in the model is split into two parts. The first part deals with parameters required to model the penetrating arteriolar or venular trunk. The second part concerns the parameters used to model the branching vessels that bifurcate from the main trunk.

4.2.1.1 Penetrating Arterioles/Venules

Penetrating arterioles and venules are randomly spaced at the pial surface and show conspicuous large arteriolar and venular columns orthogonal to the cortical surface [129]. This observation suggests that penetrating vessels can be modelled individually and the various columns combined to form a volume of the cortex. Cuboid voxels are chosen here in which to model the individual trees. The surface area density of the penetrating vessels ranges from $8.44 - 15 /\text{mm}^2$ [154] with approximately $2/3$ of these vessels being arterioles and $1/3$ venules [27,59]. As such, a surface area density of $12 \text{ vessels}/\text{mm}^2$ was chosen, in the ratio 8 arterioles to 4 venules. Assuming a square surface area at which the penetrating vessel enters the grey matter, this fixes the lengths of the square for the arterioles and venules. A square surface is chosen here as the penetrating trees are collated to form a realistic cuboidal voxel in the next chapter.

Most penetrating arterioles and venules penetrate to the middle of the cortex (although some penetrate all the way to the white matter) [42]. The depth of the cortex is approximately 2.5 mm [42], hence the depth of the cube was chosen here to be 1.25 mm . Therefore, an arteriole model will have dimensions of $0.35 \times 0.35 \times 1.25 \text{ mm}$, and a venule model will be $0.5 \times 0.5 \times 1.25 \text{ mm}$. Eight arterioles along with four

venules embedded in a capillary bed running the depth of the cortex will have dimensions of $1 \times 1 \times 2.5$ mm, the same dimensions of a typical MRI voxel.

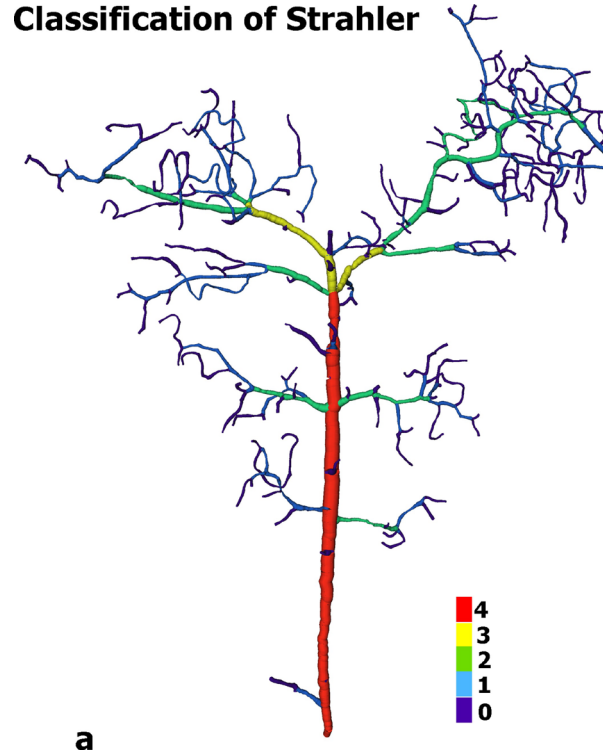


Fig 4.1 The branching pattern of an imaged cortical vein according to the diameter defined Strahler taxonomy. Reproduced with permission from [26].

The most numerous penetrating arterioles range in diameter from $15 - 40 \mu\text{m}$, with larger diameter vessels reaching $240 \mu\text{m}$ [42]. All arterioles of diameter $50 \mu\text{m}$ or less penetrate the cortex surface, with the largest diameter arterioles penetrating through to the white matter. The diameters remain approximately constant throughout the cortex, although some do have significant taper [129]. As a result, the diameter of the penetrating arterioles was chosen here to be $40 \mu\text{m}$ with the diameter remaining constant throughout the depth of the cube. Similarly, for the venules, a diameter of $110 \mu\text{m}$ was chosen in keeping with experimental observation [42].

Finally, an initial order must be assigned to the penetrating trunk, as the parameters used in the construction of the artificial trees are order-based. The order

system used here is the diameter-defined Strahler taxonomy [84] with the highest orders being assigned to the penetrating trunk and order 0 being terminal vessels (**Fig. 4.1**). Cassot et al. [26] identified between 3 – 5 orders of arterioles before the capillary bed (where a capillary is defined as a vessel with a diameter less than 10 μm). As a result, the penetrating trunk is given an initial order of 4, which is changed if the final tree density is not within 5% of the morphological value. The variable values used for the construction of the penetrating trunks, along with their justification, are given in **Table 4.1**.

Variable	Morphological Values	Model Values
Arteriole Diameter (μm)	15 – 240 ^a	40
Venule Diameter (μm)	20 – 125 ^a	110
Length (mm)	Up to 2.5 ^a	1.25
Surface density (/mm ²)	8.44 – 15 ^b	12
Penetrating Vessel Order	3 – 5 ^c	4

^a Duvernoy et al. [42] ^b Risser et al. [154] ^c Cassot et al. [26]

Table 4.1 Table of values chosen to model penetrating vessels.

4.2.1.2 Branching Vessels

A wealth of data has recently become available on the geometric properties of penetrating arterioles and venules in the cortex [27]. This provides an opportunity to generate statistically and geometrically accurate penetrating arterioles and venules – similar to the statistical model already in place for the capillary network in the cortex [43,176] – without having to make any assumptions about the form of the branching.

Area-ratios, LDRs, and bifurcation angle distributions are all available in [112] and the supplementary material of [27]. The data are recorded by order of vessel, as

well as for the total network of vessels in the cortex, making it straightforward to implement an order-based algorithm and to compare the results to the overall network parameters. The LDR distribution was found to be lognormal [112], but no data on the standard deviation and mean of each order were given (although the median at each order was given). As a result, at each order, a mean was chosen and the standard deviation was altered so that the total network LDR matched the experimental data. Normal distributions were assumed at each order.

Area ratios were given at a bifurcation between the parent branch and large daughter branch (A01), the parent and the small daughter (A02), and the large daughter and small daughter (A12). The distributions were all lognormal with given mean, median, and standard deviation [27]. Similarly, the bifurcation angles were given between the various branches ($\alpha01$, $\alpha02$, $\alpha12$). These were found to be approximately normally distributed with a given mean and standard deviation at each order (Appendix C).

4.2.1.3 Vessel Density

The algorithm developed here terminates when the appropriate vessel density has been reached for a given volume of the cortex. Cassot et al. found that in 28.6 mm^3 there were 11014 arteriolar segments and 8042 venular segments (with a ratio of 2:1 arteriolar arborisations to venular arborisations) [27]. Based on these values the vessel density required for the arterioles is $385.1 / \text{mm}^3$ and for the venules is $281.2 / \text{mm}^3$ (indicating that venules have more vessels per individual tree). These density values are used here to generate appropriate arteriolar and venular networks.

4.2.2 Algorithm

The novel order-based density-filling algorithm presented here produces statistically accurate columns of arteriolar and venular trees. The steps taken to produce these

penetrating vessels are shown in the flowchart in **Fig. 4.2**. The data used to generate these statistically accurate trees can be found in Appendix C.

The length, diameter, order, and dimensions of the column are input to generate the initial trunk of the penetrating arteriole. The trunk is then randomly seeded with nodes following the probability density function of primary branches given in Blinder et al. [20] (**Fig. C.1**). The first bifurcation generated from these nodes will always be the small daughter. The order of this branch is found by sampling from the morphological probability distribution of the connectivity of varying orders of vessel given by Cassot et al. [27] (**Fig. C.2**). The LDR, area ratio, and bifurcation angle distributions (**Figs. C.3 – C.5**) for the small daughter branch are next used to find the length, radius, and angles of the first bifurcation, determining the position of the end node of that branch. The primary branches are oriented randomly about the penetrating trunk.

For the next bifurcation, again the orders of the daughter vessels are found by sampling from the morphological probability distributions. Values of radius and length for both the large daughter and small daughter vessels are found and all 3 bifurcation angles are sampled from the normal distributions [27]. As a result, a constraint is imposed so that the sum of the three angles is always less than 2π (as the solid angle must be less than 2π). The radii, lengths, and bifurcation angles then uniquely determine the end nodes of the vessels. For arterioles, a twist about the parent branch of 90° is imposed to mimic the characteristic shape observed for arterioles. For venules, a random twist is imposed emulating their more lateral branching [42]. This branching is repeated until 4 orders of bifurcation have been reached, or all the terminal vessels are of order 0.

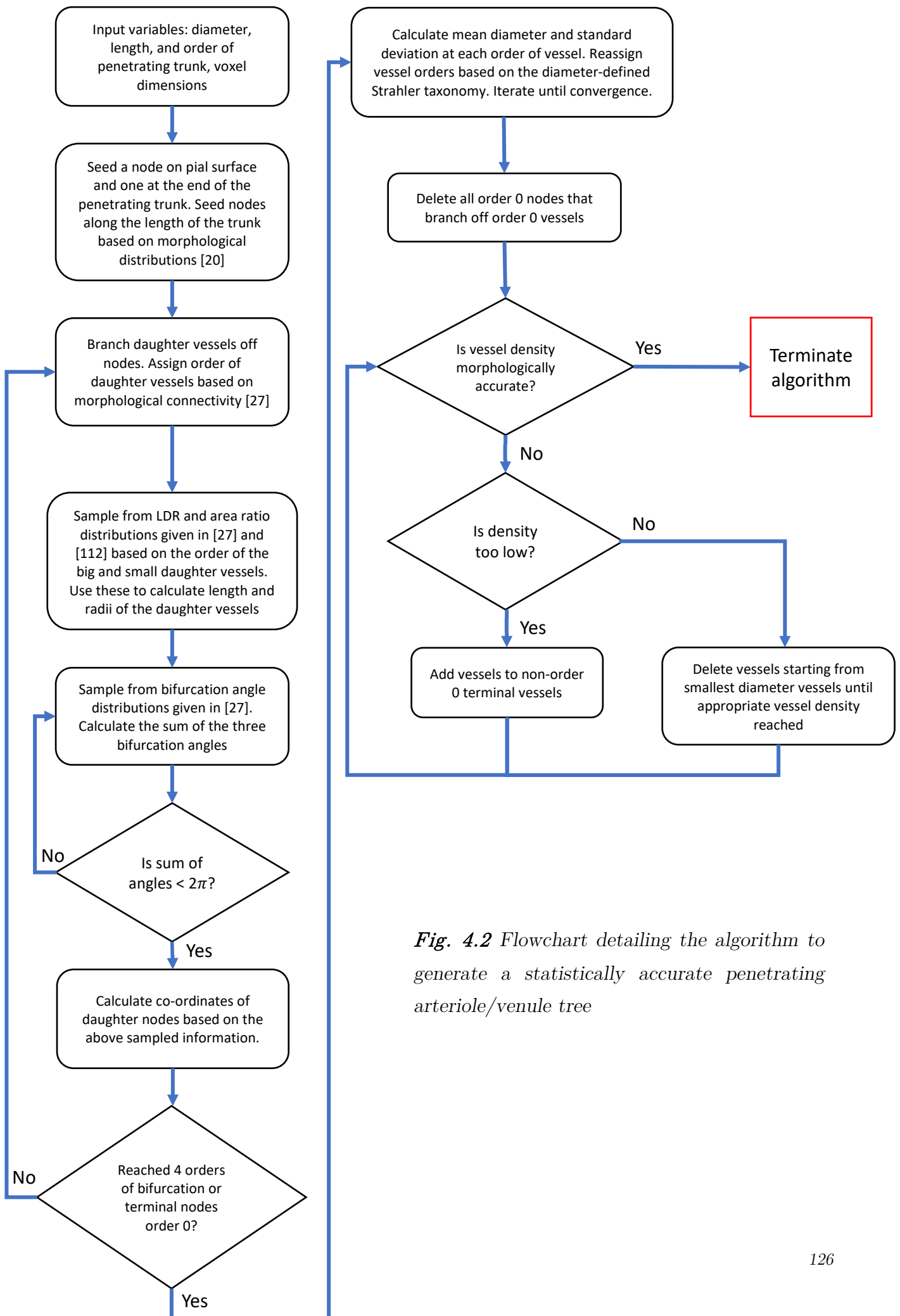


Fig. 4.2 Flowchart detailing the algorithm to generate a statistically accurate penetrating arteriole/venule tree

At this stage, the diameter-defined Strahler algorithm is used to calculate the mean and standard deviation of the diameters at each order. The vessels are then reassigned orders based on which order their diameter falls into. The density of the vessels is also calculated. Then three filters are used to ensure that the density is morphologically accurate. If a vessel is order 0 and has daughter branches, all the daughter branches are deleted. This filter is always used as an order 0 vessel must be terminal. Depending on whether the vessel density is too small or too large, two other filters are implemented. If the vessel density is too small, order 0 vessels are added to non-order 0 terminal vessels. If the density is too large, vessels are deleted starting with the smallest diameter vessels. Once a morphologically accurate density is finally reached the algorithm is terminated.

4.2.3 Vascular Resistance

The total vascular resistance of a penetrating tree is calculated and used to calculate blood perfusion as a first order approximation. Poiseuille flow is assumed in each vessel from which the resistance of the vessel can be found

$$\Delta P = RQ \quad (4.1)$$

$$R = \frac{8\mu(r)L}{\pi r^4} \quad (4.2)$$

where ΔP is the pressure drop in the vessel, Q is the flowrate, R is the resistance of the vessel, L is the length of the vessel, r is the radius of the vessel, and $\mu(r)$ is the apparent viscosity of the blood, which is dependent on the radius due to the Fåhræus-Lindqvist effect [47]. The apparent in-vivo viscosity can be calculated using the following relationship describing the variation of μ_{vivo} as a function of vessel diameter d_i (μm) and discharge haematocrit H_D (which is set here to be 0.45) [143].

$$\mu_{vitro} = \mu_p \left[1 + (\eta_{0.45} - 1) \frac{(1 - H_D)^{C_D} - 1}{(1 - 0.45)^{C_D} - 1} \right] \quad (4.3)$$

$$\eta_{0.45} = 220e^{-1.3d_i} + 3.2 - 2.44e^{-0.06d_i^{0.645}} \quad (4.4)$$

$$C_D = (0.8 + e^{-0.075d_i}) \left(-1 + \frac{1}{1 + 10^{-11} \cdot d_i^{12}} \right) + \frac{1}{1 + 10^{-11} \cdot d_i^{12}} \quad (4.5)$$

$$\mu_{vivo} = \mu_{vitro} \cdot \left(\frac{d_i}{d_{i,eff}} \right)^4 \quad (4.6)$$

μ_p is the viscosity of blood plasma (1.2 mPa s), μ_{vitro} is the in-vitro blood viscosity, and $\eta_{0.45}$ is the relative apparent viscosity of blood for a discharge haematocrit of 0.45 (defined as the ratio of the apparent viscosity to the plasma viscosity). The coefficients multiplying d_i all have units of $1/\mu\text{m}$. $d_{i,eff}$ is the effective diameter of the blood vessel that takes into account the thickness of the endothelial surface layer and is derived using correlations detailed in Pries and Secomb [143].

Using equations (4.2) – (4.6) the resistance of any given vessel can be calculated. For a given tree, the resistances at bifurcations are calculated in parallel and added to the parent vessel resistance. This is repeated until a given tree has one resistance, R_{total} . It is assumed here for simplicity that all the terminal vessels exit to one pressure P_{out} . For arterioles the pressure drop across the penetrating arteriole (ΔP) is set to be 35 mmHg, and for venules the pressure drop is set to be 15 mmHg [205]. Using the pressure drop and the total resistance, the flow rate Q through the tree can be calculated using equation (4.1) and from that the CBF using the following equation

$$CBF = \frac{Q \times 1 \times 10^6 \times 60 \times 100}{V \times \rho} \quad (4.7)$$

where V is the volume of tissue the tree feeds, ρ is the cerebral tissue density (0.96 g cm^{-3}) [36,115], 1×10^6 converts m^3 to mL, and 60 and 100 convert seconds to minutes and g to 100g respectively. The volume of tissue fed by a given tree is unknown in this model. As a result, it is chosen to be the cuboid volume that each arteriole/venule

is generated in (the cuboid having a depth of 1.25 mm). This of course may not be the region of influence of the penetrating vessel, but as I am only interested in the variation of the CBF with varying topology, this will not have an effect on the analysis.

4.2.4 Validation

Arterioles and venules are simulated over 1500 runs each. The algorithm uses statistics given at each order of vessel. Therefore, the bifurcation area ratios, bifurcation angles, and LDRs can be calculated and compared against experimental data. As well as this, the arteriole/venule trees can be combined with a statistically accurate capillary network in order to calculate total network statistics such as total length, mean segment length, and vascular volume.

4.3 Results

Examples of random arteriolar trees generated using the algorithm outlined are shown in **Fig. 4.3**. They show clearly the variability in the shape and density of the penetrating vessels that the algorithm introduces due to its use of sampling from statistical data. The blood vessels were assumed to be straight thin-walled cylinders, hence the slightly artificial look of the penetrating trees in **Fig. 4.3**. Three thousand simulations were run here in total, 1500 arterioles and 1500 venules. The results were then analysed and compared to morphological measurements.

4.3.1 LDRs, Area Ratios & Bifurcation Angles

Histograms of the generated values of LDR, area ratios, and bifurcation angles for all the bifurcations in the 3000 simulations are given in **Fig. 4.4**, along with the approximate morphological distributions calculated by Lorthois et al. and Cassot et al. [27,112]. Note that, for a given bifurcation, A0 refers to the cross-sectional area of

the parent vessel, $A1$ the cross-sectional area of the large daughter vessel, and $A2$ the cross-sectional area of the smaller daughter vessel. Similarly, $\alpha01$ is the angle between the parent and large daughter, $\alpha02$ is the angle between the parent and smaller daughter vessel, $\alpha12$ is the angle between the two daughter vessels, and the asymmetry is the difference between $\alpha01$ and $\alpha02$. The mean and standard deviations of the distributions fitted to the simulations presented here can be found in **Table 4.2**, along with the morphologically calculated parameters.

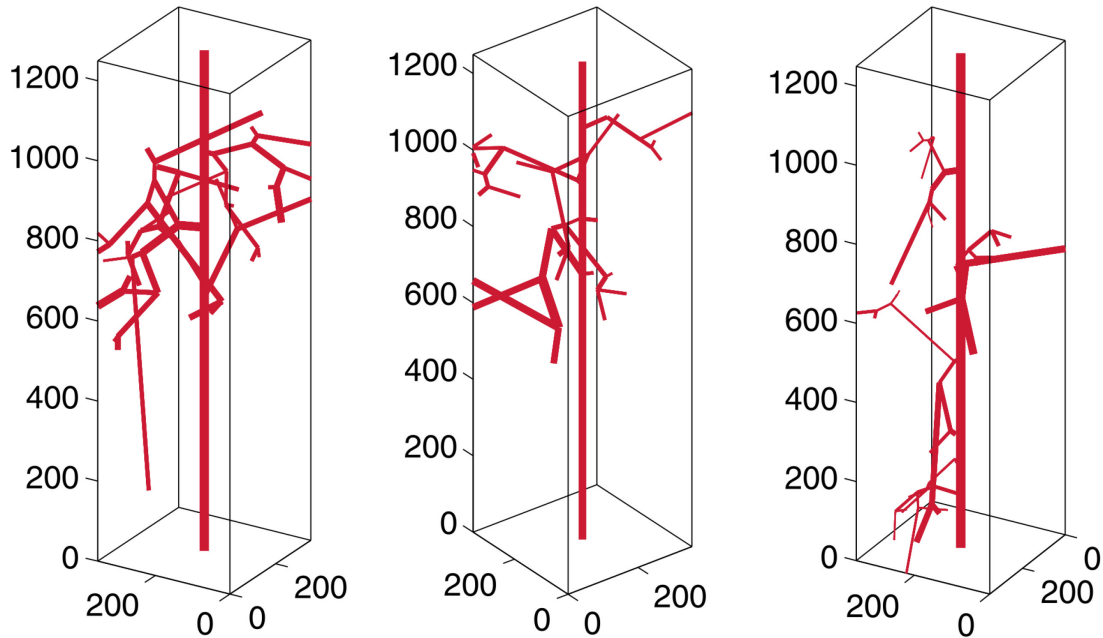


Fig. 4.3 Three examples of randomly generated morphologically accurate penetrating arterioles.

As can be seen by inspection there is, on the whole, a good fit to the morphological measurements. All the variables computed have a mean within the standard deviation of the experimental values (note that the mean vessel density values have no standard deviation as they are calculated from one 28.6 mm³ sample). No hypothesis testing was undertaken due to the large number of vessels analysed and hence vanishingly small values of standard error involved.

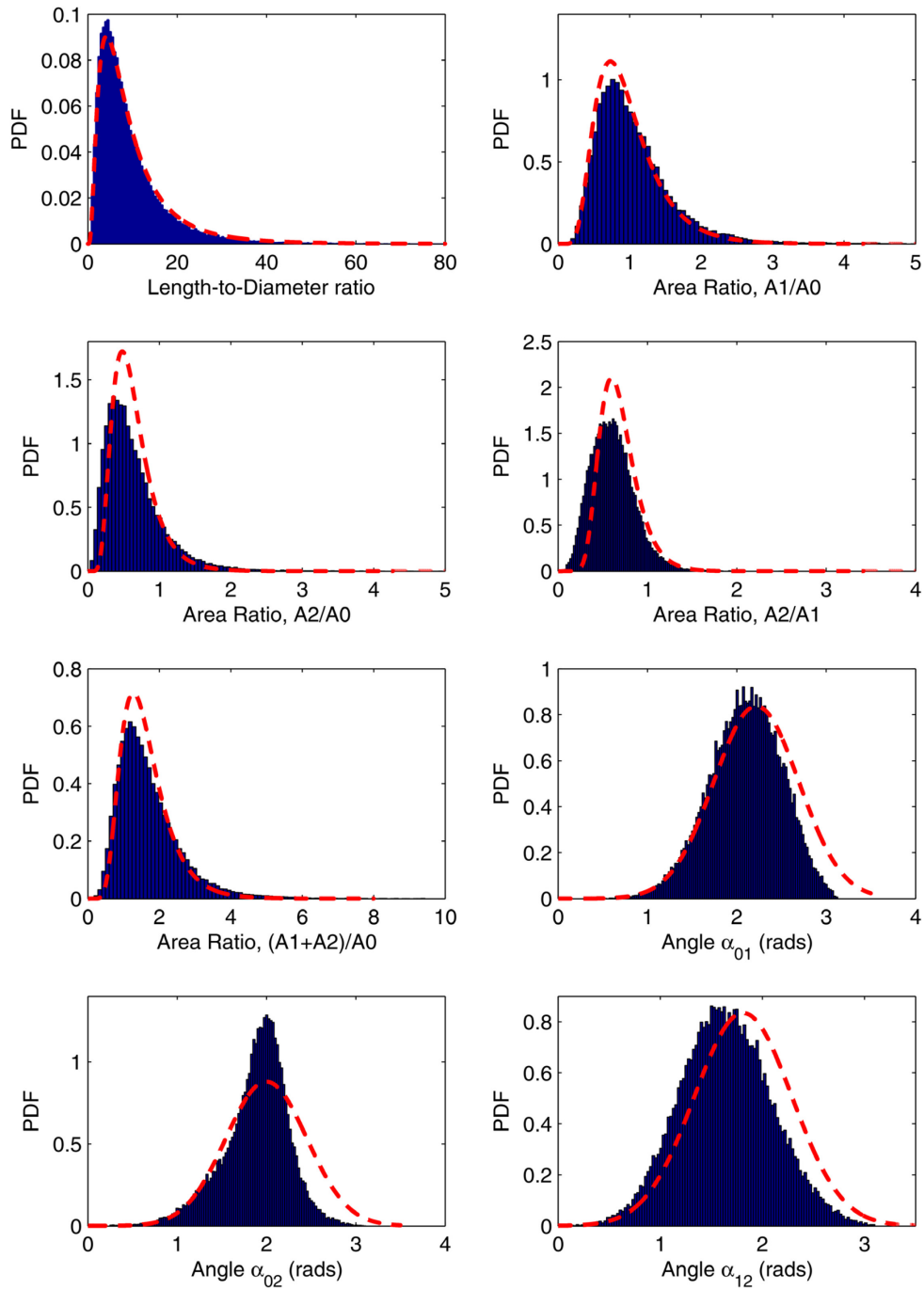


Fig. 4.4 Histograms of the simulation data (blue bars) generated using the algorithm (3000 penetrating trees). The red dotted line is the distribution approximated from the true morphological data for all the penetrating trees taken from [27,112]

	Computed Model Values	Morphological Measurements
Arteriole Mean Vessel Density (/mm ³)	382.1 ± 68.7	385.1^a
Venule Mean Vessel Density (/mm ³)	280.1 ± 42.5	281.2^a
Length-to-Diameter Ratio	9.46 ± 8.42	10.37 ± 9.41^b
A1/A0	1.06 ± 0.54	0.984 ± 0.46^a
A2/A0	0.646 ± 0.45	0.642 ± 0.294^a
A2/A1	0.614 ± 0.28	0.686 ± 0.213^a
(A1+A2)/A0	1.70 ± 0.90	1.626 ± 0.684^a
α_{01} (rads)	1.047 ± 0.20	1.109 ± 0.238^a
α_{02} (rads)	0.942 ± 0.18	0.999 ± 0.227^a
α_{12} (rads)	0.820 ± 0.223	0.906 ± 0.239^a
Asymmetry (rads)	0.106 ± 0.291	0.111 ± 0.373^a

^a Cassot et al. (including supplementary material) [27]; ^b Lorthois et al. [112]

Table 4.2 Mean and standard deviation of computed variables of interest and their corresponding morphological measurements.

