Scalable Multi-Parametric Imaging of Excitable Tissue

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Abstract

The field of cardiac electrophysiological imaging has advanced tremendously in the past three decades with developments in fluorescent dyes, photodetectors, optical filters, illumination sources, computers and electronics. This thesis describes several scalable multi-parametric imaging systems and their application to cardiac tissue preparations at various levels of complexity. Using off-the-shelf components, single-camera multi-parametric optical mapping systems are described for various fluorescent dye combinations and single-element photodiode-based fibre-optic detection systems are described for drug-testing applications. The instruments described take advantage of modern voltage-sensitive dyes, multi-band optical filters and powerful light-emitting-diodes, from the ultraviolet to the red.

The two electrophysiological parameters focused on were transmembrane voltage and the intracellular calcium concentration. Several voltage and calcium dye combinations were established, which produce no signal cross-talk. Furthermore, second- and third-generation voltage dyes were characterized in cardiac tissue, *in vitro* and *in vivo*.

The developed systems were then applied to isolated Langendorff-perfused whole-hearts, *in vivo* whole-hearts, thin ventricular tissue-slices and human induced pluripotent stem cell-derived cardiac tissue. The interventions applied include accurately-timed electrical and mechanical local stimulation of the whole-heart to generate ectopic beats, cardiotoxic drugs and flash-photolysis of caged-compounds.

With the high-throughput demands of drug discovery and testing, further development of scalable optical electrophysiological systems may prove critical in reducing attrition and costs. And for *in vivo* optical mapping, development of minimally-invasive and clinically-relevant optical systems will be essential in validating existing theories based on *in vitro* experiments and exploring cardiac function and behaviour with the heart intact in the organism.

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Abbreviations

AP	Action Potential
APD	Action Potential Duration
$[Ca^{2+}]_i$	Intracellular Calcium Concentration
CaT	Calcium Transient
CICR	Calcium Induced Calcium Release
CMOS	Complementary Metal-Oxide Semiconductor
DMSO	Dimethyl Sulfoxide
EAM	Electroanatomic Mapping
EMCCD	Electron-Multiplied Charge-Coupled Device
iPSC	Induced Pluripotent Stem Cell
iPSC-CM	Induced Pluripotent Stem Cell-Derived Cardiomyocyte
LAD	Left Anterior Descending Coronary Artery
LED	Light Emitting Diode
MEC	Mechano-Electric Coupling
MEF	Mechano-Electric Feedback
NADH	Nicotinamide Adenine Dinucleotide – Hydrogen (reduced)
NP-EGTA	*O*-Nitrophenyl Ethylene-Glycol-Tetraacetic-Acid
PIN photodiode	P-type, Intrinsic, N-type photodiode with a lightly-doped near-intrinsic semiconductor region between the p-type and n-type semiconductor regions
PDA	Photodiode Array
SAN	Sinoatrial Node
V_m	Membrane Voltage

"Authority in science exists to be questioned, since heresy is the spring from which new ideas flow."

John C. Polanyi

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*** Tentative article title and author list**

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Contents

1 Introduction

The goal of the research reported in this thesis was to advance the field of cardiac electrophysiological imaging using fluorescence methods. Various attempts are described to leverage recent advances in fluorescent dyes, photodetectors, optical filters, illumination sources, computers and electronics. First, the relevant background that was needed before embarking on these endeavours is covered. Key topics include cardiac tissue structure and electrophysiology, norm and patho-physiological properties, modern photodetectors and light-emitting-diodes (LEDs), basic mathematical characterizations of multi-cellular cardiac electrical activity, optical mapping of single-cells, monolayers and whole-hearts. Although the field of cardiac imaging has made tremendous strides over the past three decades with the introduction of voltage-sensitive dyes, there are still many areas where advances need to be made. In particular, applying developments in firstly, the optics and electronics field and secondly, the medical device industry, will enable high-throughput and *in vivo* measurements to be made.