The LDR and three area ratios were approximated as lognormal distributions by Cassot et al. [27], whilst the bifurcation angles were approximated as normal distributions. It should be noted that these are only approximations and that the real distributions from experimental measurement are neither perfectly normal or perfectly lognormal. Hence exact agreement is not expected.

The bifurcation angle distributions from experimental data are also approximated as normal by Cassot et al. and hence extend beyond a value of 180°. However, no angle can be greater than 180°, as the algorithm always takes the smaller

angle at a junction of two vessels; hence the histograms are cut off at this value (**Fig. 4.4**) whilst the experimental distribution extends beyond it.

4.3.2 Stem-Crown Analysis & Murray's Law

The normalized cumulative length ($L/L_{\max}$) and volume ($V/V_{\max}$) was computed for all the bifurcations generated by the model and these are shown in **Fig. 4.5**. $L_{\max}$ and $V_{\max}$ are the total cumulative length and the total cumulative volume respectively of a given penetrating tree. Each point in **Fig. 4.5** corresponds to a stem-crown unit on a given tree, where a vessel segment is defined as a stem and the entire tree downstream of the stem is defined as the crown [187]. A power law relationship is found between the normalized length and normalized volume of the form:

$$\frac{V}{V_{\max}} = \kappa \left(\frac{L}{L_{\max}} \right)^{\gamma} \quad (4.8)$$

with parameter values $\gamma = 1.239 \pm 0.007$ and $\kappa = 1.17 \pm 0.02$. Cassot et al. [28] had previously found $\gamma = 1.187 \pm 0.003$ and $\kappa = 0.965$ from morphological measurements. Both lines of best fit are plotted on a logarithmic graph in **Fig. 4.5**. As can be seen, there is very good agreement between the experimental and modelled results.

As well as this, the distribution of the exponent, k , of the generalized Murray's Law was calculated at every bifurcation (4.9):

$$d_o^k = d_1^k + d_2^k \quad (4.9)$$

where d_o is the parent vessel diameter, d_1 is the large daughter vessel diameter, and d_2 is the small daughter vessel diameter. According to the minimum energy principle (and hence minimum volume principle) proposed by Murray [121], the k exponent is expected to have a value of 3. The median value of k found here was 2.5 with an interquartile range (IQR) of 1.75 – 3.7. This is remarkably close to the value derived from physical principles by Murray. Cassot et al. calculated a k exponent with median

3.58 and IQR 2.28 – 14.5 from the data [28]. The spread of the k exponents over the different bifurcations is shown in **Fig. 4.6**. As can be seen, the k exponent varies from values below 1 all the way up to 25. There also appear to be peaks in the distribution at k values of 2, 3, 4, 6, 8, and 12. Despite some investigation, the reason for these peaks is uncertain.

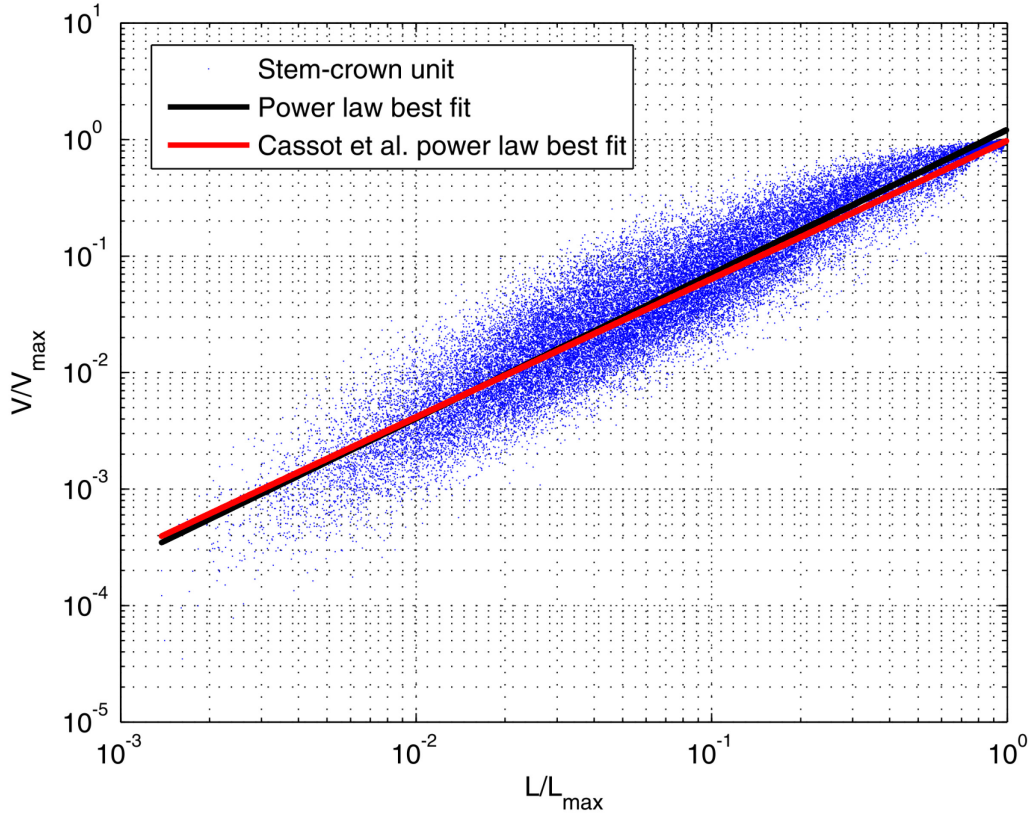


Fig. 4.5 Normalized cumulative volume–length relationships for all bifurcations simulated, along with power law fit (solid black line) and experimental fit (solid red line)

The spread in k shows that, although Murray’s Law with a k exponent of 3 is a useful modelling rule, it is likely to over-simplify the complex geometry and interactions of the microvasculature. Therefore, models that use a single k value of 3 to model the microvasculature should be used with caution.

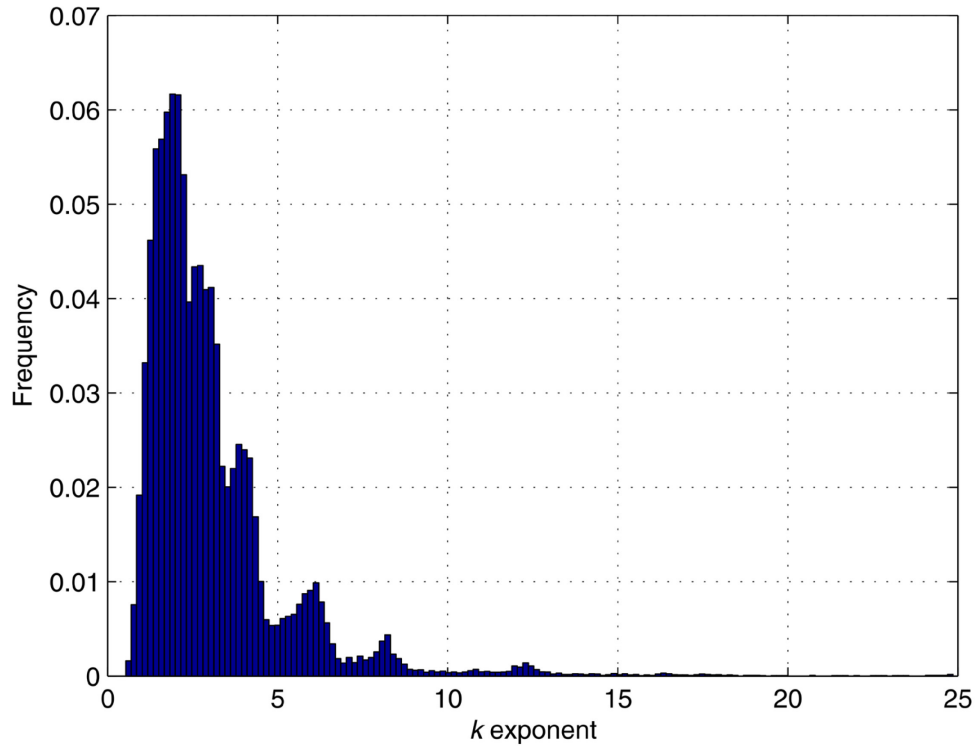


Fig. 4.6 Distribution of k exponent over all the bifurcations simulated by the algorithm.

4.3.3 Full Vascular Network

Su et al. [176] previously developed a statistically accurate capillary network of the human cortex, which was later homogenized and scaled-up in Chapter 3. The geometric properties of that network can now be combined with the geometric properties of the arteriole/venule network developed here in order to analyse statistically the full vascular network, looking at parameters such as total length/mm³, vascular surface/mm³, and vascular volume. The results are summarised in **Table 4.3**. It should be noted that the vascular volume was calculated assuming that the capillary network extended the depth of the grey matter (2.5 mm), but that the penetrating tree only extended halfway into the grey matter.

A specific morphological data set was used to compare against the model here. The data set in question had maximum vessel diameters of 25 μm [101]. Therefore, in

order to gain a meaningful comparison, the algorithm was run with penetrating arterioles of diameter $25\ \mu\text{m}$. The resulting values of total length/ mm^3 , vascular surface/ mm^3 , vascular volume, volume/surface, and mean segment length are all remarkably close to the morphologically measured values, validating the volume filling algorithm. As a comparison, Linninger et al. [107] calculated a vascular surface/ mm^3 of $13.60\ \text{mm}^2/\text{mm}^3$ and a vascular volume fraction of 2.49% using a volume minimisation algorithm (CCO) for the arterioles/venules, which are also in good agreement with the morphological parameters.

Parameter	25 μm Penetrating Arteriole	40 μm Penetrating Arteriole	Morphological Measurements ^a
Total Length/ mm^3 (mm/mm^3)	497.5 ± 12.2	527.4 ± 20.7	411.16 – 613.17
Vascular Surface/ mm^3 (mm^2/mm^3)	11.5 ± 1.18	15.9 ± 3.28	10.19 – 12.85
Vascular Volume (%) of total volume) – 2.5 mm depth	2.15 ± 0.54	4.55 ± 2.6	2.39 – 3.02
Volume/Surface (μm)	3.41 ± 1.77	5.69 ± 2.95	2.1 – 2.4
Mean Segment Length (μm)	59.7 ± 9.4	65.7 ± 15.9	52.67 ± 50.38

^a Lauwers et al. [101].

Table 4.3 Parameters calculated for the full vascular network (including capillary network) using 40 μm penetrating vessels and 25 μm penetrating vessels, as well as the equivalent morphological measurements.

I have thus shown that using order-based data extracted experimentally it is possible to reconstruct morphologically accurate penetrating arteriolar/venular trees, and full vascular networks in the cortex, without any assumptions. The algorithm is easily adaptable for different regions of the brain where the parameters may be different. As more data are obtained it will be possible to construct larger, morphologically accurate models of the blood vessels across different regions of the brain.

4.3.4 Vascular Resistance and CBF

The mean and standard deviation of the vascular resistance for the venule and arteriole trees, along with the calculated CBF is summarised in **Table 4.4**. The 110 μm venule tree gives an accurate CBF value of $46.0 \text{ mL min}^{-1} 100\text{g}^{-1}$ which is close to the CBF values observed experimentally of $54.7 \pm 6.1 \text{ mL min}^{-1} 100\text{g}^{-1}$ [131]. The 40 μm and 25 μm arterioles, however, are one and two orders of magnitude smaller respectively than the morphologically value. From equation (4.2) it can be seen that the vascular resistance is extremely sensitive to the radius as it is inversely proportional to the fourth power of the radii. This leads to substantially larger resistances for smaller diameter vessels. When calculating CBF (4.7) the only variable is V , the volume of tissue the penetrating vessel perfuses. This depends on the assumption of the ratio of arterioles-to-venules and the number of penetrating vessels per mm^2 . In this chapter a 2:1 ratio of arterioles to venules has been assumed, but other papers assume a 3:1 ratio which would lead to larger values of arteriolar CBF [42,107]. As well as this, the region of influence of the penetrating vessel is unknown – often having quite large overlapping regions of perfusion [59,110] – making it difficult to accurately calculate the CBF using tree vascular resistances. Similarly, the assumption that a 25 μm arteriole will perfuse the same volume as a 40 μm arteriole is unlikely and also leads to the non-morphological values of CBF.

Penetrating Diameter	25 μm (arteriole)	40 μm (arteriole)	110 μm (venule)
R_μ ($\text{kg m}^{-4} \text{s}^{-1}$)	3.4846×10^{14}	4.2801×10^{13}	8.9092×10^{11}
R_σ ($\text{kg m}^{-4} \text{s}^{-1}$)	7.899×10^{13}	8.6576×10^{12}	1.2307×10^{11}
Mean CBF ($\text{mL min}^{-1} 100\text{g}^{-1}$)	0.58	4.60	46.0
σ CBF(%)	36	27	18
IQR CBF (%)	80 – 108	82 - 110	89 - 106

Table 4.4 The mean tree resistance (R_μ) and standard deviation of the tree resistance (R_σ) along with the CBF for each diameter of penetrating vessel. The CBF is calculated assuming a 2:1 ratio of arterioles to venules and 12 trees/ mm^2 surface density.

Of more interest, however, is the relative variability in CBF introduced by the statistical nature of the algorithm. Regardless of the penetrating vessel diameter, 50% of the CBF values remain within 20% of the mean CBF, indicating a tight distribution of the CBF. The interquartile range shows a slight asymmetry towards smaller CBF values. Therefore, it seems to be the case that the variations in CBF in various regions of the brain are only minimally affected by the varying topology of the vessels. The diameter of the penetrating vessel conversely has a much larger impact on the CBF, and hence oxygen delivery.

4.4 Discussion

In this chapter, I have developed an algorithm to generate statistically accurate 3-dimensional models of the penetrating arterioles and venules based on data from a region of the cortex in the temporal lobe. The use of this data to generate penetrating arterioles/venules has meant that the assumptions of volume and energy minimisation previously used to model these trees [107] could be relaxed. This order-based density-

filling algorithm was used to simulate 3000 penetrating trees from which morphological parameters of interest were calculated and validated against experimental data. A stem-crown analysis was conducted showing very close agreement between the model and morphological measurements. Murray’s k exponent was found to have a median of 2.5 with an IQR 1.75 – 3.7. This is an interesting result as no assumption was made with regards to the k exponent in the algorithm, yet it came out in very good agreement with the minimum energy k exponent of 3, indicating that it is an emergent property of the model.

This penetrating vessel algorithm was then combined to a statistically accurate capillary generating algorithm [176] in order to calculate geometric parameters for the full vascular network. The resulting parameters of interest were all in very close agreement with the morphologically measured values (**Table 4.3**), validating the order-based density-filling algorithm developed here. Finally, the vascular resistance was calculated for the penetrating trees and from this, a first order approximation for CBF was calculated. The CBF was heavily affected by the radius of the penetrating vessel and the assumed volume of tissue the tree perfuses. However, the variability of the CBF with the quasi-random topology of the algorithm was $< 36\%$ of the mean CBF. This suggests that the variation of CBF in the brain is only nominally affected by the topology of the vessels, and is more likely to be affected by the varying vessel radii.

The only other 3-dimensional geometric construction algorithm for the cerebral microvasculature that I was able to find was that by Linninger et al. [107]. Minimum tree volume optimization based on CCO [86] was used to generate the penetrating arteriolar and venular trees which also provided a good match to the morphological data. This is based on the “minimum cost” concept proposed by Murray [121], a weighted minimum between the work needed to overcome the wall viscous stress and

the building costs of the blood flow system. However, it is unlikely that minimising intravascular volume is the only principle at work in the construction of the penetrating vessels [86]. The model presented in this chapter found a spread in the k exponent of less than 1 up to 25, with a median of 2.5. Therefore, a model built on this principle is likely to over-simplify the complex reality of the microvasculature. The k exponent is an emergent property of this model; it was not specifically included as a property in the construction of the model. A model based on statistical data, like the one presented here, allows there to be a much more complex representation of the microvasculature without making assumptions with regards to minimising volume or energy. Therefore, a much more general model of the arterioles and venules can easily be adapted to differing regions of the brain when statistical data become available.

One limitation of this statistical model, however, is the lack of differentiation in the statistics between the arterioles and venules. The order-based data, given in the supplementary material of Cassot et al. [27], are not segregated by type of vessel (arteriole or venule). As a result, the penetrating trees generated use ‘averaged’ properties based on both the arterioles and venules. If vessel specific order-based data become available, however, it would be straightforward to adapt the distributions being sampled from accordingly and hence to generate more accurate arterioles and venules. Another limitation of the model is a lack of tortuosity in the vessels; they are approximated as straight thin-walled cylinders, as seen in **Fig. 4.3**. The curvature of penetrating arterioles and venules has been quantified by Lorthois et al. [112] and should be included in future to generate more realistic networks, although it is anticipated that the effect on the blood flow through the vessels will be of second order due to the small curvature of individual vessels.

It should be noted that the algorithm developed in this chapter is based on one set of experimental morphometric data, 28.6 mm³ in size [27] (due to a lack of other

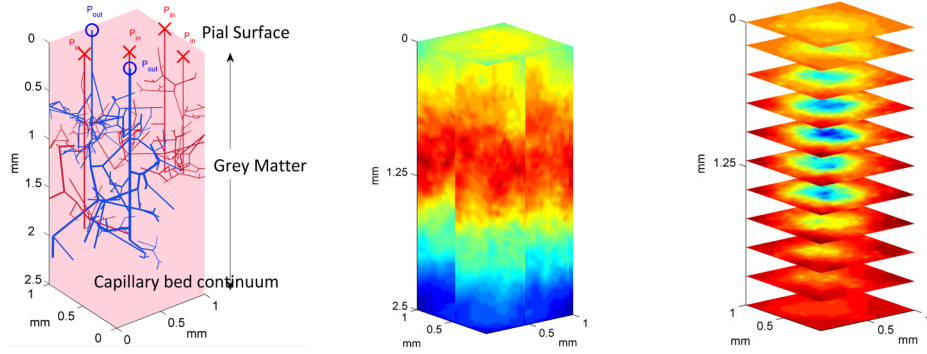
accurate data on the human cerebral microvasculature). Therefore, the penetrating vessels generated in this chapter characterize only a small region of the brain. It is very likely that different regions of the brain will have different statistics. However, once more information about the topology and geometry of the arterioles and venules in other areas of the human brain becomes available, the algorithm presented here is easily modifiable to generate realistic networks for individual regions.

The current algorithm is able to model voxel-size regions of penetrating vessels. The next step will be to couple the penetrating vessel network to the homogenized capillary network previously established [43] and to simulate blood flow. This would then be a mathematically rigorous data-driven multi-scale model of the blood flow in the cortex; where perturbation theory was used to generate statistically accurate homogenized capillary networks that can easily be scaled to large volumes; and the algorithm in this chapter produced data-driven models of the penetrating vessels. The coupling of these two models will allow for the modelling of realistically sized MRI (or perfusion imaging) voxels from which cerebral blood flow and perfusion will be measured and compared to MRI data. This would help to establish the link between the microvasculature and the macro-scale flow in the cortex. Once the blood flow model has been established, it can then be coupled to oxygen transport models, from which mean transit time (MTT) and oxygen extraction fraction (OEF) can be calculated [134,135] and compared to experimental values. This will allow us to explore the interplay between topology and nutrient delivery and its robustness, as well as the effect of ischaemic stroke on these values [65].

Chapter Summary

Through development of a novel data-driven algorithm to simulate the penetrating vessels in the microvasculature accurately, it is now possible to simulate cerebral blood

flow at both the capillary and arteriole/venule scale in a way that is not restricted to particular experimental conditions. It is also possible to easily model the penetrating vessels in varying regions of the brain due to the data-driven aspect of the model as long as the statistical data are available. The coupling of the arteriole/venule and homogenized capillary models allows for perfusion imaging voxel-scale simulations to be run efficiently. This will allow us to better assess the impact of the microvascular architecture on blood and oxygen perfusion on the global-scale. This is now explored in the next chapter.



Chapter 5

Investigating the Effects of a Penetrating Vessel Occlusion with a Multi-Scale Microvasculature Model of the Human Cerebral Cortex

The work presented in this chapter has been submitted for publication as El-Bouri, W. K. and Payne, S. J. (2017), ‘Investigating the effects of a penetrating vessel occlusion with a multi-scale microvasculature model of the human cerebral cortex’.

Abstract

The effect of the microvasculature on observed clinical parameters, such as cerebral blood flow, is poorly understood. This is partly due to the gap between the vessels that can be individually imaged in humans and the microvasculature, meaning that mathematical models are required to understand the role of the microvasculature.

As a result, a multi-scale model based on morphological data was developed here that is able to model large regions of the human microvasculature. From this model, a clear layering of flow (and 1-dimensional depth profiles) was observed within a voxel, with the flow in the microvasculature being driven predominantly by the geometry of the penetrating vessels. It also appears that the pressure and flow are decoupled, both in healthy vasculatures and in those where occlusions have occurred, again due to the topology of the penetrating vessels shunting flow between them.

Occlusion of a penetrating arteriole resulted in a very high degree of overlap of blood pressure drop with experimentally observed cell death. However, drops in blood flow were far more widespread, indicating that pericyte controlled regulation on the capillary scale likely plays a large part in the perfusion of tissue post-occlusion. CBF measured post-occlusion was found to be very robust, dropping on average by 7.5 – 8.2% demonstrating the difficulty of observing such micro-infarcts using perfusion imaging techniques.

5.1 Introduction

As previously discussed in this thesis, any reduction of blood flow to the brain can lead to tissue death at a time scale of the order of tens of seconds to minutes. Additionally, the microvasculature, and changes to its topology, has been linked to diseases such as Alzheimer's disease, vascular dementia, and brain tumours

[24,80,81,116]. For example, although Alzheimer's disease, the leading cause of dementia, has traditionally been thought to be due to the formation of amyloid plaques and neurofibrillary tangles, it is now appreciated that there is also a strong vascular component to this pathology [182].

Despite the importance of the microvasculature in maintaining a healthy supply of blood and oxygen to the brain, little is known about the specific effects that the topology of these vessels has on blood and oxygen transport. This is predominantly due to the relatively low resolution of modern clinical imaging techniques that can only discern vessels larger than approximately 0.8 – 0.9 mm in diameter [123]. There is thus an imaging gap relating to the microvasculature of the human brain, which can only currently be filled using mathematical models. These models must be based on accurate physiological data, either obtained post-mortem or from animal models. To quantify this link between the microvasculature and clinical imaging techniques, models of the scale of a standard clinical perfusion imaging voxel, i.e. with a length scale in the order of mm are required. Such models allow us to explore the links between the geometry of the microvasculature and perfusion and blood pressure measurements, as well as the relationship between flow and metabolism in more detail.

There are various smaller scale models (length scale order 100 μm or less), both cast based and statistical, modelling both human and rat cerebral capillary networks [48,157,163,176]. Although useful, these models are too small to provide a direct one-to-one comparison to clinical imaging voxel data. Most of the large-scale models (order mm length scale) that are available in the literature are based on animal models – and most of these are cast based models – due to the relative ease of imaging the microvasculature in animal models to a higher resolution than in the human microvasculature [54,58,59,161]. However, the link between these animal microvasculatures and the human microvasculature has not yet been quantified. For

example, rat brains have more penetrating venules than penetrating arterioles in the cortex [19], which is opposite to the situation in human brains [27]. As well as this, these animal models are based on casts which, although valuable in modelling flow and oxygen transport for that particular network, are specific to that given region. The simulation results are thus restricted to the experimental conditions that they were derived from and strongly dependent upon the choice of boundary conditions. Reichold et al. [149] previously attempted to overcome this by using an ‘upscaling’ approach for averaged quantities of an artificial 2-dimensional capillary network. This averaged network was then coupled to penetrating vessels in a 2-dimensional grid, demonstrating good agreement between the upscaled model and the non-upscaled model. However, since this was only performed for an artificially generated non-morphological network in 2D, the simulations did not fully represent the rat cerebrovasculature.

In comparison, large-scale models of the human microvasculature are few, mainly due to the relative scarcity (and difficulty) of obtaining physiological data compared to animal models. Lorthois et al. [110] developed a blood flow model based on available human cerebral data [26]; this discrete cast model was found to rely heavily on the prescribed boundary conditions, with heterogeneities in blood flow within the network controlled by the vascular architecture. The same cerebral data was also used by Linninger et al. [107] to generate a large-scale model that also modelled the individual vessels of the microvasculature discretely, with the capillary network statistically generated matching physiological data, and the penetrating vessels generated using constrained constructive optimization (CCO). Thus, these penetrating vessels are not directly based on physiological data. Additionally, developing larger models of the vasculature becomes very computationally expensive using discrete models such as these.

To the best of my knowledge, there are currently no models of the human microvasculature that investigate the effects of penetrating vessel occlusions on the perfusion and blood pressure of the human microvascular network. The effects of penetrating vessel occlusions (micro-infarcts) have, however, been investigated in mice [20,129] and rats [166] experimentally. The studies found clear conical regions of cerebral tissue supplied by the penetrating vessels, with robustness to blood flow drops/tissue death deeper in the cortex. This led to penetrating arterioles being labelled as a “bottleneck” in cerebral perfusion [129]. Studies in patients with dementia identified a large number of cortical micro-infarcts with diameters in the order 0.1 – 1 mm [11,85,98,186]. However, whether these are predictive of or a cause of dementia is as yet unknown due to the inability of conventional clinical imaging to resolve at the length scale of penetrating vessel diameters. As a result, computational models are required to investigate flow at this scale.

The aim of this chapter is thus to generate a multi-scale model of the human microvasculature that is able to predict the effect of a penetrating vessel occlusion on local and global flow and perfusion whilst mitigating the effects of the prescribed boundary conditions. This model is based on physiological data, combining a homogenized capillary bed (Chapter 3) with statistically generated penetrating vessels (Chapter 4), is computationally scalable, and generalizable to any available data set. The mean CBF is calculated through these voxel sized models and validated against experimental data. Pressure and volume averaged velocity are investigated, as well as layered flow within the voxels. This is done for both the healthy vasculature and for the occluded vasculature, with the effects of such occlusions compared to the experimental literature.

5.2 Materials and Methods

The data used in this chapter come from the collateral sulcus in the temporal lobe of the human brain. Thick sections of India-ink injected human brain were scanned using confocal laser microscopy and the network reconstructed [26]. Statistical data were extracted from these networks, delineated into capillary and penetrating vessel data. Full details of the data used can be found in the literature [26,27,101,112].

5.2.1 Morphometry of the Microvasculature

The data detailed above have previously been used to generate statistically accurate models of both the cerebral capillary network [176] and the cerebral penetrating vessels (Chapter 4) [44] which were morphometrically validated against experimental data. The capillary network was then mathematically homogenized (Chapter 3) [43] to extract the macro-scale properties of the capillary network and to allow these networks to be scaled up to large volumes computationally efficiently. This allows the capillary network to be characterised as a porous medium where the permeability encapsulates the morphological characteristics of the network. This permeability is calculated from the morphologically accurate networks described above, as detailed previously in Chapter 3.

A large-scale model (1 x 1 x 2.5 mm) of a clinically sized perfusion imaging voxel is generated here by combining the models mentioned above. The depth of the model (2.5 mm) runs from the pial surface down to the grey matter/white matter interface [101]. The porous capillary bed is assumed to cover the entire volume with a constant, isotropic permeability tensor as previously calculated [43].

The penetrating vessels start at the pial surface and penetrate into the cortex. Their terminal nodes terminate in the porous capillary bed and act as sources and sinks delivering and removing blood to and from the capillary bed. It is assumed that

there are 12 penetrating vessels /mm² in the ratio 2:1 arterioles-to-venules, as found morphologically [27,154]. As the precise spacing of these vessels is uncertain, and this model is statistical, the 1 mm² area is split into 4 quadrants, each containing 2 arterioles and 1 venule randomly placed on the pial surface (**Fig. 5.1a**).

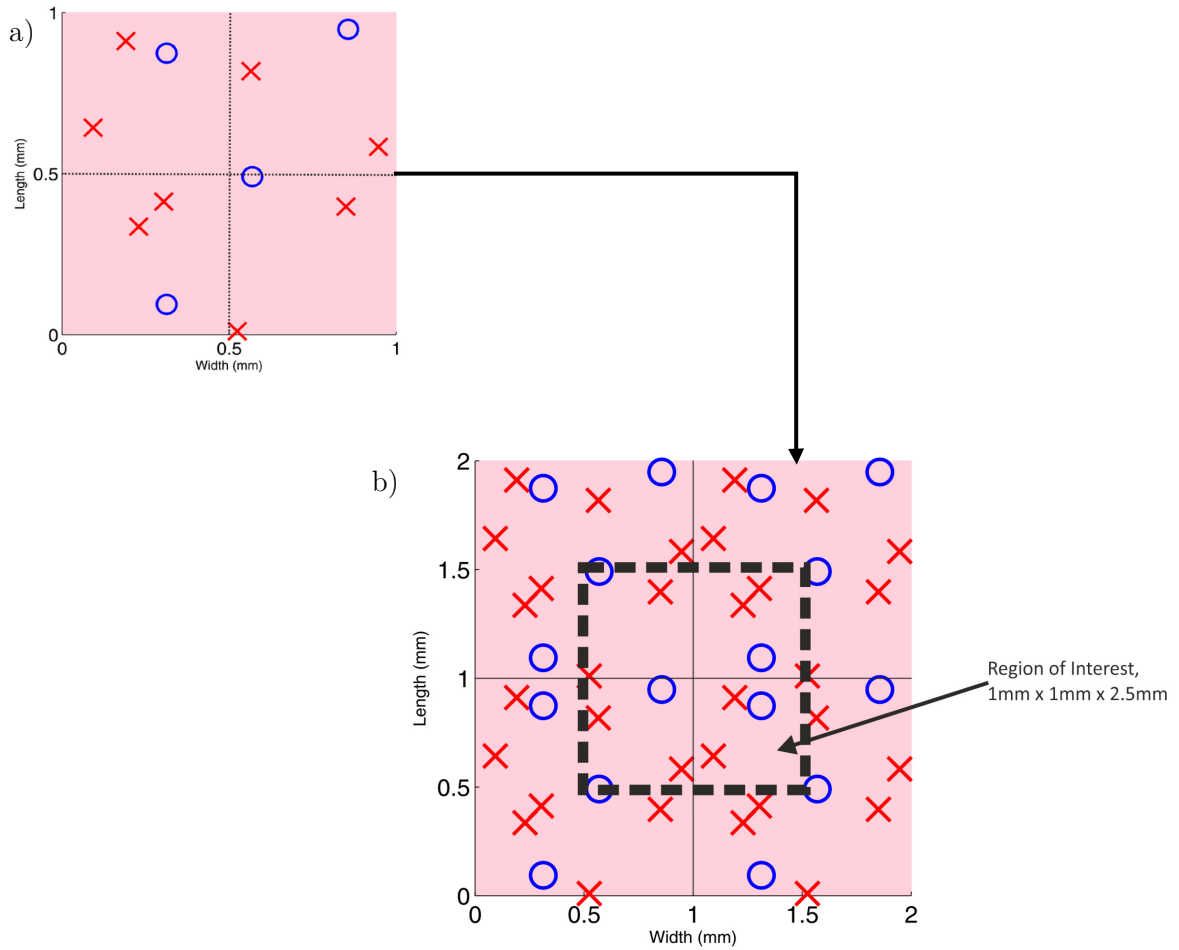


Fig. 5.1 *a)* Top down view of a 1 x 1 x 2.5 mm voxel, split into 4 quadrants each containing 2 arterioles and 1 venule. *b)* The same voxel as in a) repeated 4 times to form a 2 x 2 x 2.5 mm voxel. The region of interest is shown as the black dashed line.

Two voxel cases are chosen: case 1 with mean arteriole diameters of 20 μm and mean venule diameters of 50 μm ; and case 2 with mean arteriole diameters of 40 μm and mean venule diameters of 110 μm . The latter case is chosen to provide a direct comparison to a previous model of the human microvasculature which used mean

arteriole diameters of $40\ \mu\text{m}$ and mean venule diameters of $115\ \mu\text{m}$ [107]. In order to investigate the effect of penetrating vessel diameter on flow and pressure pre- and post-occlusion in the network, case 1 is then chosen to have arteriolar diameter half that of the latter case to provide a comparison. The depths of the penetrating vessels were assumed to be linearly related to their diameters, with larger diameters penetrating deeper (as observed experimentally) [42]. The arteriole depths were centred on a mean $1.25\ \text{mm}$ depth for the mean diameter vessel, and the venules similarly on a $1.75\ \text{mm}$ depth.

Variable	Morphological Values	Model Values
Arteriole Diameter (μm)	15 – 240 [42]	Case 1: 20 ± 5 Case 2: 40 ± 8
Venule Diameter (μm)	20 -125 [42]	Case 1: 50 ± 10 Case 2: 110 ± 16
Arteriole Length (mm)	Up to 2.5 [42,101]	Case 1: 1.25 ± 0.31 Case 2: 1.25 ± 0.25
Venule Length (mm)	Up to 2.5 [42,101]	Case 1: 1.75 ± 0.35 Case 2: 1.75 ± 0.25
Surface Density ($/\text{mm}^2$)	8.44 – 15 [154]	12
Arteriole Pial Pressure (mmHg)	65 – 90 [175,177]	75
Venule Pial Pressure (mmHg)	15 – 25 [175,177]	15
Capillary Bed Permeability ($\text{mm}^3\ \text{s}\ \text{kg}^{-1}$)	N/A	4.28×10^{-4} (Chapter 3)
Voxel Dimensions (Length x Width x Depth) – (mm)	-	1 x 1 x 2.5

Table 5.1 Values used to model blood flow through the voxel.

A full list of the parameters used in this voxel model is shown in **Table 5.1**. 100 simulations were run for each voxel case, and the density of the penetrating vessel network validated against experimental data. It should be noted that all other attributes of the capillary network and penetrating vessels, such as connectivity, length distributions, area ratios, and bifurcation angles, have previously been validated (Chapters 3 and 4) [43,44,176].

5.2.2 Simulating Blood Flow through the Voxel

5.2.2.1 Coupled ODE/PDE Model of the Blood Flow

Using homogenization theory it was previously shown that the capillary bed can be modelled as a porous medium using a volume averaged form of Darcy's law (Chapter 3) [43]:

$$\langle \mathbf{u}_c \rangle = -\mathbf{K} \nabla p_c \quad (5.1)$$

where $\langle \mathbf{u}_c \rangle$ is the volume averaged capillary velocity, $\mathbf{K}$ is the permeability tensor, and ∇p_c is the pressure gradient across the capillary bed. The permeability tensor was shown to be isotropic and diagonally dominant in Chapter 3 indicating that a constant value of permeability can be used. For this region of the brain this was found in Chapter 3 to be $4.28 \times 10^{-4} \text{ mm}^3 \text{ s kg}^{-1}$. This equation can be combined with that of mass conservation:

$$\nabla \cdot \langle \mathbf{u}_c \rangle = S \quad (5.2)$$

to form:

$$\nabla \cdot (\mathbf{K} \nabla p_c) = -S \quad (5.3)$$

which is Poisson's equation in 3D, that can be solved for capillary bed pressure. The S term is the source/sink term that comes from the terminal arterioles/venules. This couples the capillary and penetrating vessel models.

The Poiseuille flow equation is then used to model the blood through the penetrating vessels:

$$Q = G\Delta P \quad (5.4)$$

where:

$$G = \frac{\pi r^4}{8\mu(r)L} \quad (5.5)$$

where ΔP is the pressure drop in the vessel, Q is the flowrate, G is the conductance of the vessel, L is the length of the vessel, r is the radius of the vessel, and $\mu(r)$ is the apparent viscosity of the blood, which is dependent on the radius due to the Fåhræus-Lindqvist effect and the haematocrit [47]. The Fåhræus-Lindqvist effect is accounted for here through a correction for in-vivo viscosity taking into account the endothelial surface layer [143] (see (4.3) – (4.6)).

As flow is conserved at each bifurcation node, the net flow at each node i is equal to zero, except for at the terminal source and sink nodes. Therefore, for each vessel, flow can be modelled as

$$\sum_i^n (P_i - P_j)G_{ij} = S_i \quad (5.6)$$

where G_{ij} is the conductance of the vessel linking node i to node j and is zero unless the connectivity is equal to 1. The source term S_i is zero for all bifurcations except for terminal sources and sinks, and at the inlet to the penetrating vessels. At the pial surface the penetrating arterioles were assigned a pressure of 75 mmHg and the penetrating venules a pressure of 15 mmHg leading to a pressure drop across the voxel of 60 mmHg, based on a previously proposed physiological range [175,177]. This Poiseuille flow model (5.6) for the bifurcating trees is then coupled to the discretized

form of (5.3) through the source/sink terms S_i . This coupled model is then solved using a standard linear solver (LU decomposition).

A finite volume discretization is used for (5.3) with volume sources representing the terminal arterioles and venules. The finite volumes have dimensions of $50 \times 50 \times 50 \text{ } (\mu\text{m})^3$ as this was found to give a good balance between the distribution of sources and sinks within the model and the speed of solution. This choice of grid size also avoids the difficulty in coupling the terminal vessel point sources to a coarser grid representation of the capillary bed – with the grid size chosen here being the same order as the arteriole/venule diameters. The coupled model (5.3) and (5.6) is solved yielding the nodal pressures in the network from which the Darcy velocity can be found using the discrete form of (5.1).

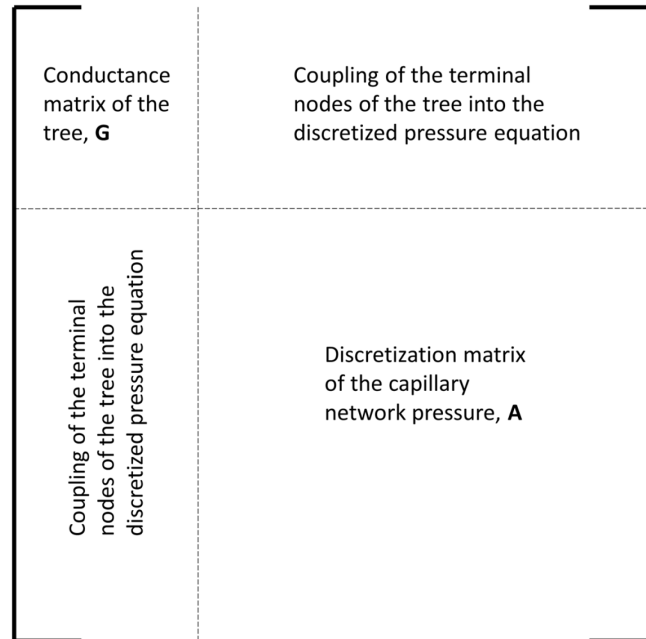


Fig. 5.2 Schematic of the expanded matrix that couples the penetrating vessel pressures to the capillary bed pressures through the terminal nodes.

The coupling of the arterioles/venules into the discretized pressure equation for the capillaries is shown in **Fig. 5.2**. The conductance matrix of the penetrating

trees (based on (5.6)) is concatenated onto the standard discretized mesh of the capillary network (5.3). The terminal nodes, S_i , of the penetrating vessels are then reassigned to a node in the discretized capillary mesh that is closest to their position in the capillary bed. In this way, the capillary bed is directly coupled to the penetrating vessels spatially, allowing for a direct solution of the pressure equation (5.3) using a linear solver.

5.2.2.2 Boundary Conditions

The results of blood flow simulations in cast models have previously been shown to have a very strong dependence on the boundary conditions [59,110]. However, this issue is mitigated here due to the homogenization of the capillary network. The capillary network is assumed to be periodic (a reasonable assumption due to the randomness of the network [13]) in the homogenization procedure, allowing the permeability to be calculated. The value of permeability is, as a result, independent of the pressure gradient. Therefore, the 4 sides of the voxel perpendicular to the pial surface are also given periodic boundary conditions. The pial surface of the voxel is given a no-flow boundary condition, as is the grey matter/white matter interface at the bottom of the voxel. These were considered appropriate boundary conditions as no blood vessels can penetrate beyond the pial surface, and the white matter is much less vascularized than the grey matter [42] (see **Fig. 5.3**).

To reduce further the effects of the boundary conditions, the voxel is repeated 4 times to form a larger 2 x 2 x 2.5 mm voxel (repeating the voxel being mathematically acceptable due to the periodic boundary conditions). A region of interest is then extracted from this enlarged voxel; a cuboid removed from the centre of size 1 x 1 x 2.5 mm (see **Fig. 5.1b**). This central region is the one used for analysis.

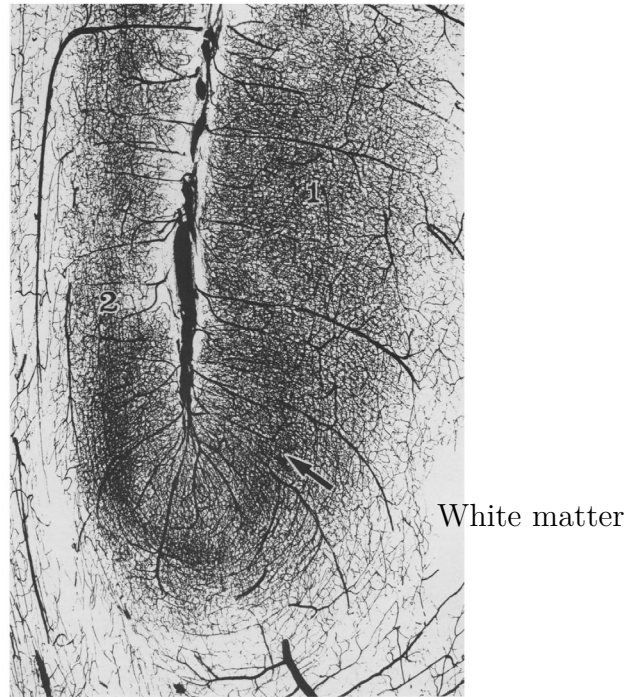


Fig. 5.3 The central sulcus of a 54-year-old male. Notice the very thinly vascularised white matter region at the bottom of the image. Adapted with permission from [42].

5.2.3 Occlusion of a Penetrating Arteriole

The effects of occluding one penetrating vessel in the rat cortex have previously been studied experimentally [20,129,166]. The model proposed here can also easily be used to study occlusions of penetrating vessels in the human microvasculature. This allows us to determine how much of what can be seen post-occlusion is due to the topological or passive effects of certain regions losing flow, and how much can be better explained using an active response model (which is not modelled here). The comparison between experimental penetrating occlusions and this model will thus be discussed in more detail later.

The method of occlusion used here involved identifying the nearest arteriole to the centre of the larger (2 x 2 x 2.5 mm) voxel. The region of interest (a cuboid with dimensions 1 x 1 x 2.5 mm) is then centred over this penetrating arteriole. The blood flow simulation is run twice for this region: once for the healthy vasculature, and once

for when the central arteriole is occluded (the upper portion of the arteriole trunk is occluded so that there is no flow downstream anywhere in the penetrating tree). The difference in the solution between the healthy vasculature and occluded vasculature is taken and this difference is averaged over 100 simulations to observe the averaged effect of a penetrating vessel occlusion on blood pressure and velocity.

5.3 Results

5.3.1 Morphometric Validation

One hundred simulations were run to generate statistically accurate voxels of the penetrating vessels; this was done for both voxel cases. The densities of the vessel networks were 674 ± 43 , and 659 ± 45 /mm³, matching morphological densities [27]. The arterioles and venules also matched their respective morphological densities separately (281 /mm³ and 385 /mm³). One example of a statistically generated voxel is given in **Fig. 5.4**.

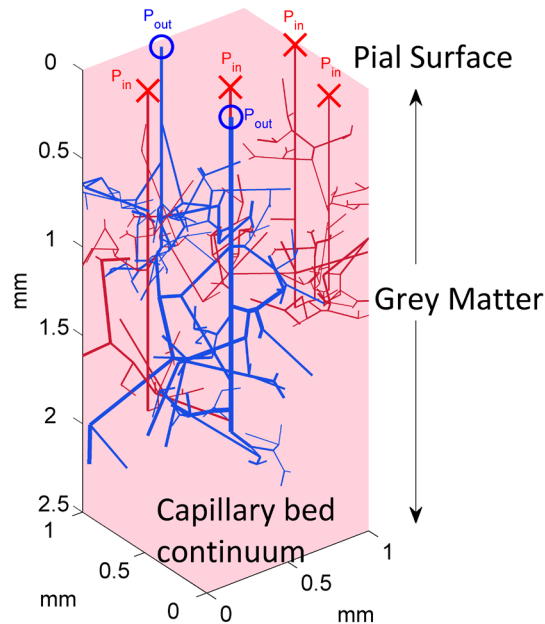


Fig. 5.4 An example voxel showing half the penetrating vessels for easier viewing. The penetrating vessels enter from the pial surface into the grey matter, coupling to the capillary bed continuum via the terminal vessels.

5.3.2 Cerebral Blood Flow

The CBF is calculated for each voxel by summing the flow leaving the terminal arterioles within the region of interest. This led to a CBF of $39.4 \pm 13.9 \text{ mL min}^{-1} 100\text{g}^{-1}$ for the case 1 voxels ($20 \text{ }\mu\text{m}$ arterioles; $50 \text{ }\mu\text{m}$ venules), and a CBF of $93.75 \pm 69.7 \text{ mL min}^{-1} 100\text{g}^{-1}$ for the case 2 voxels ($40 \text{ }\mu\text{m}$ arterioles; $110 \text{ }\mu\text{m}$ venules), indicating, as expected, a strong influence of the penetrating vessel diameters on CBF. It is highly likely that such large diameter arterioles supply a volume of tissue larger than the voxel considered [61]. The model developed by Linninger et al. [107] had a CBF of $64 \text{ mL min}^{-1} 100\text{g}^{-1}$. This lower value of CBF is to be expected, however, due to the lower surface density of the penetrating vessels used (5.33 /mm^2 average in comparison to the 12 /mm^2 used in this chapter).

Post-occlusion, the CBF drops across all voxel simulations (see **Fig. 5.5**). However, somewhat surprisingly, many of the voxel simulations seem highly robust to the occlusion of a penetrating arteriole, with CBF values dropping on average by only -7.5% of the mean CBF value for the case 1 voxels, and -8.2% for the case 2 voxels (with 60% of the voxels having a CBF drop of less than 5%). If each arteriole contributed equally to the CBF then we would, naively, expect a 12.5% drop in CBF post-occlusion. However, the majority of voxels have drops less than half of this. These relatively modest average drops in CBF indicate that a single occlusion has only a small effect, one that would be challenging to observe with the resolution of current CBF perfusion imaging. The distribution of the absolute values of the CBF drop can be characterised as lognormal with mean 1.70 and standard deviation 0.89 for case 1, and mean 1.33 and standard deviation 1.27 for case 2. A Wilcoxon signed rank test was run for both size configurations determining that there is a statistically significant drop in flow post-occlusion. However, when comparing the mean drop across both cases, it is found not to be statistically significant ($p = 0.165$).

In order to determine whether there were any boundary effects from the choice of penetrating arteriole to occlude, the distance from the centre of the large cuboid (2 x 2 x 2.5 mm) to the position of the chosen penetrating arteriole is plotted against drop in CBF post occlusion (Appendix D, **Fig. D.6** and **Fig. D.12**). No boundary effects were discovered. As well as this, in order to determine what characteristic of the occluded tree most affected the drop in CBF, various properties such as tree volume, penetrating vessel radius, and total tree length were plotted against drops in CBF (Appendix D). It was found that whilst the density of the tree and the number of terminal nodes had little effect, the volume of the tree (along with the total length and penetrating vessel radius) had the largest effects on drops in CBF as has been previously documented [44,166]

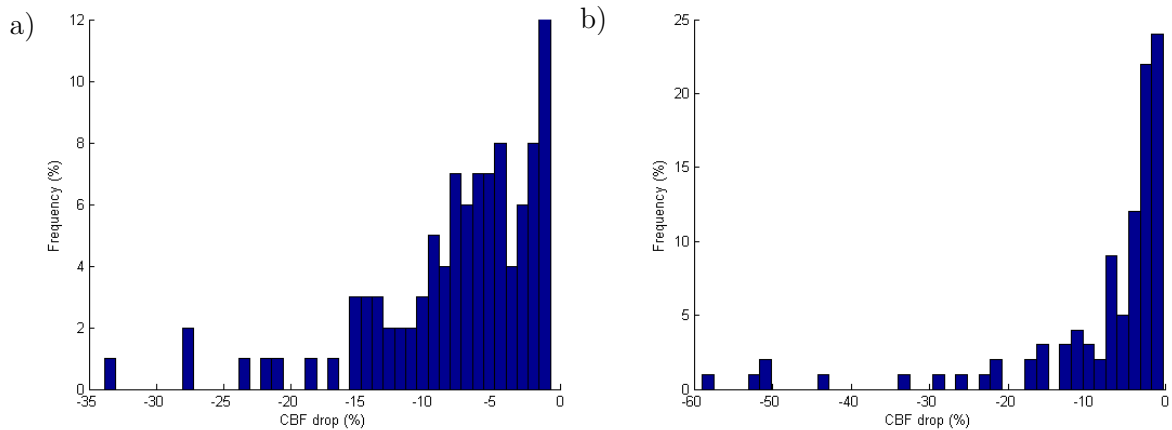


Fig. 5.5 Histogram of the change in CBF post-occlusion of a central arteriole in every voxel. **a)** Case 1: 20 – 50 μm vessels; **b)** Case 2: 40 – 110 μm vessels.