For a majority of the experiments, the simple, but effective, Langendorff-perfused isolated whole-heart preparation was used. As the bulk of the described research focused on developing and validating various methodologies, rats were mainly used because of their low cost. Ventricular tissue slices were then extensively explored as a more physiologically-relevant 2D model compared to the more popular neonatal rat ventricular myocyte (NRVM) monolayers. In collaboration with the University of Michigan and the University of Wisconsin, monolayers formed from human induced-pluripotent-stem-cell-derived cardiomyocytes (iPS-CM) were explored as an exciting new 2D model that can be electrically and mechanically stimulated and imaged using similar approaches developed for the Langendorff-perfused heart. In collaboration with Stanford University, *in vivo* multi-parametric imaging through blood-perfused tissue was explored to make the transition to studying cardiac electrophysiology with the heart still intact in the animal. Various voltage- and calcium-sensitive dye combinations were explored to establish negligible crosstalk and novel dye-combinations for multi-parametric imaging. Local electrical and mechanical stimulation, drugs and caged-compounds were used to perturb electrophysiological parameters.

Individual projects (culminating in manuscripts) are then discussed:

Project 1

Ratiometric imaging of voltage and calcium transients is a method that was developed to eliminate/minimize the effects of uneven dye loading, photobleaching and motion artifacts. Ratiometric approaches also permit quantification of transient amplitudes. This project describes a system for ratiometric imaging of voltage and calcium, using a single camera system and multi-band emission filters. For locally perturbing the heart, ECG-coordinated local electrical and mechanical stimulators were developed and applied while imaging. It was found that downstream consequences of local electrical and mechanical stimulation are indistinguishable.

Project 2

With the recent commercial availability of human iPS-CMs, patient specific studies are now possible. In this study, the formation of large confluent monolayers was explored. Monolayers were formed on silicone elastomer membranes firstly, to enable future studies on stretching and secondly, because past studies have shown differences in cell development trajectories between rigid and "soft" environments. It was found that monolayers formed on silicone membranes have higher electrical conduction speeds than those formed on rigid plastic petri dishes. A multi-parametric imaging system using a popular voltage dye and low-affinity ratiometric calcium dye was developed and applied to imaging these monolayers. The same system was also applied to NRVM monolayers, rat ventricular tissue-slices and rat whole-hearts, demonstrating the system's versatility.

Project 3

With the advent of near-infrared 2nd-generation voltage sensitive dyes, cardiac researchers have demonstrated high signal quality in blood-perfused isolated heart preparations. Imaging of red calcium dyes has also been done in blood-perfused isolated hearts. Taking advantage of the isosbestic point of one of these 2nd-generation voltage dyes, a simultaneous voltage and calcium imaging system for blood-perfused tissue was developed and applied to an *in vivo* rat heart model. Use of such voltage dyes for angiography and contrasting infarct versus non-infarct tissue was also established.

Project 4

The photodetectors used in cardiac imaging are typically very expensive, making high-throughput measurements impractical. The goal of this study was to develop systems based on low-cost scientific cameras, high-speed consumer digital cameras, optical-fibres and single-element photodiodes for multi-parametric imaging/detection in whole-hearts and tissue-slices. Drugs were then applied to demonstrate the sufficient signal quality yielded by such inexpensive systems for high-throughput drug testing applications.

Project 5

Taking advantage of the isosbestic point of two near-infrared 2nd-generation voltage sensitive dyes, two different voltage- and calcium-dye imaging systems were developed. A proof-of-principle three-parameter (voltage, calcium and NADH) imaging system was also developed using a single camera and triple-band emission filter. Using mirrors, a voltage and calcium panoramic imaging system for the whole heart was also developed. And as an optical perturbation tool, flash-photolysis of a caged-compound was demonstrated using ultraviolet LEDs.

Conclusions and a discussion of future work complete the thesis. References and appendices containing software and electronic circuit schematics can be found in appendices.