5.3.3 Pressure and Velocity in the Healthy Vasculature

The averaged pressure and velocity maps of the region of interest (the central section in **Fig. 5.1b**) are shown in **Fig. 5.6** and **Fig. 5.7** for case 1 and case 2 respectively. These show 3D maps of pressure and velocity averaged over the 100 voxels for each configuration, as well as a planar slice taken halfway into the grey matter (**Fig. 5.6c**, **5.6d** and **Fig. 5.7c**, **5.7d**). It should be noted that the velocity values quoted here

are the volume averaged total velocities (the Darcy velocity) of the capillary network. Depending on the volume fraction of the vasculature in the voxel, this leads to an average velocity in the capillaries in the order of $0.1 - 1 \text{ mm s}^{-1}$, similar to values found experimentally [62,95,166,171]. Additionally, if the volume averaged velocity is multiplied by the discretized area, we obtain a Darcy flux across the case 1 voxel of $1 - 15 \text{ pL s}^{-1}$ and $1 - 18 \text{ pL s}^{-1}$ across the case 2 voxel, which compares very favourably to the observed fluxes calculated for the deep microvessels (with diameters $< 10 \text{ }\mu\text{m}$) in rat cortex of $1 - 50 \text{ pL s}^{-1}$ [166].

The first thing to note from these figures is the clearly planar nature of both the pressure and velocity. There appear to be layers of relatively constant pressure and velocity with predominantly a depth-wise gradient. This layering, or shunting, of flow is also seen experimentally [20] and in simulations on casts [161]. What is particularly interesting to note is that the capillary network has been assumed here to be homogenous and isotropic (encapsulated in the constant $\mathbf{K}$ tensor). This indicates that this layered flow within the cortex is a function of the geometry of the penetrating vessels rather than that of the capillary network, with preferential movement of blood flow between the terminal arterioles and terminal venules. This further supports the idea that the capillary bed can be treated as a porous, homogenous, isotropic medium with a given value of permeability. The planar nature of the blood flow can further be seen in **Fig. 5.8** where planar and depth-wise velocity are plotted for the case 1 averaged voxel (the case 2 voxel results being similar). The planar velocity is defined as $\sqrt{u_x^2 + u_y^2}$, where u_x and u_y are the velocities in the x and y directions respectively. The depth-wise velocity is defined as the absolute values of the z -direction velocity. Blood flow is approximately twice as large in the planar direction compared to depth-wise, further supporting the validity of layer specific analysis.

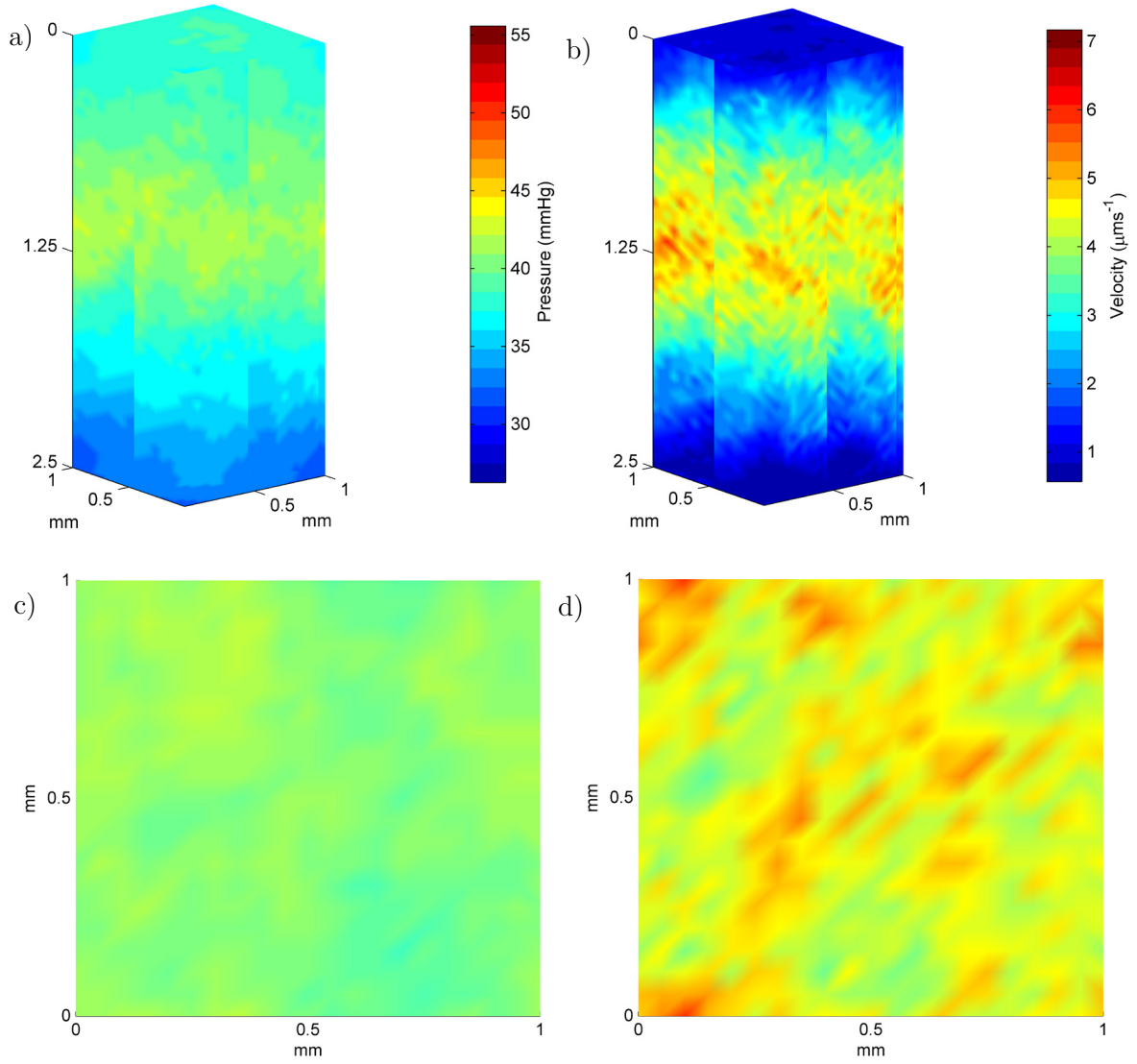


Fig. 5.6 Pressure and velocity maps for Case 1 ($20 - 50 \mu\text{m}$) configuration – averaged over 100 voxel realisations: **a)** 3D map of pressure in the region of interest; **b)** 3D map of Darcy velocity in the region of interest. **c)** Planar slice of pressure at 1.25 mm depth; **d)** Planar slice of velocity at 1.25 mm depth.

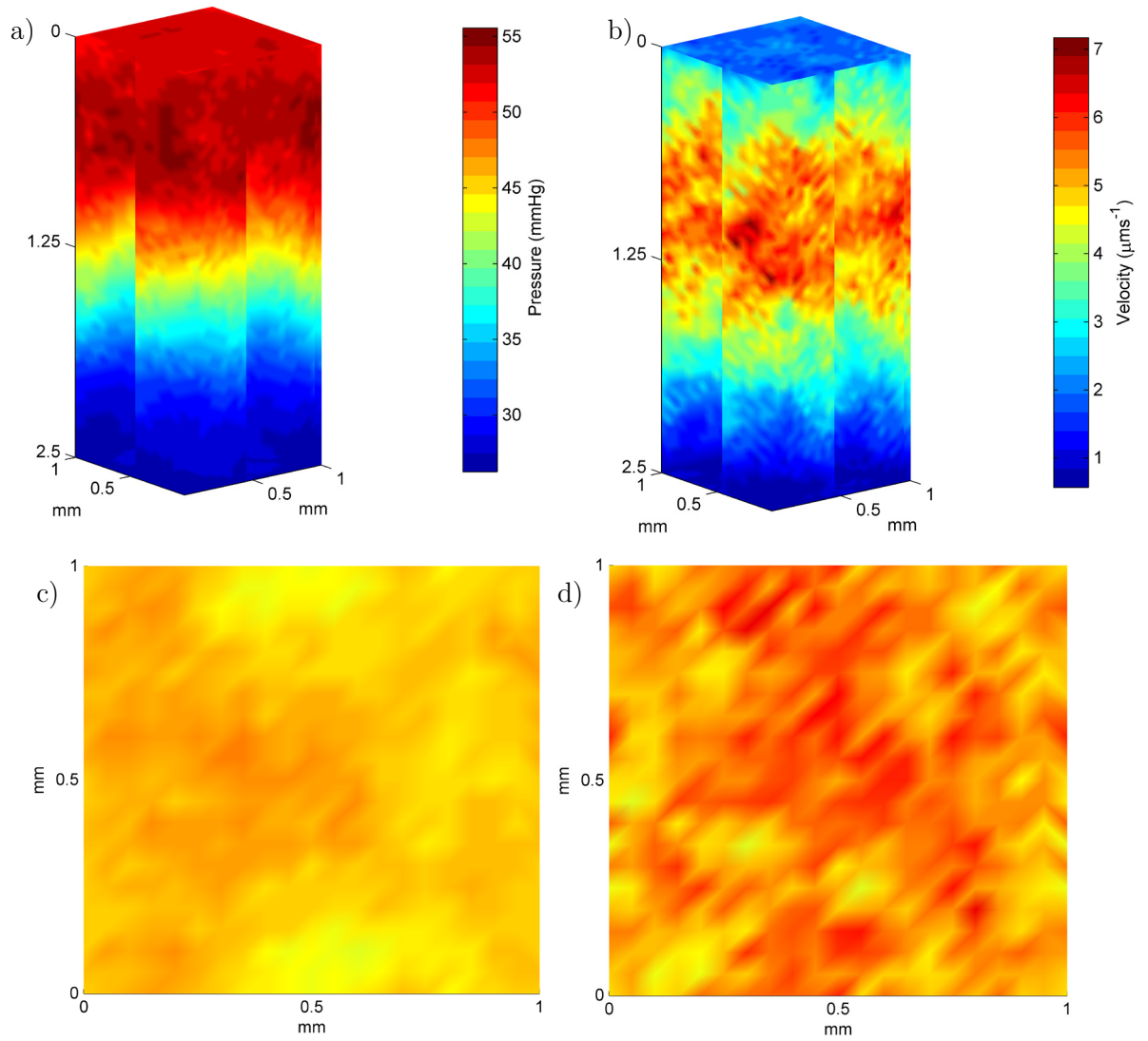


Fig. 5.7 Pressure and velocity maps for Case 2 (40 – 110 μm) configuration – averaged over 100 voxel realisations: **a)** 3D map of pressure in the region of interest; **b)** 3D map of Darcy velocity in the region of interest. **c)** Planar slice of pressure at 1.25 mm depth; **d)** Planar slice of velocity at 1.25 mm depth.

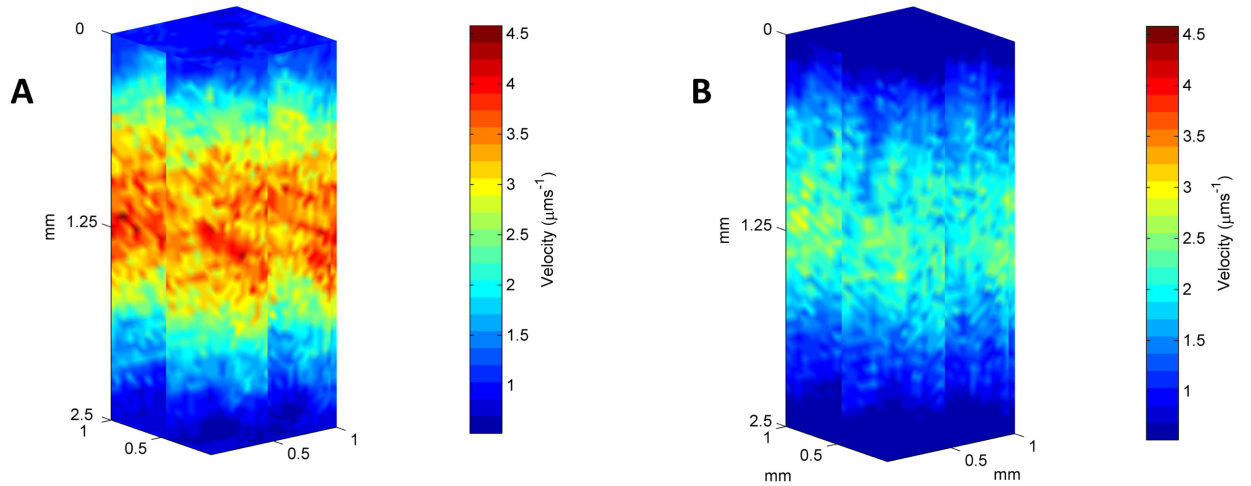


Fig. 5.8 Case 1 voxel – averaged over 100 voxel realisations: **a)** Planar Darcy velocity; and **b)** depth-wise Darcy velocity.

It can also be seen (**Fig. 5.7a**) that larger diameter vessels (as found in the case 2 voxel) impose a much larger pressure gradient over the depth of the voxel – from the pial surface to the white/grey matter interface – than the smaller diameter vessels (as found in the case 1 voxel). However, despite the increased pressure gradient, we do not see this gradient in the velocity profiles (**Fig. 5.6b**, **Fig. 5.7b**). There thus appears to be a clear decoupling of the pressure gradient and flow within the voxels due to the terminal penetrating vessel nodes acting as sources and sinks within the capillary bed. In other words, the flow is driven by the geometry of the penetrating vessels – the flow is not predominantly pressure gradient driven.

The mean capillary bed pressure is 38 mmHg for the case 1 voxel, and 43 mmHg for the case 2 voxel. This is consistent with experimental values in the cat mesentery of 35 – 45 mmHg [108], and with models with capillary bed pressures of 30 mmHg [110] and 35 – 40 mmHg [21].

5.3.4 Effects of Occlusion on Blood Pressure

I next plot the effects of a central arteriole occlusion on blood pressure in **Fig. 5.9** and **Fig. 5.10** for the case 1 and case 2 voxels respectively. What is immediately

apparent is the central region of reduced blood pressure, with only a small decrease in blood pressure outside this region. As well as this, the central region of reduced blood pressure forms a conical shape, with the area of the region getting smaller with depth until the deep layers exhibit very little pressure drop. This is seen in both the smaller (case 1) and larger (case 2) vessel diameters. This region of reduced blood pressure shows very good agreement with the region of hypoxia post-occlusion that has been identified experimentally in rats [166]: although what was being measured experimentally was the region of tissue hypoxia and necrotic cells, it is shown here that this also overlaps with a region of substantially reduced blood pressure.

The radius of the blood pressure drop is approximately $250 - 300 \mu\text{m}$ for the case 1 vessels and between 350 and $450 \mu\text{m}$ for the (larger) case 2 vessels at their maximum (**Fig. 5.9c**, **Fig. 5.10c**). Again, this shows good agreement with the experimentally measured region of tissue hypoxia observed post penetrating vessel occlusion of $230 - 250 \mu\text{m}$ [166] and the region of observed flow drop of $350 \mu\text{m}$ [129].

Therefore, there appears to be a clear relationship between regions of cell death and regions of reduced blood pressure. Also of interest is the volume of tissue where there is a blood pressure drop. Both the case 1 and case 2 configurations have similar penetrating depths for the blood pressure drop, but the lateral area affected by the occlusion is greater for the case 2 vessels (**Fig. 5.9d**, **Fig. 5.10d**). This has been shown experimentally by Shih et al., where they also find that occluding larger – hence larger flux – penetrating vessels leads to larger micro-infarcts [166]. It also points towards the robustness of the deep microvasculature in the cortex, a characteristic demonstrated in marmoset cerebral tissue [61]. It should be noted that although the model in this chapter assumes a homogenous, isotropic capillary network, it does again reproduce results found experimentally, seeming to indicate the validity of assuming a homogenous, isotropic capillary network in the grey matter.

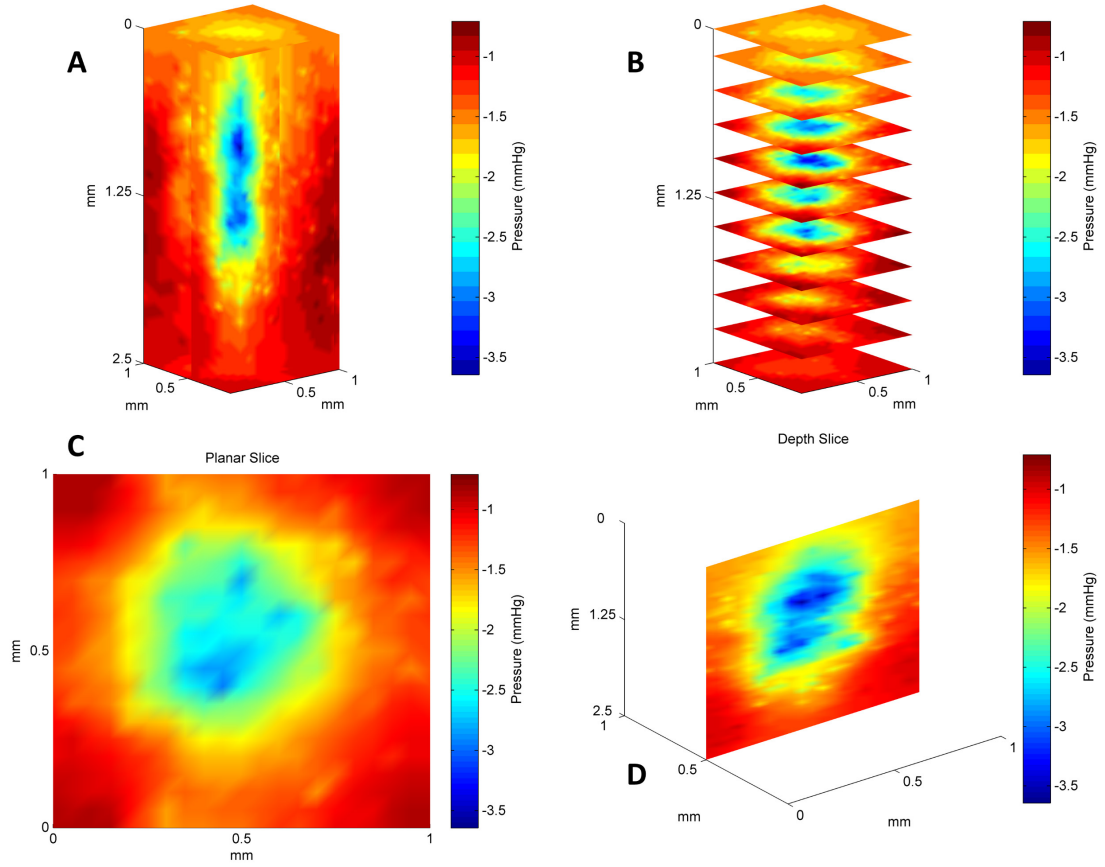


Fig. 5.9 Pressure difference between the averaged healthy and occluded vasculature for the Case 1 configuration – averaged over 100 voxel realisations. **a)** 3D map of pressure difference displaying a clear central region of blood pressure drop. **b)** Slices of the pressure map taken at 250 μm intervals through the depth of the voxel, clearly displaying the conical nature of the blood pressure drop. **c)** Planar slice taken 1.25 mm down the voxel showing a circular region of pressure drop, with a radius of around 250 μm . **d)** Planar slice taken depth-wise through the voxel 0.5 mm along the width of the voxel, again showing the conical nature of the pressure drop.

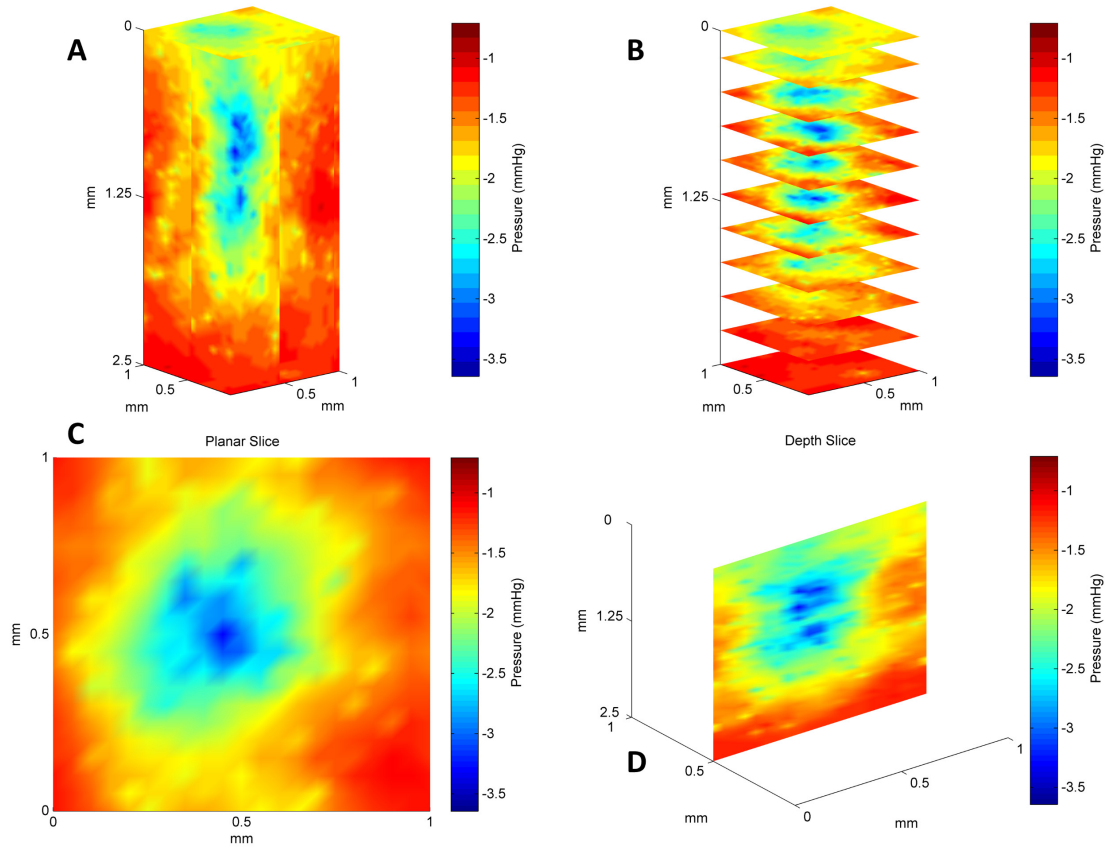


Fig. 5.10 Pressure difference between the averaged healthy and occluded vasculature for the Case 2 configuration – averaged over 100 voxel realisations. **a)** 3D map of pressure difference displaying a clear central region of blood pressure drop. **b)** Slices of the pressure map taken at $250\ \mu\text{m}$ intervals through the depth of the voxel, clearly displaying the conical nature of the blood pressure drop. **c)** Planar slice taken $1.25\ \text{mm}$ down the voxel showing a circular region of pressure drop, with a radius of around $350\ \mu\text{m}$. **d)** Planar slice taken depth-wise through the voxel $0.5\ \text{mm}$ along the width of the voxel, again showing the conical nature of the pressure drop.

Despite the apparent collateral compensation deeper in the cortex, it should also be noted that the topology of the penetrating vessels strongly affects the local blood pressure, with the occlusion of these vessels causing large drops in blood pressure in the surrounding tissue. This has also been observed in mouse and marmoset

microvasculatures [20,59]. This provides further support for the idea proposed by Nishimura et al. that the penetrating vessels are “the bottleneck of perfusion” [129].

5.3.5 Effects of Occlusion on Blood Velocity

I next plot the effects of an arteriole occlusion on total volume averaged velocity in **Fig. 5.11** and **Fig. 5.12** for the case 1 and case 2 voxel configurations respectively. There is clearly a central region of reduced flow, matching the blood pressure drop, but the drop in flow is less spatially confined than the drop in pressure. The drop in flow affects a larger volume of tissue, spreading out further and deeper than the drop in blood pressure. The micro-infarct volume dependence on vessel diameter is again observed, with the larger case 2 vessels having larger regions of flow drop. This difference between the pressure and flow drops again indicates a decoupling of the pressure and flow within the voxel due to the topology of the penetrating vessels – with the flow being driven by the geometry of the terminal vessels more than the voxel-scale pressure gradient.

The spatial region of flow drop has a clear central core, but spreads through a large region of the voxel (with the case 2 vessels spreading through most of the voxel). This contrasts with the red blood cell velocities measured by Nishimura et al. who found that preservation of average flow emerges at a radius of $350\ \mu m$ away from the occluded penetrating vessel [129]. A potential reason for this discrepancy might be accounted for by the active regulation of the capillary network and neighbouring penetrating vessels, through pericyte contraction and neurotransmitter release, leading to increased perfusion in that region compensating for the widespread drop seen here. It should be noted again that the model presented in this chapter is passive and thus cannot account for either the local or the global regulation of CBF. A potential modelling reason for this discrepancy is due to the representative voxels in **Fig. 5.11** and **Fig. 5.12** being averaged over 100 simulations, leading to a smearing

of the velocities across the voxel, although this is not seen with the blood pressure drops.

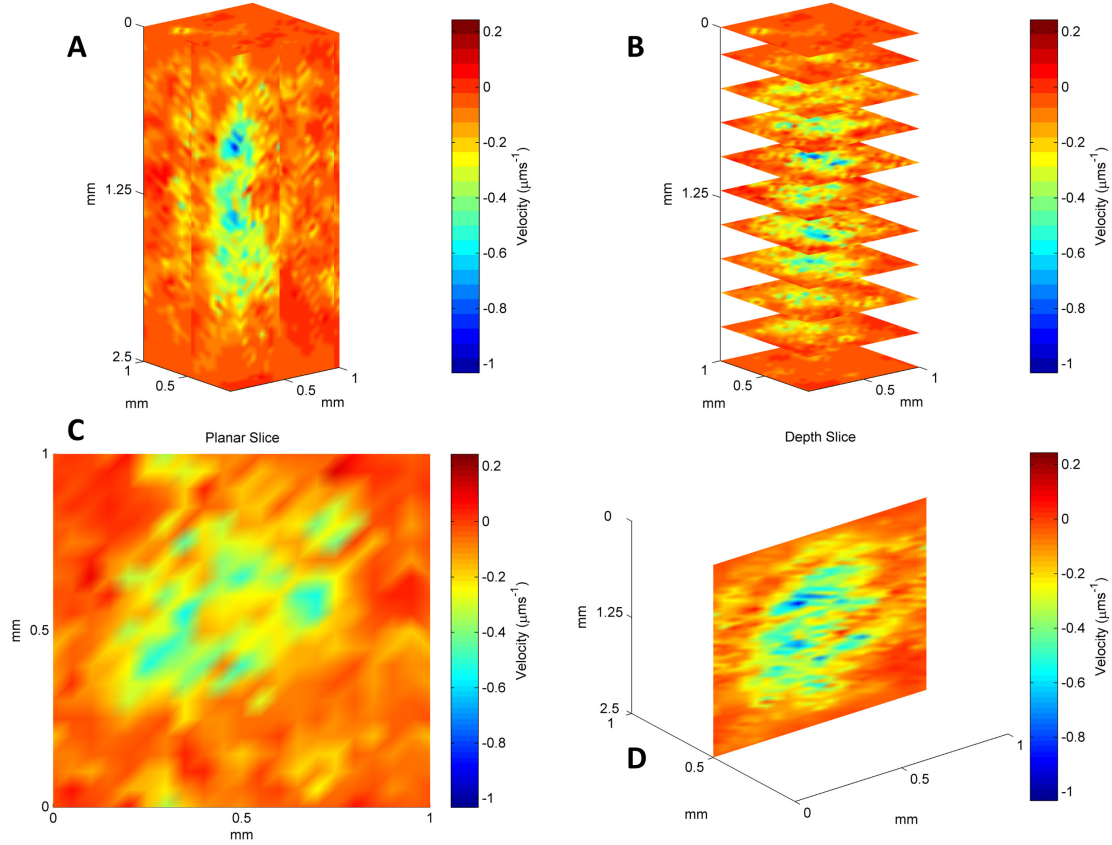


Fig. 5.11 Volume averaged velocity difference between the averaged healthy and occluded vasculature for the Case 1 configuration – averaged over 100 voxel realisations. **a)** 3D map of velocity difference displaying a central region of blood velocity drop. **b)** Slices of the velocity map taken at 250 μm intervals through the depth of the voxel. **c)** Planar slice taken 1.25 mm down the voxel. **d)** A planar slice taken depth-wise through the voxel 0.5 mm along the width of the voxel, showing an expansive region of blood velocity drop.

It can also be seen that there are small regions/vessels that increase in flow post-occlusion, despite this model being passive. This was also observed in the rat cortex post-occlusion of a penetrating arteriole [129], where a large variability of flow was observed, with both increases and decreases in flow in vessels surrounding the

occlusion. This large variability in flow is also observed in this model from one voxel simulation to the next. Due to the finite number of voxel simulations, it is thus very likely that this large variability will manifest itself in small regions of increased flow. However, further experiments and more simulations will be required to determine whether this is a robust phenomenon or a result of statistical variability (even with our 100 networks).

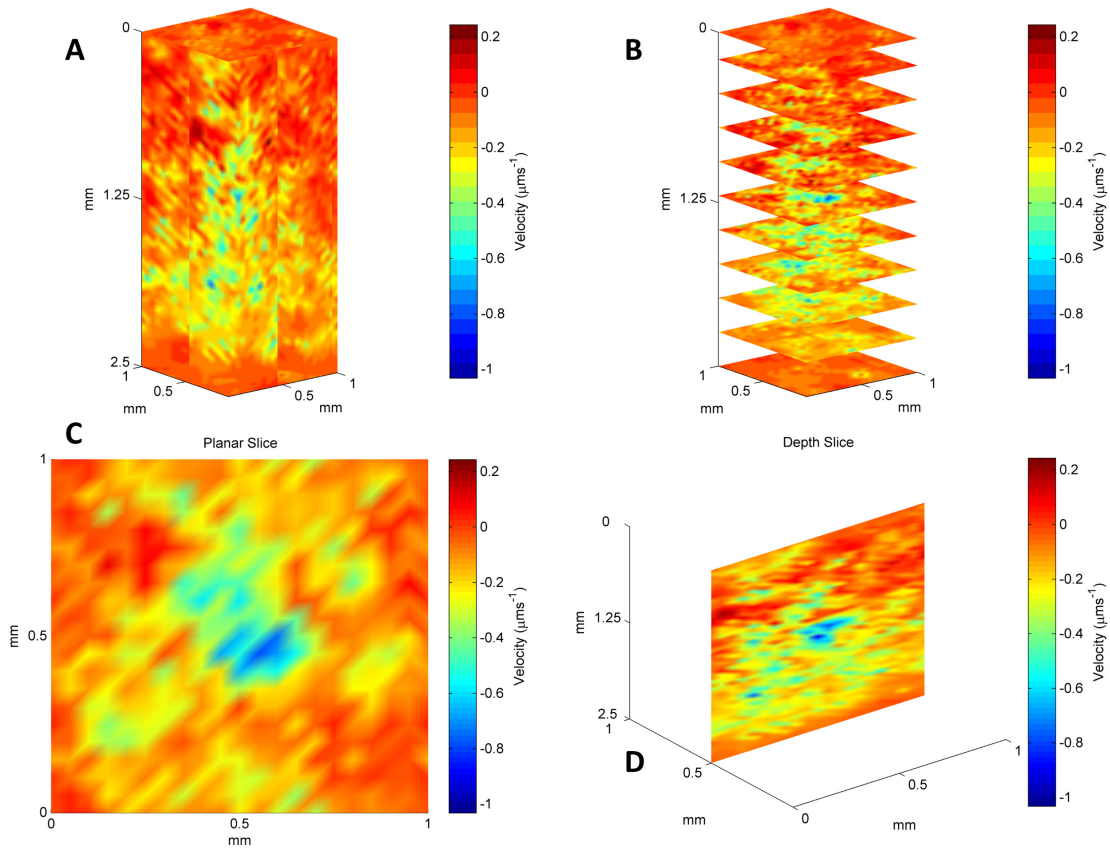


Fig. 5.12 Volume averaged velocity difference between the averaged healthy and occluded vasculature for the Case 2 configuration – averaged over 100 voxel realisations. **a)** 3D map of velocity difference displaying a more diffuse region of blood velocity drop. **b)** Slices of the velocity map taken at 250 μm intervals through the depth of the voxel. **c)** Planar slice taken 1.25 mm down the voxel. **d)** A planar slice taken depth-wise through the voxel 0.5 mm along the width of the voxel, showing an expansive region of blood velocity drop.

5.3.6 One-dimensional Depth Profiles

Based on the planarity shown earlier, it is possible now to look at depth profiles of both the pressure and total velocity. These are shown in **Fig. 5.13**, where each layer is $100\ \mu\text{m}$ thick averaged across the plane. The pressure and velocity are again found to be decoupled for both the healthy and occluded vasculature due to the geometry of the penetrating vessels. As well as this, despite the larger vessels in the case 2 voxels, and the larger pressure gradient, the planar averaged velocity is similar to the planar averaged velocity in the case 1 voxels. This suggests that the flow in the voxel is predominantly set by the pressure boundary conditions rather than the penetrating vessel diameters.

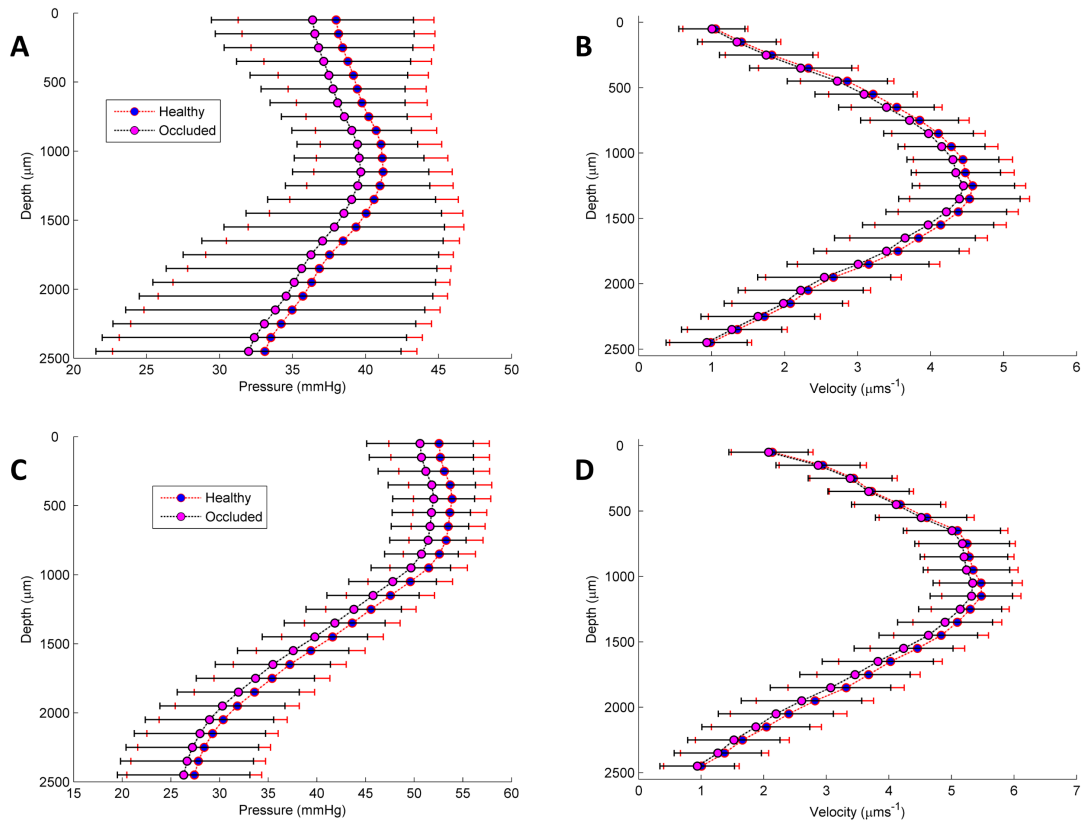


Fig. 5.13 *a,b)* 1-D planar averaged depth profiles of pressure and total velocity for the case 1 configuration. *c,d)* 1-D planar averaged depth profiles of pressure and total velocity for the case 2 configuration. Blue solid circles – Healthy vasculature; Magenta solid circles – Occluded vasculature.

The difference between the healthy and occluded vasculature pressure and velocity distributions is minimal. This means that the effects of a penetrating vessel occlusion are predominantly local and cannot be seen when averaged over a planar area, as much of the area will not be affected. This is relevant to imaging measurements of CBF as this indicates that penetrating vessel occlusions will be very difficult to observe, due to the minimal effects that they have on averaged flow quantities.

5.3.7 Venous Occlusions

All of the above analysis was also run for penetrating venule occlusions (results not shown). The results are similar to those for the arterioles, with conical pressure change regions, except when occluding the venule there is a conical pressure increase as opposed to a decrease. The drops in flow, however, are still diffuse across the voxel. As the venules are larger in size (mean of 50 μm for case 1 and 110 μm for case 2) the drops in CBF in the voxels are larger (case 1: -13.9% average, case 2: -19.7% average) with 50% of voxels having a CBF drop of less than 10%. A Wilcoxon signed-rank test was used to determine that there is a statistically significant difference in the CBF drops between occluding an arteriole and occluding a venule at each case.

This indicates that a penetrating venule occlusion is more likely to be detectable than a penetrating arteriole due to the size in flow drop. These results correlate well with those in Shih et al. who also occluded penetrating venules and found that they “generated a microinfarction with notable similarity to that caused by occlusion of a penetrating arteriole” [166]. As well as this, larger volume, and hence larger flux, penetrating vessels also led to larger volumes of infarction, which again has been observed here with the occlusion of the larger volume penetrating venules.

5.4 Discussion

The effects of the human microvasculature topology on macro-scale CBF, blood pressure and velocity remain relatively poorly understood. In particular, little is known about the effects of penetrating vessel occlusions in the human microvasculature on pressure and CBF due to the inability of conventional imaging techniques to resolve these small-scale vessels. As a result, I have developed a multi-scale model of the human microvasculature in this chapter to analyse the flow properties in voxel sized models for both the healthy and occluded microvasculature. This model was validated by calculating the average CBF for voxels where the arteriolar diameters are 20 μm average ($39.4 \text{ mL min}^{-1} 100\text{g}^{-1}$) and 40 μm average ($93.75 \text{ mL min}^{-1} 100\text{g}^{-1}$) indicating the very large effect that penetrating vessel diameter has on CBF - an important effect for autoregulation and neurovascular coupling. The range of velocities and fluxes in the capillary network was also found to be between $0.1 - 1 \text{ mm s}^{-1}$ and $1 - 18 \text{ pL s}^{-1}$ which show good agreement with experimental data [62,95,166,171].

One of the differences between this model and other large-scale models of the cerebral microvasculature is the treatment of the capillary bed as one homogenous, isotropic, porous medium, characterised by a single value of permeability. In contrast, other models, whether statistical or cast reconstructions, model capillaries individually [20,107,110,161]. From these models, it has been shown that RBC flow tends to be layered, with shunting of flow between penetrating arterioles and venules terminating at the same depth. This layering is also observed in this model, with very clear planar regions of velocity and pressure, indicating that the layering and shunting of flow is a direct result of the geometry of the penetrating vessels as opposed to the capillary network topology. It also suggests that modelling the capillary network as homogenous

and isotropic is a valid assumption, opening up the possibility of further large-scale modelling of the human cerebral microvasculature.

One benefit that a model such as this has is its ability to provide greater detail than a standard perfusion imaging voxel, allowing us to observe sub-voxel level detail, whilst also being able to average the flow properties for comparison with conventional imaging voxels. This is seen with the observation that flow and pressure are decoupled within the voxel – with flow preferentially travelling in the planar direction despite a depth-wise pressure gradient. This is due, again, to the geometry of the penetrating arterioles and venules, particularly their terminal vessels that act as sources and sinks within the capillary bed.

Another benefit of the model presented here is the ability to simulate penetrating arteriole occlusions, and to observe how such occlusions affect the behaviour of the cerebral microvasculature. Post-occlusion the CBF was found to drop on average between 7.5 – 8.2%. Post-occlusion of a venule the CBF was found to drop on average between 13.9 – 19.7%. The CBF robustness to an arteriole occlusion demonstrates that such micro-infarcts will be very difficult to pick up using current MRI perfusion imaging. This agrees with what was previously found in Chapter 4: i.e. that the topology of the penetrating vessels contributes only a small amount to the CBF variability; the radius on the other hand has a much larger effect on the CBF.

Within the voxel, the difference in pressure between the occluded and healthy vasculature shows clear conical regions of blood pressure drop, which overlie very well with the regions of tissue hypoxia observed post-occlusion in rats [166]. Larger diameter vessels, hence larger flux vessels, exhibited larger volumes of blood pressure drop – again correlating with what is found experimentally [166]. Blood pressure deeper into the grey matter was less affected by the occlusion than that at the surface

layers. As few penetrating vessels reach the deepest layers, this is likely to be a topological effect, with the drop in pressure in the voxel being very heavily dependent on the location of the penetrating vessels and their terminal nodes. This backs up the idea that the penetrating vessel is “the bottleneck of perfusion” [129]. The reproduction of these experimental results again demonstrates the validity of assuming a homogenous, isotropic capillary network.

The decoupling in pressure and flow was also observed with the occluded microvasculature. The conical regions of pressure drop were not emulated in perfusion, with perfusion drop being far more diffuse across the voxel. Nishimura et al. found that the RBC velocity returns to baseline beyond a 350 μm radius around the occluded vessel [129]. This is not seen in this model, indicating that there is an active response in the capillary network around the penetrating vessel that restores flow up to this point. However, in the direct vicinity of the penetrating vessel flow cannot be restored, due to the reliance on that region of tissue on the topology of particular penetrating vessels.

This highlights one of the major limitations of the model which is the lack of an active response. It is currently thought that capillary pericytes actively regulate capillary dilation, with capillary dilation occurring before arteriolar dilation, and contributing up to 84% of the blood flow increase in that region [63]. However, this is a topic of ongoing debate, with other mechanisms proposed as potential pathways in capillary dilation [49,50,67]. Other potential discrepancies between modelled and experimental findings may also be due to other biological mechanisms that are not modelled, such as the adhesion of leukocytes [197]. Regardless, it seems that the capillary bed plays an active role in the regulation of cerebral blood flow through the pericytes. The model presented here is purely passive and so cannot predict changes in the microvasculature post-occlusion. Therefore, the next step will be to model an

active response to see how changes in the microvasculature affect post-clot perfusion and blood pressure, and how much affect the active response has in comparison to the passive response.

This active response will need to be modelled with feedback based on (most likely) a hypoxia model to determine where the response is activated. This would be incorporated via a temporally and spatially varying permeability that would be pre-computed from the simulation of dilating vessels in statistically accurate anatomical models (as in Chapter 3). Although it is thought that dilation of vessels will increase permeability, the exact relationship between dilation and its effect on permeability is not yet known and this will be the subject of future work. Such simulations of autoregulation on this homogenized model can then be cross-validated against more detailed anatomical models. As well as this, density variations within the cortex can also be incorporated via a spatially varying permeability straightforwardly. These density variations, which have not been modelled, may account for some of the discrepancy between the flow model and experimental work.

Beyond these improvements, a variable haematocrit model should be included in future, as currently a constant haematocrit is assumed, although it is likely that this will have only a second order effect [59]. Simulation of this bi-phasic flow can be performed in a similar fashion to that in Gould and Linninger [57] in order to pre-compute the permeability tensor which can then be used in the multi-scale model presented in this Chapter.

The model can be further validated in future against MRI voxels once an oxygen transport model has been added. Perfusion MRI imaging can also be used to derive experimentally a transit time distribution (TTD), which could be calculated using the model presented here along with an oxygen transport mechanism [134,135].

The TTD could then be calculated across a number of simulated voxels and compared to human data for the same region of brain. Such a model would be valuable in helping us further to observe the layered effects of blood flow, as well as the effects of flow regulation on blood pressure and perfusion post-occlusion. It would also be valuable in comparing oxygen transport in this homogenous capillary bed with oxygen transport in cast based models [107,161]. This will aid in determining whether there is capillary flow homogenization in passive and active states. A further benefit of this model is that it will be able to potentially predict MRI resolutions that will be able to pick up a penetrating vessel occlusion, although the exact methods for this analysis is a subject for future work. Most importantly, however, this model will allow us to draw a clearer link between the ‘unseen’ microvasculature and MRI scans in future, helping to bridge the ‘imaging gap’ that currently exists.

Chapter Summary

The final content chapter of this thesis brings together the previous two chapters into one complete multi-scale model of the human cerebral microvasculature. The porous capillary bed is coupled to the statistically generated penetrating arterioles and venules through their terminal vessels. This model is able to simulate healthy and occluded vasculatures at various length scales (due to the homogenized capillary bed), whilst minimising the effects of boundary conditions. Such a multi-scale model is very useful when investigating various effects of regulation and occlusion (amongst others) on the cerebral blood flow, pressure, and velocity.

The observation that this model reproduced numerous experimental and cast-simulated results in both healthy and occluded vasculature not only indicates its usefulness, but also backs up the assumption that the capillary bed can be modelled as a homogenous, isotropic, porous medium.

Additionally, penetrating vessel occlusions in the human cerebral microvasculature have been modelled here for the first time. These models indicated a decoupling of pressure and perfusion in the vasculature, with clear conical regions of pressure drop that overlie with regions of cell death observed in rats. These results pointed towards the likely regulation of the capillary bed, potentially through pericyte contraction.

Chapter 6

Conclusions and Future Work

In this chapter I will summarise the results of this thesis and discuss the implications of the findings, as well as the limitations. I will then propose future work that could build upon what was presented here and potentially further our understanding of the role of the microvasculature in health and disease, and better link the behaviour on the micro-scale to clinical imaging results.

6.1 Summary of Results & Discussion

The primary aim of this thesis was to generate statistical large-scale models of the human cerebral microvasculature in order to link the micro-scale behaviour to macro-scale observable properties, such as CBF. This large-scale model was then used to analyse the cerebral microvasculature in both health and disease (penetrating vessel

occlusions). The analysis of this large-scale model provided insight into the role of the capillaries and penetrating arterioles in perfusing the parenchyma, the variation of pressure and flux within an MRI-sized voxel, and the effects of occlusions on observable properties such as CBF. The steps taken to generate and to analyse such a novel, statistical, large-scale model are given below.

In Chapter 3 the theoretical framework for homogenizing the blood flow and oxygen transport in the capillary network was developed and presented. To ‘scale-up’ the microvasculature to large volumes, it was necessary to find a way to generate large models of the capillary network computationally efficiently (as they are by far the most numerous vessels in the microvasculature) [155]. This was achieved by applying homogenization theory (assuming the capillary network could be approximated as periodic) to the Navier-Stokes equation and mass transport equation in the capillary bed. The result of the homogenization resulted in the flow through the capillary network being approximated as Darcy flow (the capillary bed approximated as a porous medium), with the permeability tensor $\mathbf{K}$ encapsulating the geometry of the micro-scale capillary network. The mass transport equation homogenization resulted in an averaged form of the mass transport equation, with both the interstitial and capillary domains coupled. For oxygen transport, it was found that advection and metabolism dominate over diffusion on the macro-scale, resulting in the mass transport equation for oxygen being a simple advection-reaction equation.

This theory was then applied to a statistically accurate model of the capillary network in a region of the human cortex [176], with permeability calculated for voxel sizes ranging from $125\ \mu\text{m}$ to $625\ \mu\text{m}$. The model was modified to impose periodicity. The permeability was found to converge to an ‘effective’ permeability for all three principal directions of the 3-dimensional tensor. The permeability was highly isotropic, with less than 1.2% variation between the 3 principal directions. Off-diagonal values

of permeability were found to be two orders of magnitude smaller than the principal directions. A representative elementary volume of $375\ \mu\text{m}$ was calculated giving a 4.5% error from the converged effective permeability. Larger cube sizes can of course be used with smaller errors, depending on the application. Finally, the permeability was validated by calculating CBF, giving a value of around $55\ \text{mL min}^{-1} 100\text{g}^{-1}$, in remarkably good agreement with observed experimental values [131].

Chapter 4 dealt with the next scale up from the capillary network – the penetrating vessels. In order to generate large-scale models of the microvasculature it was necessary to have a statistical model of the penetrating arterioles and venules that could then be combined with the capillary network. As a result, a novel order-based density-filling algorithm was developed to generate statistically accurate 3-dimensional models of the penetrating arterioles and venules. This algorithm was based on sampled morphological data, readily available in the literature [27,112], from the collateral sulcus in the right temporal lobe. The algorithm sampled bifurcation data to grow trees at each bifurcation, before employing simple filters to either add or prune vessels depending on whether a morphological density had been reached. Using such an algorithm meant that previous assumptions – such as volume and energy minimisation – traditionally used when modelling such vascular trees could be relaxed [86,107,122]. Additionally, such a model is generalizable to any available dataset of any vascular trees, demonstrating the flexibility of such statistical modelling techniques.

The results of this algorithm displayed a very good fit to all morphological parameters. An analysis of Murray’s k exponent found a median of 2.5 with interquartile range of 1.75 – 3.7. This is interesting as it is an emergent property of the model – no assumptions with regards to the k exponent were made when developing the algorithm. This indicates that a model built using Murray’s Law may

oversimplify the complex reality of the microvasculature. The vascular resistance of the trees was calculated, and from this a first approximation for CBF was also calculated. It was found that by far the largest impact on CBF was the diameter of the penetrating vessels. In contrast, the quasi-random topology of the penetrating vessels contributed $< 34\%$ of the variability from the mean CBF value. This is valuable in understanding that the topology of the penetrating vessels has a nominal impact on macro observable properties, such as CBF. The diameters have a much larger effect.

Finally, Chapter 5 brought together the work in the previous two chapters into one unifying large-scale model of the human cerebral microvasculature. The capillary bed was a continuum, characterised by the permeability calculated in Chapter 3, and the penetrating vessels (generated in Chapter 4) were coupled to the continuum via their terminating vessels. The effects of boundary conditions were mitigated due to the permeability of the capillary network being calculated from a periodic model, effectively giving a true permeability of the network. 100 simulations were run for $2 \times 2 \times 2.5$ mm voxels (comprised of a repeating, periodic $1 \times 1 \times 2.5$ mm voxel) from which the central $1 \times 1 \times 2.5$ mm region was used for analysis. Two cases were considered with varying penetrating vessel diameter sizes.

The model was validated using a CBF calculation, and calculating the velocities and fluxes through the homogenous capillary network. The CBF gave physiological values, but was again highly influenced by diameter choice of the penetrating vessels. The model displayed a clear layering of flow and pressure, something that has been observed experimentally and in simulations [20,161]. The model also displayed a decoupling of pressure and flow across the voxel, where the depth-wise pressure gradient contrasted to the greater lateral, layered flow. This was a direct result of the topology of the penetrating vessels shunting flow between them.

The model was also used to simulate penetrating vessel occlusions, with a single arteriole occluded and the difference between the healthy and occluded vasculature analysed. A clear conical region of pressure drop was observed, which overlay very well with regions of tissue hypoxia observed post-occlusion in rats [129]. Occlusion of larger diameter vessels resulted in larger regions of pressure drop. There was also a clear robustness of pressure drop with depth, as also observed experimentally and in simulations [61]. This was deemed likely to be due to the rarefaction of penetrating vessels with depth, again pointing towards the topology of the penetrating vessels being the determinants of flow and pressure in a voxel. The drops in flow, however, were far more diffuse across the voxel, indicating that in-vivo there may be an active response which is compensating for flow where the penetrating vessels have been occluded. The post-occlusion CBF, on the other hand, displayed a robustness; only dropping on average between 7.5 – 8.2% of the mean healthy CBF. This implies that any micro-infarct in the microvasculature would be very difficult to detect using current clinical perfusion imaging.

The very good agreement of the large-scale model presented in this thesis with experimental and simulated data indicates the validity of approximating the capillary bed as a homogenous, isotropic, porous medium. This, of course, is a very important result, as it opens up the potential to model further large volumes of cerebral tissue efficiently. This in turn will improve our understanding of the effects of the microvasculature on what can be observed through clinical imaging.

The large-scale model presented in this thesis has the ability to provide greater detail than a standard perfusion imaging voxel. This allows us to observe sub-voxel level detail, whilst also being able to derive averaged parameters (such as CBF and TTD) that can be compared with conventional imaging voxels. In this way, this model makes it possible to simulate disease in the microvasculature in a way that can be

used in conjunction with clinical imaging to help to further diagnose and understand pathological conditions.

6.2 Limitations

This model is, of course, not without its limitations. Firstly, throughout the thesis a constant haematocrit has been assumed. This is not physiologically likely due to the phase separation effect at bifurcations in the network (as discussed in Section 2.2.3.3). However, the simulation of models that account for this phase separation has shown that “phase separation has a minor function in the global microvascular haemodynamic behaviour” [59].

A further limitation comes from the data on which this model is based. The data used to generate the microvasculature come from 3 casts imaged from a small section of the collateral sulcus in the right temporal lobe of one brain [26] (due to the lack of other accurate data on the human cerebral microvasculature). Therefore, the model here characterizes only a small region of the human brain. The network topology and density will likely vary quite substantially in other regions of the brain. The statistical nature of the model may account for some of this variability across the cortex, but ultimately more data will be required through ex-vivo imaging in order to generate models for other regions of the brain. The model presented in this thesis would be very easily adaptable to other datasets once they become available however, demonstrating a strength of the statistical modelling approach.

One limitation specific to the capillary network is the assumption of periodicity. This assumption was necessary to simplify the derivation of the homogenized equations (by aiding in the removal of secular terms). However, it is not known whether such an assumption is valid for the capillary network. It is possible to argue that for a sufficiently random microstructure, the approximation of periodicity

is valid [13,69]. In the absence of experimental data determining the randomness or periodicity of the capillary networks, periodicity is used to simplify the derivation.

A limitation concerning the penetrating vessel algorithm is the lack of differentiation in the statistics between arterioles and venules. The order-based data available (given in the supplementary material of [27] – Appendix C) are not segregated by type of vessel (arterioles or venules). As a result, the penetrating trees generated use ‘averaged’ properties of the trees that are based on both arterioles and venules. The only way they are differentiated in the model presented in this thesis is through their density and major diameters. Bifurcation angles, area ratios, and length-to-diameter ratios all come from the same statistics. However, it would be straightforward to adapt this statistical algorithm to any data that may become available in future that differentiate between the statistics of arterioles and venules.

Throughout this thesis, I have assumed straight blood vessels, neglecting their tortuosity. In order to produce more realistic networks, the tortuosity of these vessels should be modelled, although it is anticipated that the effect on the blood flow through the vessels will be of second order due to the small curvature of the individual vessels. Despite this, it has been observed that penetrating vessel sinuousness increases with age [42], potentially indicating that the increased tortuosity is a sign of potential neurovascular degeneration.

Finally, as demonstrated in Chapter 5, it seems that an active response on the capillary and penetrating vessel scale may account for the diffuse drops in flow observed post penetrating arteriole occlusion. The lack of such an active response is the major limitation of this model, meaning that post-occlusion it is unable to predict changes in the microvasculature. This can be remedied, however, by incorporating an active response into the model, and this will be discussed in the next section.

6.3 Future Work

Simple changes that can be made to the model include introducing tortuosity to the vessels and a phase separation model. These would of course be valuable, but as mentioned above, are likely to have second order effects on the simulation results.

The model developed here has the potential to be used alongside clinical imaging techniques to ‘discover’ the role and effects of the microvasculature in health and diseases. Currently, it is only possible to model blood flow (and pressure) in this model. This can be linked back to perfusion clinical imaging via the calculation of CBF, as has been previously demonstrated. A perfusion clinical imaging voxel also produces a transit time distribution (TTD). Therefore, the next step would be to calculate this TTD from the blood flow model and validate it against perfusion clinical images [134].

It is then envisaged that an oxygen transport model coupled to the blood flow model developed here would be incorporated. The oxygen transport homogenization theory has already been developed for the capillary network in Chapter 3, only requiring implementation on the statistical capillary network. This oxygen transport PDE can then be coupled to oxygen transport in the penetrating vessels to produce a full oxygen transport model in a large-scale voxel-sized model of the human microvasculature. Not only would this be valuable in further validating the model against perfusion clinical imaging data, it would also allow us to calculate oxygen extraction factors (OEF) and oxygen consumption in the tissue (CMRO_2) [135]. This could then be further used to validate the model. This would also provide an oxygen transport model in a homogenous capillary bed that could be compared to oxygen transport simulations in cast based models [107].

The oxygen transport model would also be very useful when simulating penetrating vessel occlusions (and other cerebrovascular diseases). Currently, this model is only able to assess changes in blood flow and blood pressure, but with an oxygen transport model, it would be possible to observe oxygen starved tissue. This, in turn, would provide a much better comparison to the effects of experimentally observed penetrating vessel occlusions in rodents, where regions of necrosis and hypoxia are measured [166].

As mentioned in the previous section, this model is purely passive; it does not model the effects of microvascular regulation. This means that the response to changes in the microvasculature, such as an occlusion, is not properly modelled. Therefore, a necessary next step, in order to make the model more physiologically relevant, is to include an active response model. Modelling this active response can be done in a number of ways, for example by modelling neurogenic nitric oxide [181]. Once an oxygen model has been implemented, however, it could be possible to model the active response using feedback based on a hypoxia model post penetrating vessel occlusion. This in turn will result in a dilation/contraction in either or both the penetrating arterioles and capillaries. The dilation/contraction in capillaries could be incorporated via a spatially and temporally varying permeability tensor which is calculated from the micro-scale REV (as in Chapter 3).

It is hoped that the model developed in this thesis already goes some way in helping to bridge the ‘imaging gap’ that currently exists with clinical imaging techniques. By introducing the future work described above, the model will be able to draw a much clearer link between the ‘unseen’ microvasculature and clinical imaging. This will help to elucidate the role of microvascular topology on macro-scale oxygen transport in health and disease.

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Appendix A

Proof of Local-Scale Invariance of Leading Order Interstitial Pressure and Concentration

The homogenization of the interstitial domain and mass transport equation was presented in Chapter 3. It was stated that the leading order interstitial pressure ($p_t^{(0)}$) and the leading order concentration ($c^{(0)}$) are locally constant. The proof of this is presented below.

A.1 Locally Constant Interstitial Pressure

Multiplying (3.51) by $p_t^{(0)}$ and integrating over the interstitial domain we get:

$$\int_{\Omega_t} p_t^{(0)} \nabla_{\mathbf{x}}^2 p_t^{(0)} dV = 0 \quad (\text{A.1})$$

Using the product rule for the divergence operator this can be rewritten as:

$$\int_{\Omega_t} \left(\nabla_{\mathbf{x}} \cdot (p_t^{(0)} \nabla_{\mathbf{x}} p_t^{(0)}) - |\nabla_{\mathbf{x}} p_t^{(0)}|^2 \right) dV = 0 \quad (\text{A.2})$$

Using the divergence theorem on the first term in (A.2) this then becomes:

$$\int_{\Gamma} (p_t^{(0)} \nabla_{\mathbf{x}} p_t^{(0)}) \cdot \mathbf{n} dS - \int_{\Omega_t} |\nabla_{\mathbf{x}} p_t^{(0)}|^2 dV = 0 \quad (\text{A.3})$$

where the surface integral in the first term is over the capillary walls as, due to periodicity, the surface integral over the boundary cell walls is 0.

From the boundary condition (3.52) it can be seen that the first term is equal to zero and hence (A.3) reduces to:

$$\int_{\Omega_t} |\nabla_{\mathbf{x}} p_t^{(0)}|^2 dV = 0 \quad (\text{A.4})$$

This is only true when $p_t^{(0)}$ is locally constant, i.e. $\nabla_{\mathbf{x}} p_t^{(0)} = 0$. As a result, this proves $p_t^{(0)} = p_t^{(0)}(\mathbf{x})$.

A.2 Locally Constant Concentration

The mass transport equation is homogenized for 7 different cases in this thesis. For all seven cases the leading order concentration $c^{(0)}$ is locally constant. The proof of this is presented below. It should be noted that the proof will deal with the $\mathcal{L}_0$ operator as defined in (3.90). However, the analysis is just as valid for the $\mathcal{L}_1$ operator as defined in (C.10) which is effectively the $\mathcal{L}_0$ operator without the advection term.

In a similar fashion to the analysis of the interstitial pressure above, multiply (3.91) by $c^{(0)}$ and integrate over the entire domain

$$\int_{\Omega} c^{(0)} \mathcal{L}_0 c^{(0)} dV = 0 \quad (\text{A.5})$$

Expanding the $\mathcal{L}_0$ operator:

$$\int_{\Omega} \left(D c^{(0)} \nabla_{\mathbf{X}}^2 c^{(0)} - Pe_d c^{(0)} \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(0)}) \right) dV = 0 \quad (\text{A.6})$$

The first term in (A.6) is the same form as (A.1) and hence can be rewritten as:

$$\int_{\Omega} D (\nabla_{\mathbf{X}} \cdot (c^{(0)} \nabla_{\mathbf{X}} c^{(0)}) - |\nabla_{\mathbf{X}} c^{(0)}|^2) dV \quad (\text{A.7})$$

Using the divergence theorem, splitting the diffusion term into the interstitial and capillary domains, and using periodicity on the first term (such that the surface integral is only over the capillary walls):

$$\int_{\Gamma} (D_c c_c^{(0)} \nabla_{\mathbf{X}} c_c^{(0)} - D_t c_t^{(0)} \nabla_{\mathbf{X}} c_t^{(0)}) \cdot \mathbf{n} dS - \int_{\Omega} D |\nabla_{\mathbf{X}} c^{(0)}|^2 dV \quad (\text{A.8})$$

Applying the flux boundary condition (3.92) to the first term in (A.8) makes this surface integral zero and therefore the first term in (A.6) has been reduced to:

$$\int_{\Omega} D |\nabla_{\mathbf{X}} c^{(0)}|^2 dV \quad (\text{A.9})$$

Now, consider the advection term (second term) in (A.6). Using the divergence theorem and periodicity this becomes:

$$\int_{\Gamma} (Pe_d (c^{(0)})^2 \mathbf{u}^{(0)}) \cdot \mathbf{n} dS \quad (\text{A.10})$$

This surface integral is equal to zero using the fluid flux boundary condition $\mathbf{u}^{(0)} \cdot \mathbf{n} = 0$ from (3.24) and (3.50). Therefore, the problem has reduced to

$$\int_{\Omega} D |\nabla_{\mathbf{x}} c^{(0)}|^2 dV = 0 \quad (\text{A.11})$$

This is only true if both $c_t^{(0)}$ and $c_c^{(0)}$ are locally constant. As $c_c^{(0)} = c_t^{(0)}$ on Γ , then $c^{(0)} = \bar{c}(\mathbf{x}, t)$ throughout the volume Ω .

Appendix B

Homogenization of the Mass Transport Equation at Different Scalings

The homogenization of the mass transport of oxygen was presented in Chapter 3 (Section 3.2.3). This was found to be when advection and metabolism dominate over diffusion on the macro-scale. There are 6 other mass transport cases assuming an $O(\varepsilon)$ difference between the relative transport terms. They are presented below.

B.1 Advection dominates on the macro-scale

The characteristic time for when advection dominates over metabolism and diffusion is $t_c = t_L^{adv} = \varepsilon t_L^{reac} = \varepsilon t_L^{dif}$. This gives $P_L = O(\frac{1}{\varepsilon})$, $Pe_L = O(\frac{1}{\varepsilon})$, and $Da_L = O(1)$.

Therefore, on the local-scale $P_d = O(\varepsilon)$, $Pe_d = O(1)$, and $Da_d = O(\varepsilon^2)$. Hence (3.80) becomes

$$\begin{aligned} \varepsilon \overline{P_d} \frac{\partial c}{\partial t} + Pe_d (\nabla_{\mathbf{X}} \cdot (c \mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c \mathbf{u})) &= D \nabla_{\mathbf{X}}^2 c + \\ &2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c + \varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon^2 \overline{Da_d} c \end{aligned} \quad (\text{B.1})$$

The boundary conditions are the same as in Chapter 3 (no-slip, no-leak). Substituting the multiple-scales expansion of $\mathbf{u}$ (3.83) and c (3.84) into (B.1) and again equating powers of ε we get at $O(\varepsilon^0)$:

$$D \nabla_{\mathbf{X}}^2 c^{(0)} - Pe_d \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(0)}) = 0 \quad (\text{B.2})$$

Therefore, the $O(\varepsilon^0)$ system can be written as

$$\mathcal{L}_0 c^{(0)} = 0 \quad (\text{B.3})$$

as previously defined in (3.91). This is subject to the same boundary conditions as in the first case. As in the first case, the system only has constant solutions in $\mathbf{X}$, i.e. $c^{(0)} = \bar{c}(\mathbf{x}, t)$ (Appendix A).

Next at $O(\varepsilon^1)$:

$$\begin{aligned} \overline{P_d} \frac{\partial c^{(0)}}{\partial t} + Pe_d \{ \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(1)}) + \nabla_{\mathbf{X}} \cdot (c^{(1)} \mathbf{u}^{(0)}) + \nabla_{\mathbf{x}} \cdot (c^{(0)} \mathbf{u}^{(0)}) \} &= \\ D \nabla_{\mathbf{X}}^2 c^{(1)} + 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(0)} \end{aligned} \quad (\text{B.4})$$

Using the same reasoning as in Case 1 (Section 3.2.3) the equation simplifies to

$$\mathcal{L}_0 c^{(1)} = \overline{P_d} \frac{\partial c^{(0)}}{\partial t} + Pe_d (\mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)}) \quad (\text{B.5})$$

The solvability condition (using the Fredholm alternative) is the volume average over the local domain, as demonstrated in [167]. The solvability condition is implemented as in (3.99). Therefore (B.5) becomes:

$$\overline{P_d} \frac{\partial c^{(0)}}{\partial t} + Pe_d (\tilde{\mathbf{u}}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)}) = 0 \quad (\text{B.6})$$

where $\tilde{\mathbf{u}}^{(0)}$ is defined as in (3.101).

Re-dimensionalizing the variables gives us the homogenized equation for advection dominated flow on the global-scale to leading order:

$$\frac{\partial c'}{\partial t'} + \tilde{\mathbf{u}}' \cdot \nabla c' = O(\varepsilon) \quad (\text{B.7})$$

In this case, continuing to the $O(\varepsilon)$ correction will bring the effects of diffusion and metabolism into the equation.

B.2 Metabolism dominates on the macro-scale

Advection is no longer the dominant mass transport mechanism in this case. Based on previous case results it is expected that the homogenized equation for this case will only include the reaction term with an unsteady term. The characteristic time is now $t_c = t_L^{reac} = \varepsilon t_L^{adv} = \varepsilon t_L^{dif}$ and on the local-scale $P_d = O(\varepsilon)$, $Pe_d = O(\varepsilon)$, and $Da_d = O(\varepsilon)$. Hence (3.80) becomes:

$$\begin{aligned} \varepsilon \overline{P_d} \frac{\partial c}{\partial t} + \varepsilon \overline{Pe_d} (\nabla_{\mathbf{x}} \cdot (c\mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c\mathbf{u})) &= D \nabla_{\mathbf{x}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c + \\ &\varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon \overline{Da_d} c \end{aligned} \quad (\text{B.8})$$

Substituting the expansions of $\mathbf{u}$ (3.83) and c (3.84) into (B.8) and equating powers of $O(\varepsilon^0)$ gives:

$$D \nabla_{\mathbf{x}}^2 c^{(0)} = 0 \quad (\text{B.9})$$

Defining the new operator

$$\mathcal{L}_1 \star = D \nabla_{\mathbf{x}}^2 \star \quad (\text{B.10})$$

then the $O(\varepsilon^0)$ equation can be written as

$$\mathcal{L}_1 c^{(0)} = 0 \quad (\text{B.11})$$

This system again only has constant solutions in $\mathbf{X}$, i.e. $c^{(0)} = \bar{c}(\mathbf{x}, t)$ (proof can be found in Appendix A whilst neglecting the advection term).

At $O(\varepsilon^1)$:

$$\overline{P_d} \frac{\partial c^{(0)}}{\partial t} + \overline{Pe_d} \{ \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(0)}) \} = D \nabla_{\mathbf{X}}^2 c^{(1)} + 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(0)} - \overline{Da_d} c^{(0)} \quad (\text{B.12})$$

As $c^{(0)}$ is locally constant the cross-diffusion term goes to 0. The advection term also goes to zero using the fact that $\nabla_{\mathbf{X}} \cdot \mathbf{u}^{(0)} = 0$ (3.23). Therefore, (B.12) becomes:

$$\mathcal{L}_1 c^{(1)} = \overline{P_d} \frac{\partial c^{(0)}}{\partial t} + \overline{Da_d} c^{(0)} \quad (\text{B.13})$$

Implementing the solvability condition gives:

$$\overline{P_d} \frac{\partial c^{(0)}}{\partial t} + \overline{Da_d} \phi_t c^{(0)} = 0 \quad (\text{B.14})$$

as expected. Re-dimensionalizing the variables gives us the homogenized equation for metabolism dominated flow on the macro-scale to leading order

$$\frac{\partial c'}{\partial t'} = -\lambda \phi_t c' + O(\varepsilon) \quad (\text{B.15})$$

In this case, continuing to the $O(\varepsilon)$ correction will bring in the effects of diffusion and advection.

B.3 Diffusion dominates on the macro-scale

In this case diffusion is assumed to play a dominant role in mass transport on the global-scale. In order for diffusion to appear on the global-scale one must go a further step when comparing orders of ε (in comparison to the previous 3 cases) by also comparing $O(\varepsilon^2)$. This introduces the complication of having to solve for $c^{(1)}$.

Going through the same analysis as the previous cases, the characteristic time scale is now $t_c = t_L^{dif} = \varepsilon t_L^{adv} = \varepsilon t_L^{reac}$. This yields $P_L = O(1)$, $Pe_L = O(\varepsilon)$, and

$Da_L = O(\varepsilon)$, and hence $P_d = O(\varepsilon^2)$, $Pe_d = O(\varepsilon^2)$, and $Da_d = O(\varepsilon^3)$. Therefore (3.80) becomes:

$$\varepsilon^2 \overline{P_d} \frac{\partial c}{\partial t} + \varepsilon^2 \overline{Pe_d} (\nabla_{\mathbf{X}} \cdot (c\mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c\mathbf{u})) = D \nabla_{\mathbf{X}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c + \varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon^3 \overline{Da_d} c \quad (\text{B.16})$$

with the same boundary conditions as in the previous cases. Performing the multiple-scales expansion and equating terms of $O(\varepsilon^0)$ gives:

$$D \nabla_{\mathbf{X}}^2 c^{(0)} = 0 \quad (\text{B.17})$$

and so the $O(\varepsilon^0)$ is given by:

$$\mathcal{L}_1 c^{(0)} = 0 \quad (\text{B.18})$$

This again yields $c^{(0)}$ as being locally constant, i.e. $c^{(0)} = \bar{c}(\mathbf{x}, t)$. Equating powers of $O(\varepsilon^1)$ gives:

$$D \nabla_{\mathbf{X}}^2 c^{(1)} + 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(0)} = 0 \quad (\text{B.19})$$

with the boundary conditions the same as those in (3.95) and (3.96).

The second term in (B.19) is zero, as $c^{(0)}$ is only a function of the macro length scale, and so what remains is:

$$\mathcal{L}_1 c^{(1)} = 0 \quad (\text{B.20})$$

This, however, does not yield locally constant solutions for $c^{(1)}$ due to the non-homogenous boundary condition (3.95) which also involves the macro length scale. As a result, in a similar fashion to Shipley and Chapman [168], a solution is proposed of the form:

$$c^{(1)}(\mathbf{x}, \mathbf{X}, t) = \chi(\mathbf{x}, \mathbf{X}) \cdot \nabla_{\mathbf{x}} \bar{c} + \bar{c}^{(1)}(\mathbf{x}, t) \quad (\text{B.21})$$

where χ is the cell variable. Inserting (B.21) into (B.20), (3.95), and (3.96) gives the stationary cell problem

$$\mathcal{L}_1 \chi = 0 \quad (\text{B.22})$$

$$(D_c(\nabla_X \chi_c)^T - D_t(\nabla_X \chi_t)^T) \cdot \mathbf{n} = (D_t - D_c) \cdot \mathbf{n} \quad \text{on } \Gamma \quad (\text{B.23})$$

$$\chi_c = \chi_c \quad \text{on } \Gamma \quad (\text{B.24})$$

In order to uniquely determine χ a condition is chosen which ensures that the $O(\varepsilon)$ correction is a function of the macro-scale variable only:

$$\int_{\Omega} \chi dV = 0 \quad (\text{B.25})$$

Finally, equating the $O(\varepsilon^2)$ terms:

$$\begin{aligned} \overline{P_d} \frac{\partial c^{(0)}}{\partial t} + \overline{Pe_d} \{ \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(0)}) \} &= D \nabla_{\mathbf{X}}^2 c^{(2)} + 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(1)} + \\ &D \nabla_{\mathbf{x}}^2 c^{(0)} \quad \text{in } \Omega \end{aligned} \quad (\text{B.26})$$

$$(D_t \nabla_{\mathbf{X}} c_t^{(2)} + D_t \nabla_{\mathbf{x}} c_t^{(1)}) \cdot \mathbf{n} = (D_c \nabla_{\mathbf{X}} c_c^{(2)} + D_c \nabla_{\mathbf{x}} c_c^{(1)}) \cdot \mathbf{n} \quad \text{on } \Gamma \quad (\text{B.27})$$

$$c_c^{(2)} = c_t^{(2)} \quad \text{on } \Gamma \quad (\text{B.28})$$

The advection term in (B.26) clearly goes to zero and so:

$$\mathcal{L}_1 c^{(2)} = \overline{P_d} \frac{\partial c^{(0)}}{\partial t} - 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(1)} - D \nabla_{\mathbf{x}}^2 c^{(0)} \quad (\text{B.29})$$

The solvability condition will be determined by the Fredholm alternative, however, unlike in previous cases, the flux boundary condition (B.27) now depends on the macro-scale. Therefore, the Fredholm alternative is used to derive a new solvability condition which is found to be

$$\int_{\Omega} (g_2 + D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(1)}) dV = 0 \quad (\text{B.30})$$

where

$$g_2 = \overline{P_d} \frac{\partial c^{(0)}}{\partial t} - 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c^{(1)} - D \nabla_{\mathbf{x}}^2 c^{(0)} \quad (\text{B.31})$$

Inserting g_2 into the solvability condition gives

$$\int_{\Omega} \left(\overline{P_d} \frac{\partial c^{(0)}}{\partial t} - D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c^{(1)} - D \nabla_{\mathbf{x}}^2 c^{(0)} \right) dV = 0 \quad (\text{B.32})$$

Integrating the first term in (B.32) over the whole domain, bearing in mind that $c^{(0)}$ is independent of the local-scale, yields the same term multiplied by $|\Omega|$. For the second term, the proposed solution for $c^{(1)}$ (B.21) is used to make the integral part of the cell problem. Taking the global-scale terms out of the integral (as the integral is over the local domain) and rearranging gives

$$\int_{\Omega} (D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c^{(1)}) dV = \nabla_{\mathbf{x}} \cdot \left(\int_{\Omega} D (\nabla_{\mathbf{x}} \chi)^T dV \right) \nabla_{\mathbf{x}} \bar{c} \quad (\text{B.33})$$

where $\bar{c} = c^{(0)}$. Finally, the last term is independent of the local-scale and so the integral is split over the capillary and interstitium and integrated to find

$$\begin{aligned} \int_{\Omega} (D \nabla_{\mathbf{x}}^2 c^{(0)}) dV &= \nabla_{\mathbf{x}}^2 c^{(0)} \int_{\Omega} D dV = \nabla_{\mathbf{x}}^2 c^{(0)} \left(\int_{\Omega_c} D_c dV + \int_{\Omega_t} D_t dV \right) \\ &= \nabla_{\mathbf{x}}^2 c^{(0)} (D_c |\Omega_c| + D_t |\Omega_t|) \end{aligned} \quad (\text{B.34})$$

Therefore, putting all the terms together and dividing through by $|\Omega|$ gives

$$\overline{P_d} \frac{\partial c^{(0)}}{\partial t} - \nabla_{\mathbf{x}} \cdot \left(\underline{\underline{D_{dif}^*}} \nabla_{\mathbf{x}} c^{(0)} \right) = 0 \quad (\text{B.35})$$

Where $\underline{\underline{D_{dif}^*}}$ is the non-dimensional diffusion tensor defined as

$$\underline{\underline{D_{dif}^*}} = (D_c \phi_c + D_t \phi_t) \mathbf{I} + \frac{1}{\Omega} \left(\int_{\Omega} D (\nabla_{\mathbf{x}} \chi)^T dV \right) \quad (\text{B.36})$$

where $\mathbf{I}$ is the identity tensor. Re-dimensionalizing the variables using $\underline{\underline{D_{dif}^*}} = D_{ch} \underline{\underline{D_{dif}}}$ gives the homogenized equation for diffusion dominated flow on the macro-scale to leading order

$$\frac{\partial c'}{\partial t'} - \nabla_{\mathbf{x}'} \cdot \left(\underline{\underline{D_{dif}'}} \nabla_{\mathbf{x}'} c' \right) = O(\varepsilon) \quad (\text{B.37})$$

As can be seen the final homogenized equation is simply an unsteady mass transport equation with diffusion, with the diffusion tensor dependent on the local cell problem concentration.

B.4 Diffusion/Advection dominate on the macro-scale

The previous case showed how to obtain a homogenized mass transport equation when diffusion plays a dominant role in the macro-scale, with the difference being that it is necessary to go to $O(\varepsilon^2)$. It is therefore now straightforward to analyse other cases where diffusion and other mechanisms also play dominant roles. When advection and diffusion dominate on the macro-scale the characteristic time is $t_c = t_L^{dif} = t_L^{adv} = \varepsilon t_L^{reac}$. This produces the scalings $P_d = O(\varepsilon^2)$, $Pe_d = O(\varepsilon)$, and $Da_d = O(\varepsilon^3)$. Hence the mass non-dimensional mass transport equation (3.80) becomes

$$\varepsilon^2 \overline{P_d} \frac{\partial c}{\partial t} + \varepsilon \overline{Pe_d} (\nabla_{\mathbf{X}} \cdot (c \mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c \mathbf{u})) = D \nabla_{\mathbf{X}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c + \varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon^3 \overline{Da_d} c \quad (\text{B.38})$$

This gives the same $O(\varepsilon^0)$ and $O(\varepsilon^1)$ equations as in the previous case and so $c^{(0)} = \bar{c}(\mathbf{x}, t)$ and $c^{(1)}$ is as defined in (B.21). At $O(\varepsilon^2)$:

$$\overline{P_d} \frac{\partial c}{\partial t} + \overline{Pe_d} \left(\nabla_{\mathbf{x}} \cdot (c^{(0)} \mathbf{u}^{(0)}) + \nabla_{\mathbf{X}} \cdot (c^{(1)} \mathbf{u}^{(0)}) + \nabla_{\mathbf{X}} \cdot (c^{(0)} \mathbf{u}^{(1)}) \right) = D \nabla_{\mathbf{X}}^2 c^{(2)} + 2 \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(1)} + D \nabla_{\mathbf{x}}^2 c^{(0)} \quad (\text{B.39})$$

with the same boundary conditions as in the previous case. There is now the addition of $c^{(1)}$ and $\mathbf{u}^{(1)}$ advection terms on the local-scale. In order to simplify the equation it

is now useful to be reminded of the incompressibility, no-slip, and Darcy's Law equations derived in Chapter 3.

$$\nabla_{\mathbf{X}} \cdot \mathbf{u}^{(0)} = 0, \quad \nabla_{\mathbf{X}} \cdot \mathbf{u}^{(1)} = -\nabla_{\mathbf{x}} \cdot \mathbf{u}^{(0)} \quad (\text{B.40})$$

$$\nabla_{\mathbf{x}} \cdot \langle \mathbf{u}^{(0)} \rangle = 0, \quad \langle \mathbf{u}^{(0)} \rangle = \mathbf{K} \nabla_{\mathbf{x}} p^{(0)} \quad (\text{B.41})$$

$$\mathbf{u}^{(0)} = \mathbf{u}^{(1)} = 0 \quad \text{on } \Gamma \text{ (no slip)} \quad (\text{B.42})$$

Expanding out the advection terms in (B.39):

$$\begin{aligned} \overline{Pe_d} (c^{(0)} \nabla_{\mathbf{x}} \cdot \mathbf{u}^{(0)} + \mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)} + c^{(1)} \nabla_{\mathbf{X}} \cdot \mathbf{u}^{(0)} + \mathbf{u}^{(0)} \cdot \nabla_{\mathbf{X}} c^{(1)} + \\ c^{(0)} \nabla_{\mathbf{X}} \cdot \mathbf{u}^{(1)} + \mathbf{u}^{(1)} \cdot \nabla_{\mathbf{X}} c^{(0)}) \end{aligned} \quad (\text{B.43})$$

Using (B.40) and the fact that $c^{(0)}$ is not a function of the local-scale, the advection terms simplify to:

$$\overline{Pe_d} (\mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)} + \mathbf{u}^{(0)} \cdot \nabla_{\mathbf{X}} c^{(1)}) \quad (\text{B.44})$$

Because the operator $\mathcal{L}_1$ and the boundary conditions are the same as for the previous case, the solvability condition is the same. Therefore

$$\int_{\Omega} \left(\overline{P_d} \frac{\partial c^{(0)}}{\partial t} + \overline{Pe_d} (\mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)} + \mathbf{u}^{(0)} \cdot \nabla_{\mathbf{X}} c^{(1)}) - \right. \\ \left. D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{X}} c^{(1)} - D \nabla_{\mathbf{x}}^2 c^{(0)} \right) dV = 0 \quad (\text{B.45})$$

The analysis of the diffusion terms are the same as the previous case and yield the same D_{dif}^* . Applying the solvability condition to the $c^{(1)}$ advection term, and using the fluid equations (B.41), the divergence theorem, and periodicity gives

$$\int_{\Omega} \mathbf{u}^{(0)} \cdot \nabla_{\mathbf{X}} c^{(1)} dV = \int_{\Omega} \nabla_{\mathbf{X}} \cdot (\mathbf{u}^{(0)} c^{(1)}) dV = \int_{\Gamma} (\mathbf{u}^{(0)} c^{(1)}) \cdot \mathbf{n} dS = 0 \quad (\text{B.46})$$

Therefore, only the first term from (B.44) is left. Applying the solvability condition, taking global terms out of the integral, and applying the incompressibility condition on the macro-scale (B.41) it is found that

$$\int_{\Omega} \mathbf{u}^{(0)} \cdot \nabla_{\mathbf{x}} c^{(0)} dV = \left(\int_{\Omega} \mathbf{u}^{(0)} dV \right) \cdot \nabla_{\mathbf{x}} c^{(0)} = \nabla_{\mathbf{x}} \cdot \left(\left(\int_{\Omega} \mathbf{u}^{(0)} dV \right) c^{(0)} \right) \quad (\text{B.47})$$

Here $\mathbf{u}^{(0)}$ is a general velocity, but in the specific case of non-leaky capillary networks the velocity is set to $\mathbf{u}_c$ and the integral is only over the capillary domain Ω_c . Putting these terms together, dividing through by $|\Omega|$, and re-dimensionalizing, the full homogenized equation is obtained for diffusion and advection dominated flow on the macro-scale to leading order

$$\frac{\partial c'}{\partial t'} + \nabla_{\mathbf{x}} \cdot (\langle \mathbf{u}'_c \rangle_c c') - \nabla_{\mathbf{x}} \cdot (\underline{\underline{D'_{dif}}} \nabla_{\mathbf{x}} c') = O(\varepsilon) \quad (\text{B.48})$$

B.5 Diffusion/Metabolism dominate on the macro-scale

In this case the metabolism term takes the place of the advection term and the characteristic time is $t_c = t_L^{dif} = t_L^{reac} = \varepsilon t_L^{adv}$. The appropriate scalings are thus $P_d = O(\varepsilon^2)$, $Pe_d = O(\varepsilon^2)$, and $Da_d = O(\varepsilon^2)$. Hence (3.80) becomes

$$\varepsilon^2 \overline{P_d} \frac{\partial c}{\partial t} + \varepsilon^2 \overline{Pe_d} (\nabla_{\mathbf{x}} \cdot (c \mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c \mathbf{u})) = D \nabla_{\mathbf{x}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c + \varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon^2 \overline{Da_d} c \quad (\text{B.49})$$

Again, the analysis of the first two orders of ε are the same as in the previous two cases. At $O(\varepsilon^2)$:

$$\overline{P_d} \frac{\partial c^{(0)}}{\partial t} + \overline{Pe_d} \{ \nabla_{\mathbf{x}} \cdot (c^{(0)} \mathbf{u}^{(0)}) \} = D \nabla_{\mathbf{x}}^2 c^{(2)} + 2D \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c^{(1)} + D \nabla_{\mathbf{x}}^2 c^{(0)} - \overline{Da_d} c^{(0)} \quad (\text{B.50})$$

The advection term drops out and so the only new term is the reaction term. As the solvability condition is the same as previously the diffusion terms are homogenized as in Section B.3, so all that is left to homogenize is the reaction term. Applying the solvability condition

$$\int_{\Omega} \overline{Da_d} c^{(0)} dV = \overline{Da_d} c^{(0)} \int_{\Omega} dV = \overline{Da_d} c^{(0)} |\Omega_t| \quad (\text{B.51})$$

So, putting the terms together, dividing through by $|\Omega|$, and re-dimensionalizing, the full homogenized equation for diffusion/metabolism dominated flow at the macro-scale is obtained to leading order

$$\frac{\partial c'}{\partial t'} - \nabla_{\mathbf{x}} \cdot \left(\underline{\underline{D'_{dif}}} \nabla_{\mathbf{x}} c' \right) = -\lambda \phi_t c' + O(\varepsilon) \quad (\text{B.52})$$

B.6 All three mechanisms dominate on the macro-scale

Finally, when all three mechanisms balance on the macro-scale the characteristic time is $t_c = t_L^{dif} = t_L^{reac} = t_L^{adv}$. Therefore, (P_L, Pe_L, Da_L) are all $O(1)$ and hence $P_d = O(\varepsilon^2)$, $Pe_d = O(\varepsilon)$, and $Da_d = O(\varepsilon^2)$. Applying these scalings to (3.80)

$$\begin{aligned} \varepsilon^2 \overline{P_d} \frac{\partial c}{\partial t} + \varepsilon \overline{Pe_d} (\nabla_{\mathbf{x}} \cdot (c \mathbf{u}) + \varepsilon \nabla_{\mathbf{x}} \cdot (c \mathbf{u})) &= D \nabla_{\mathbf{x}}^2 c + 2\varepsilon \nabla_{\mathbf{x}} \cdot \nabla_{\mathbf{x}} c + \\ &\quad \varepsilon^2 D \nabla_{\mathbf{x}}^2 c - \varepsilon^2 \overline{Da_d} c \end{aligned} \quad (\text{B.53})$$

The analysis of this case follows the previous 3 cases, with the result being a linear combination of the homogenized advection, metabolism, and diffusion terms.

$$\frac{\partial c'}{\partial t'} + \nabla_{\mathbf{x}} \cdot (\langle \mathbf{u}'_c \rangle_c c') - \nabla_{\mathbf{x}} \cdot \left(\underline{\underline{D'_{dif}}} \nabla_{\mathbf{x}} c' \right) = -\lambda \phi_t c' + O(\varepsilon) \quad (\text{B.54})$$

Appendix C

Data Sampled in the Statistical Penetrating Vessel Algorithm (Chapter 4)

This appendix contains a collection of the data used to generate the statistical penetrating vessels in Chapter 4. The data is freely available in the literature but has been collected here as a convenient reference.

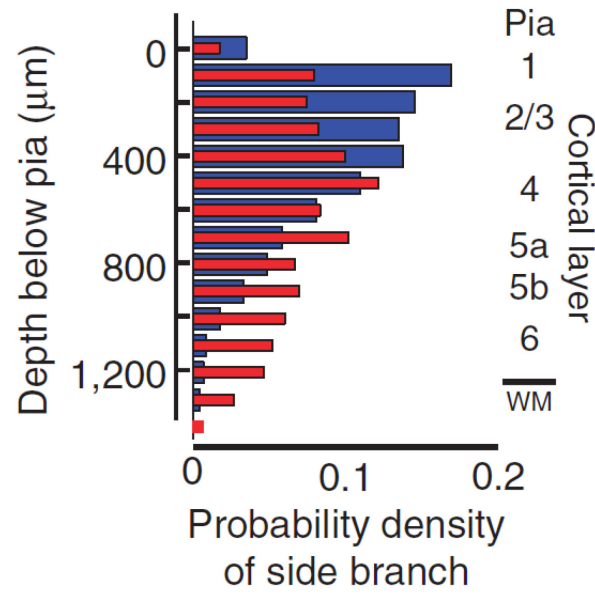


Fig. C.1 The distribution of bifurcations along the main trunk of the penetrating arterioles and venules. This probability distribution is used to initially seed the penetrating vessel with nodes for side branches. Reproduced with permission from [20].

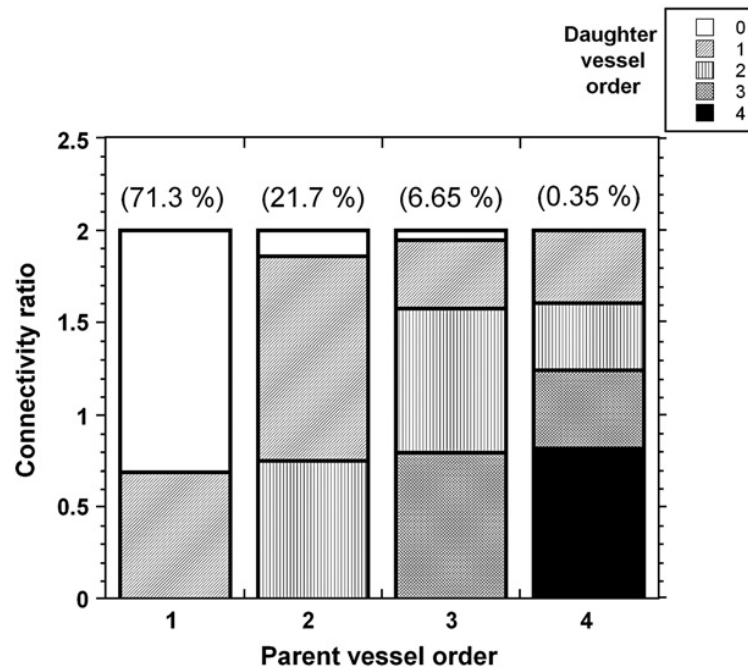


Fig. C.2 Morphological connectivity distributions. Based on the parent vessel order, the order of the two daughter vessels is determined by sampling twice from the relevant column. Reproduced with permission from [27].

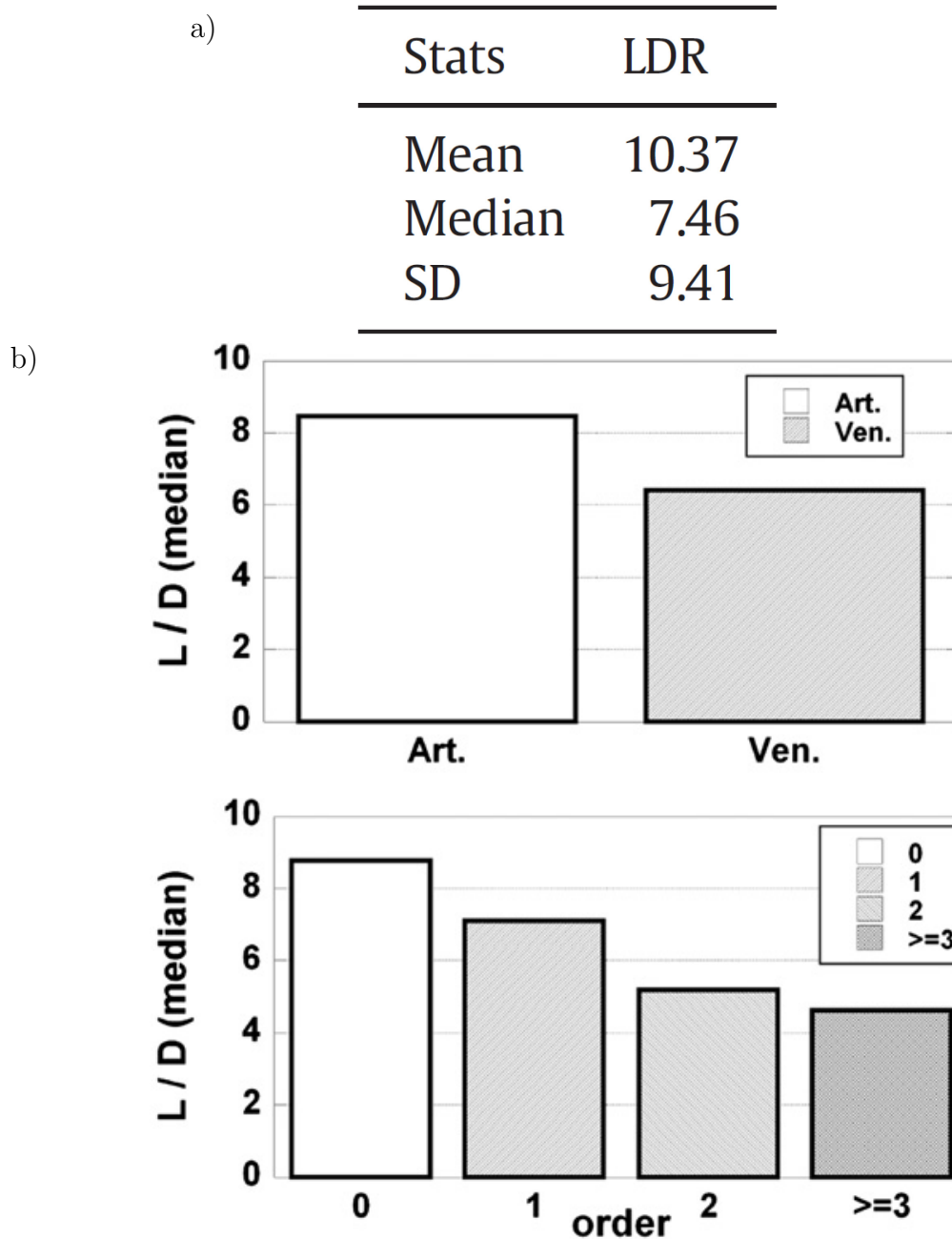


Fig. C.3 a) The mean, median, and standard deviation of the length-to-diameter ratio (LDR) of all the vascular trees analysed in [112]. b) The median LDR split by type of vessel or by order of vessel. As no standard deviations are given by order, a standard deviation was imposed for each order that resulted in a matched LDR median and matched total network properties (mean, median, SD) as in a). Adapted with permission from [112].

		A1/A0			
Group	Size	Mean	Median	SD	IQR
Arterioles	5431	1.0039	0.9198	0.4772	0.7032 1.1864
Veinules	3983	0.9560	0.8864	0.4339	0.6923 1.1144
Order 1	6710	0.9579	0.8953	0.3950	0.6991 1.1295
Order 2	2043	0.9984	0.8952	0.5026	0.6716 1.1895
Order 3	627	1.2049	1.0228	0.7871	0.7906 1.4014
Order 4	34	1.0794	1.0000	0.3884	0.8683 1.1663
ALB	5084	1.0247	0.9303	0.5073	0.7148 1.1959
HSB	3524	0.8954	0.8430	0.3513	0.6555 1.0740
IEB	806	1.1101	0.9890	0.4976	0.8049 1.2613

		A2/A0					A2/A1				
Group	Size	Mean	Median	SD	IQR	P	Mean	Median	SD	IQR	P
Arterioles	5431	0.6564	0.6217	0.3042	0.4508 0.8128	}<.0001	0.6876	0.7201	0.2099	0.5444 0.8604	}<.879
Veinules	3983	0.6227	0.5906	0.2787	0.4320 0.7774		0.6841	0.7243	0.2176	0.5385 0.8657	
Order 1	6710	0.6799	0.6526	0.2624	0.4988 0.8320	}<.0001	0.7356	0.7645	0.1805	0.6162 0.8827	}<.0001
Order 2	2043	0.5382	0.4822	0.2923	0.3436 0.6583		0.5796	0.5742	0.2305	0.3917 0.7673	
Order 3	627	0.5893	0.4902	0.4734	0.3230 0.7039	}<.0711	0.5187	0.5095	0.2482	0.3079 0.7096	}<.0001
Order 4	34	0.4161	0.3815	0.2714	0.1640 0.5424		0.4053	0.3663	0.2556	0.1869 0.5583	
ALB	5084	0.6099	0.5676	0.3145	0.3997 0.7583	}<.0001	0.6303	0.6493	0.2266	0.4563 0.8201	}<.0001
HSB	3524	0.6620	0.6364	0.2581	0.4760 0.8206		0.7586	0.7887	0.1726	0.6530 0.8983	
IEB	806	0.7600	0.7194	0.2694	0.5703 0.9058	}<.0001	0.7226	0.7448	0.1796	0.5999 0.8717	}<.0001

Fig. C.4 Main statistics of the branching area-ratios. Based on the order of the parent vessel, the appropriate distribution of the area-ratios could be sampled from. The area-ratio distributions were all log-normal. Adapted with permission from [27].

		$\alpha 01 (^{\circ})$					$\alpha 02 (^{\circ})$				
Group	Size	Mean	Median	CSD	IQR	P	Mean	Median	CSD	IQR	P
Arterioles	5431	125.81	127.89	28.12	107.56 146.11	}<.0001	111.64	112.19	26.97	93.66 130.12	}<.0001
Veinules	3983	128.73	131.09	26.01	113.6 145.93		118.22	120.03	24.06	104.22 133.9	
Order 1	6710	123.42	125.7	26.11	107.35 141.29	}<.0001	114.45	115.65	24.92	98.55 131.38	}<.0001
Order 2	2043	134.32	138.11	27.79	117.76 153.49		116.83	116.34	10.63	97.65 132.9	
Order 3	627	141.83	146.51	28.04	128.54 160.54	}<.0001	113.31	113.99	31.34	93.9 135.41	}<.0009
Order 4	34	149.00	152.595	22.06	137.94 165.42		110.92	109.92	30.75	85.85 136.66	
ALB	5084	129.64	132.42	27.92	112.33 149.38	}<.0001	113.98	114.88	26.58	97.08 131.89	}<.0023
HSB	3524	123.93	126.48	26.32	107.48 141.95		114.70	116.17	25.27	98.86 131.71	
IEB	806	124.66	127.0	25.63	110.13 141.09	}<.0036	115.04	119.2	17.29	99.76 133.23	}<.016

		$\alpha 12 (^{\circ})$					Asym ($^{\circ}$)				
Group	Size	Mean	Median	CSD	IQR	P	Mean	Median	CSD	IQR	P
Arterioles	5431	107.42	109.53	28.81	89.49 127	}<.0001	14.44	14.89	44.56	-15.36 44.53	}<.0001
Veinules	3983	98.97	97.95	24.49	84.28 113.6		10.43	10.11	39.98	-15.08 37.29	
Order 1	6710	107.09	107.49	26.26	90.93 124.41	}<.0001	8.91	9.05	41.22	-17.92 36.74	}<.0001
Order 2	2043	97.56	96.62	27.83	79.79 115.84		20.04	21.48	43.82	-9.74 48.58	
Order 3	627	88.90	87.53	28.78	71.39 107.01	}<.0001	29.93	31.99	46.42	-1.86 61.42	}<.0001
Order 4	34	82.55	82.36	23.99	64.46 98.58		41.55	46.10	45.90	9.15 72.43	
ALB	5084	101.86	101.88	27.68	84.95 120.79	}<.0001	15.93	15.72	43.91	-13.09 45.48	}<.0001
HSB	3524	105.75	106.45	27.23	88.89 123.89		9.17	9.05	40.96	-17.95 36.73	
IEB	806	107.11	106.71	24.97	90.52 123.74	}<.0054	8.33	8.37	40.57	-16.76 33.60	}<.0057

Fig. C.5 Main statistics of the bifurcation angles. Based on the order of the parent vessel, the appropriate distribution of the bifurcation angles could be sampled from. The bifurcation angle distributions were all normally distributed. Adapted with permission from [27].

Appendix D

Penetrating Vessel Characteristics and their Relationship with CBF Drops Post-Occlusion (Chapter 5)

This appendix contains a collection of figures analysing the trends in penetrating arteriole characteristics and their effects (if any) on CBF drops post-occlusion. This is done by plotting the CBF drop against: radius of tree occluded, total tree volume, total tree length, number of terminal vessels, total number of vessels in tree, and the distance of the occluded arteriole from the centre of the overall cuboid (2 x 2 x 2.5 mm) in order to analyse boundary effects on the flow solution.

D.1 Case 1 Vessels: 20 μm arterioles, 50 μm venules

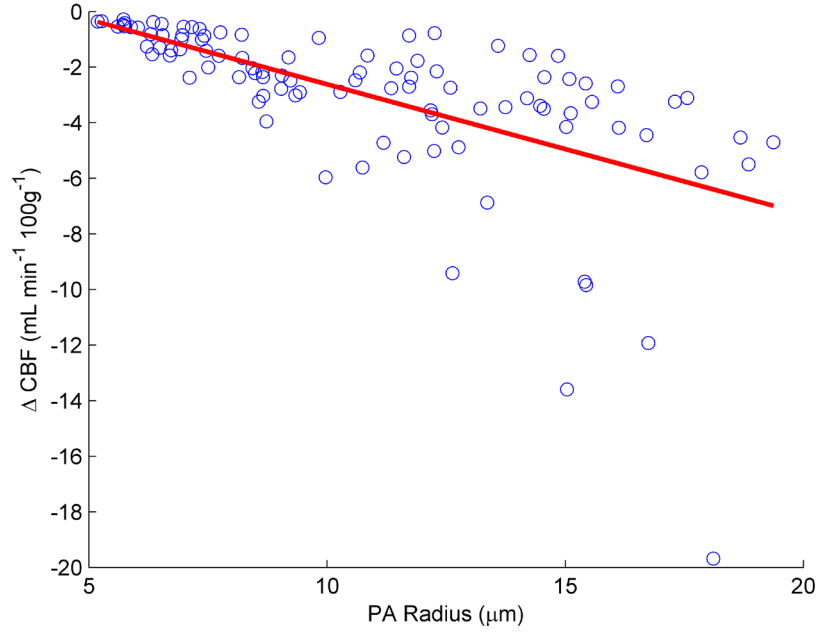


Fig. D.1 Radius of the occluded penetrating arteriole vs. drop in CBF for that simulation. Normalized gradient = -0.459

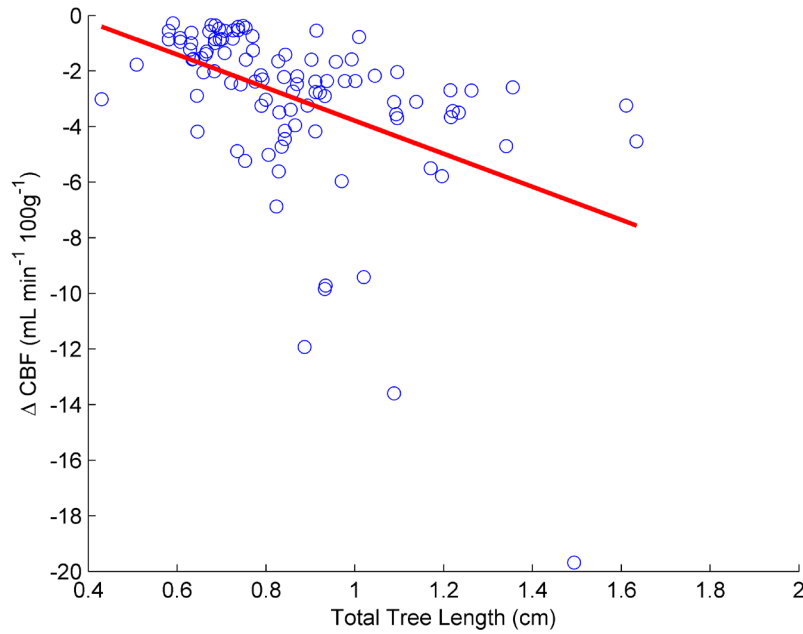


Fig. D.2 Total length of the occluded tree vs. drop in CBF. Normalized gradient = -0.49

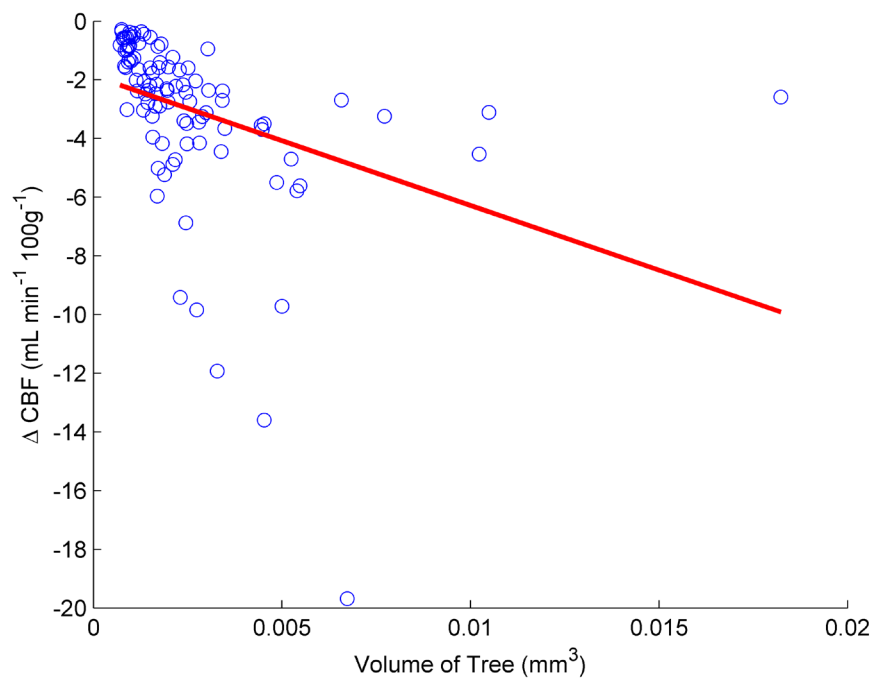


Fig. D.3 Volume of occluded tree vs. drop in CBF. Normalized gradient = -0.4088

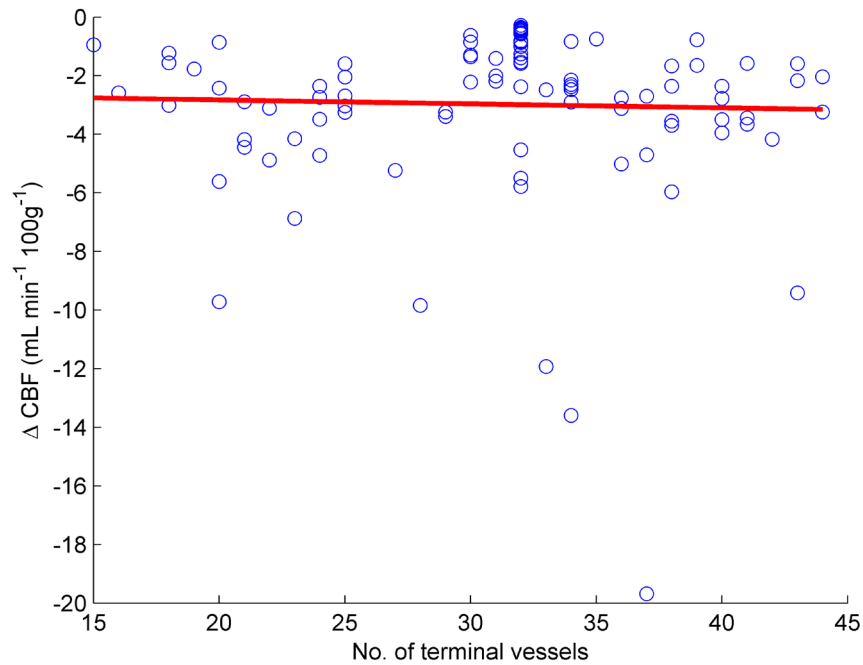


Fig. D.4 Number of terminal vessels per tree vs. drop in CBF. Normalized gradient = -0.030

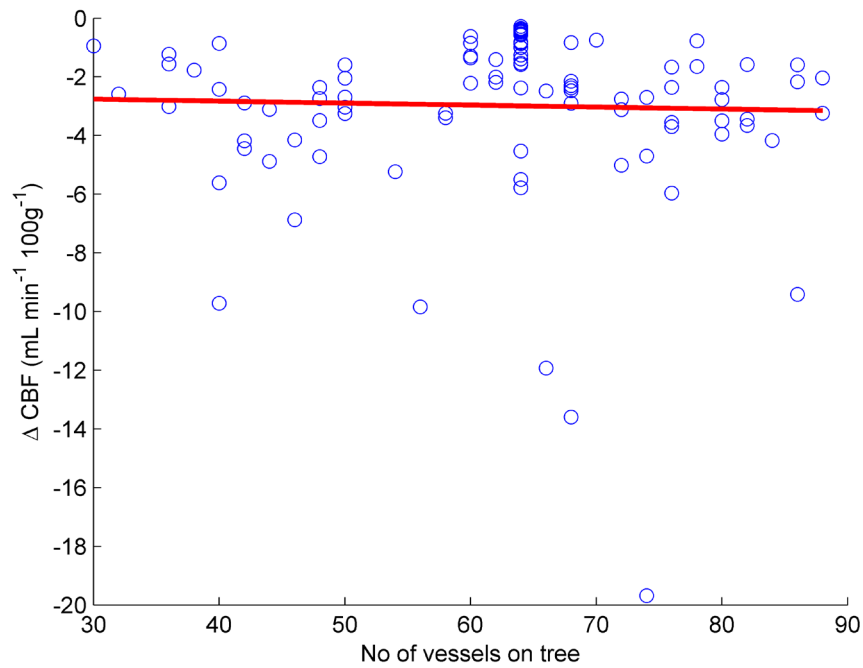


Fig. D.5 Total number of vessels on tree vs. drop in CBF. Normalized gradient = - 0.030

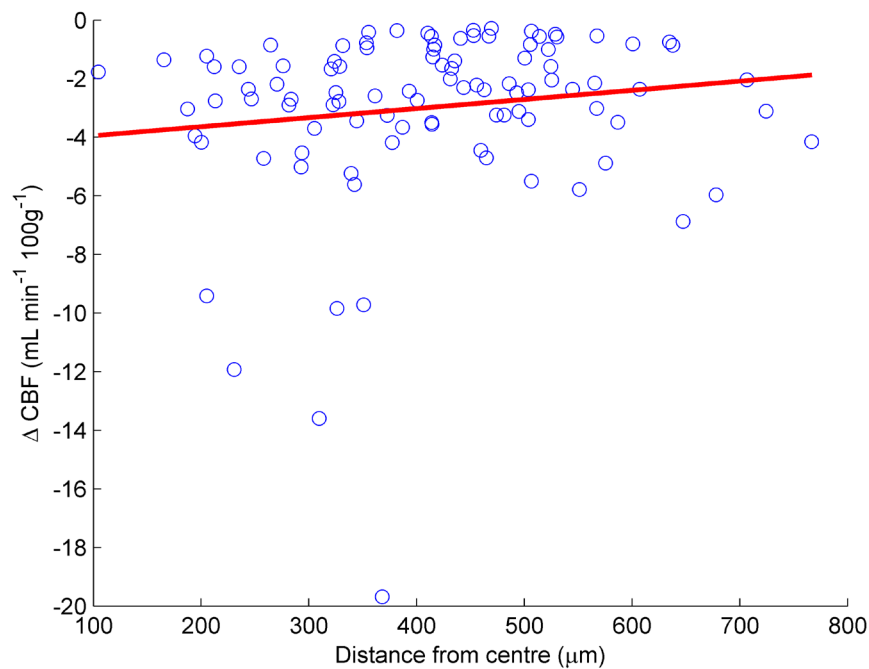


Fig. D.6 Occluded penetrating arteriole distance from the centre of the overall simulated cuboid vs. drop in CBF. Normalized gradient = 0.1210

D.2 Case 2 Vessels: 40 μm arterioles, 110 μm venules

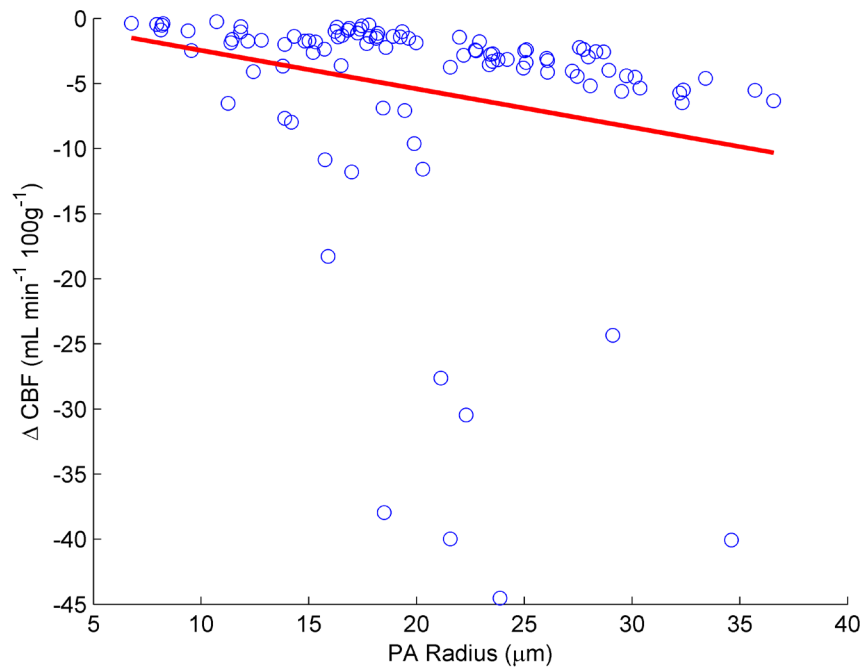


Fig. D.7 Radius of the occluded penetrating arteriole vs. drop in CBF for that simulation. Normalized gradient = -0.2426

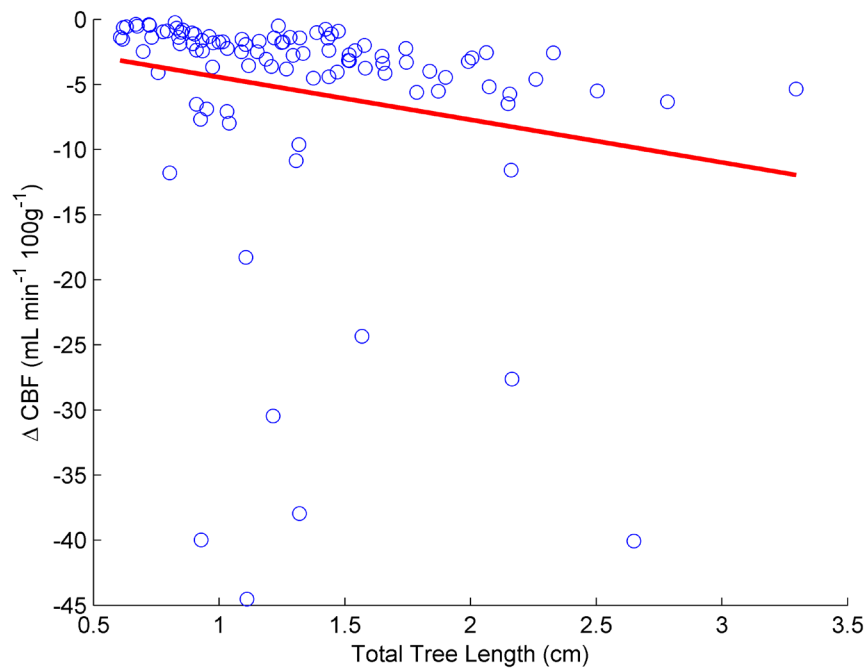


Fig. D.8 Total length of the occluded tree vs. drop in CBF. Normalized gradient = -0.2419

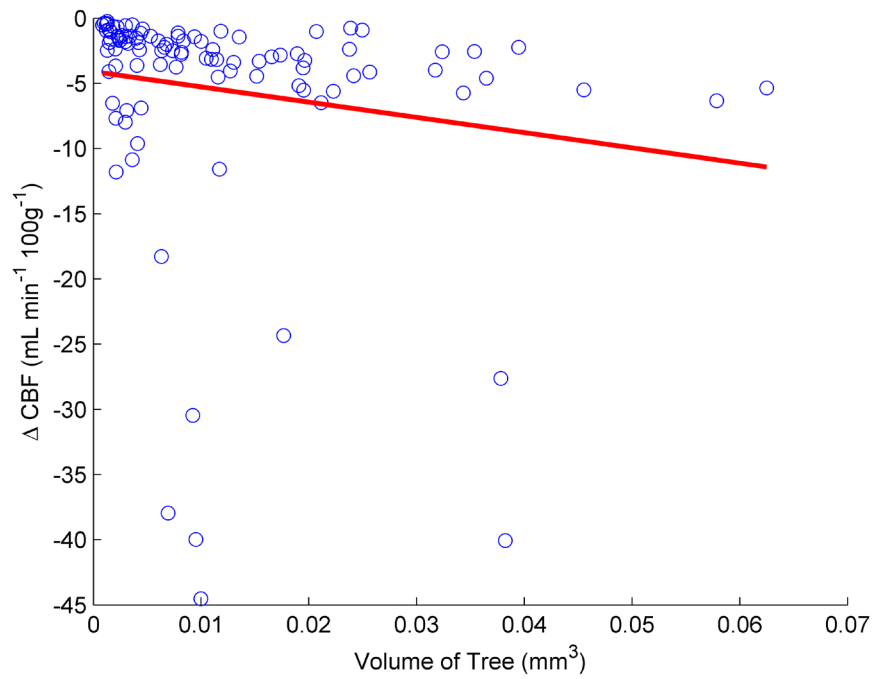


Fig. D.9 Volume of occluded tree vs. drop in CBF. Normalized gradient = -0.1641

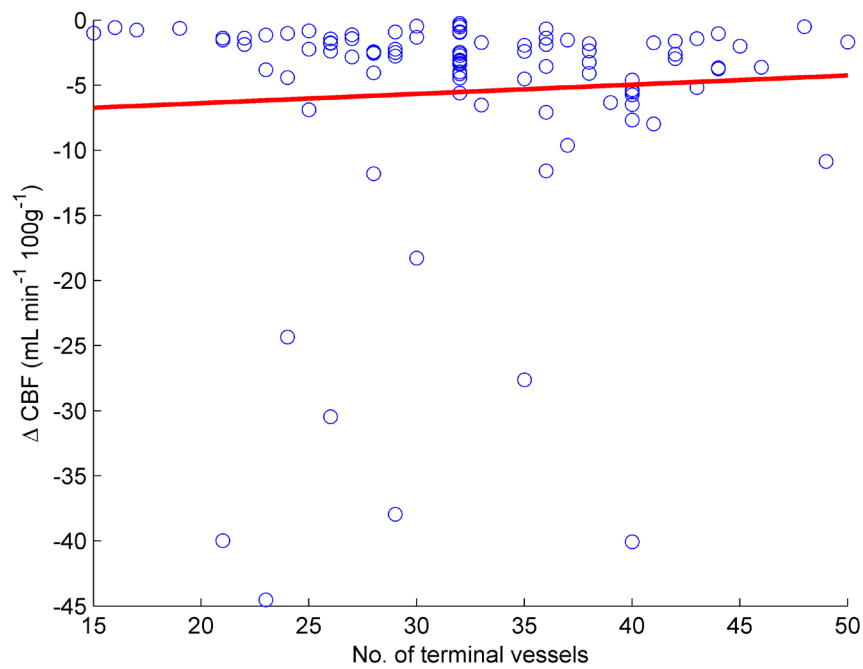


Fig. D.10 Number of terminal vessels per tree vs. drop in CBF. Normalized gradient = 0.0797

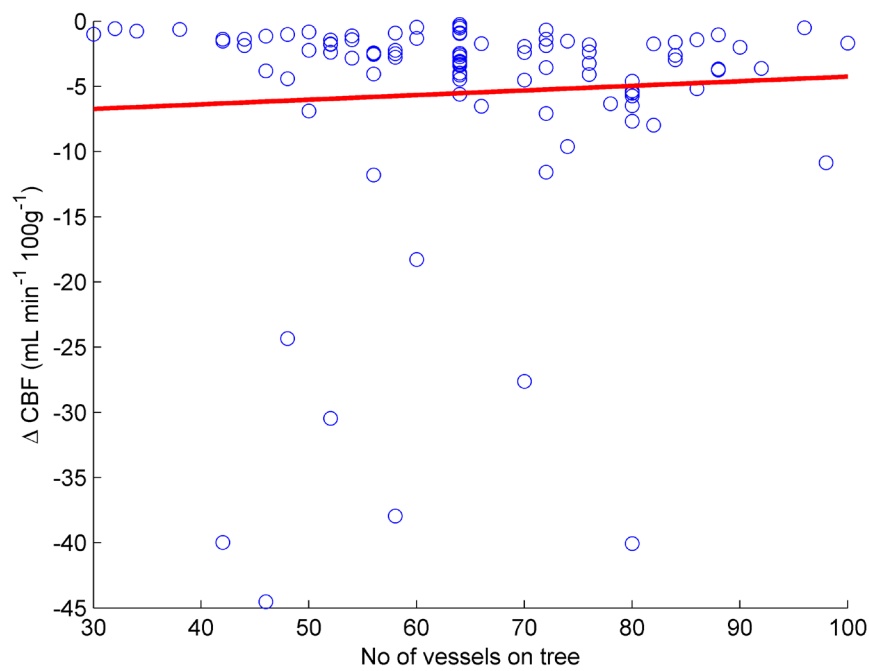


Fig. D.11 Total number of vessels on tree vs. drop in CBF. Normalized gradient = 0.0797

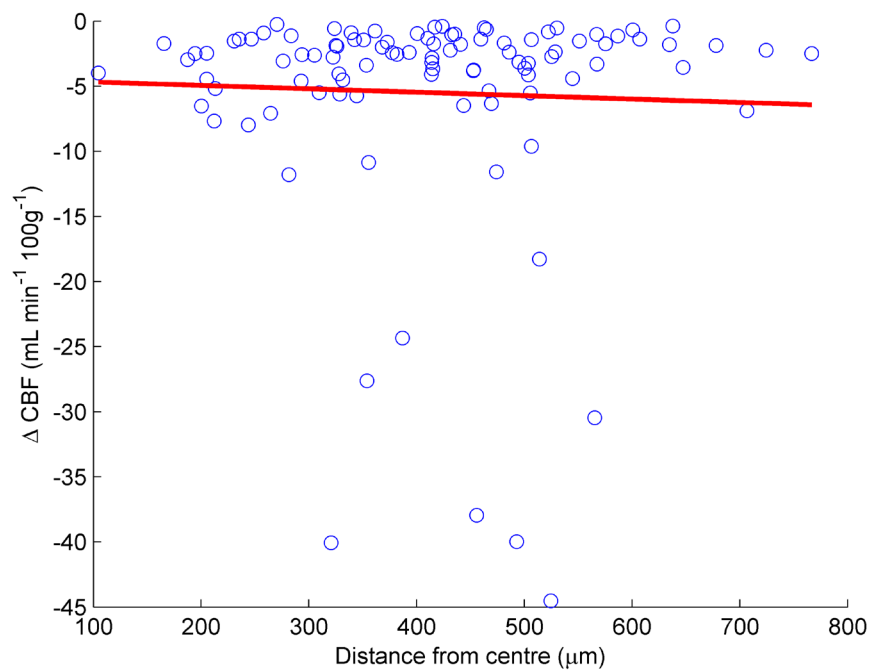


Fig. D.12 Occluded penetrating arteriole distance from the centre of the overall simulated cuboid vs. drop in CBF. Normalized gradient = -0.0453

Glossary

AD – Alzheimer’s disease

BBB – Blood brain barrier

BOLD – Blood oxygen level dependent

CBF – Cerebral blood flow

CBFV – Cerebral blood flow velocity

CCO – Constrained constructive optimization

CMRO₂ – Cerebral metabolic rate of oxygen

CNS – Central nervous system

CSF – Cerebrospinal fluid

ESL – Endothelial surface layer

fMRI – Functional magnetic resonance imaging

ICA – Internal carotid artery

ICP – Intracranial pressure

IQR – Inter-quartile range

LDR – Length-to-diameter ratio

MAP – Mean arterial pressure

MPM – Multi-photon laser scanning microscopy

MRI – Magnetic resonance imaging

OCT – Optical coherence tomography

ODE – Ordinary differential equation

OEF – Oxygen extraction fraction

PAI – Photoacoustic imaging

PDE – Partial differential equation

RBC – Red blood cell

REV – Representative elementary volume

TCD – Transcranial Doppler

TIA – Transient ischaemic attack

TPM – Two-photon microscopy

TTD – Transit time distribution

VA – Vertebral artery

VaD – Vascular dementia

WBC – White blood cell

Haematocrit – Volume percentage of red blood cells in blood

Haemorrhage – A bleed within or on the surface of the brain from a ruptured blood vessel

Infarction – A region of tissue that has died due to prolonged shortage of oxygen

Interstitium – The porous medium in which the capillaries are embedded i.e. brain parenchyma

Ischaemia – A reduction of blood supply to tissue causing a shortage of oxygen

Occlusion – Blockage of a blood vessel

Scalability – The ability to model large regions of blood flow without the associated larger increases in computational complexity and time

Secular terms – Mathematical artefacts introduced through the implementation of the multi-scale homogenization method

Statistically accurate – Matches the measured properties of microvascular networks statistically e.g. matching the diameter and length distributions of the networks

Voxel-sized – A model of the size of a clinical MRI voxel i.e. order of mm