

Dear Editor,

The bold claims to provide 'novel identification of the capillary-to-fiber interface' and being 'the first method capable of deciphering the complex fiber-type specific capillary network' [1] are erroneous, justified by suggesting other software is unable to identify the fiber-type specific capillary network in an automated manner. This is demonstrably incorrect for integer-based [2], as well as area-based fibre type-specific analysis of local capillarity [3]. Such a disingenuous representation of resources available also devalues a program recently published in this journal [4].

Other programs may not calculate precisely the same indices of capillarity being promoted, but such parameters can easily be derived if required from the outputs of e.g. OTM [4]. We chose not to implement indices such as CC and SF [5] as they are relatively insensitive to physiologically relevant stimuli [6]. Discontinuity of integer-based indices inevitably provide lower analytical resolution than non-integer values [7]. As such, area-based indices of local capillary supply have superior descriptive power [8].

Muscle2View [1] includes objects touching the image border, violating the unbiased counting rule essential for robust data analysis [9]. Detection errors inherent with thresholding limits automation, while human pattern recognition and integration of contextual information may avoid ambiguity: Muscle2View discarded some fibres due to poor circularity, but the effect was unquantified. Intrinsic variability of fibre type or capillary staining is recognised to be likely beyond the scope of thresholding algorithms for error-free implementation. For example, it is well known that a range of endothelial cell makers provide different estimates of capillarity [10]. It is no surprise that unsupervised analysis yields up to a 15% difference from manual counting. While possibly acceptable for studies of gross capillary supply, analysis of local capillary supply (which the study aims to facilitate) is more sensitive to erroneous data in calculating e.g. tissue oxygenation [11].

A choice of methods is to be applauded, and we do not underestimate the effort to generate this alternative (development and validation of OTM took over a decade). While Muscle2View may prove useful in some circumstances and has much to commend it, e.g. application of multiple detection routines to reduce detection error, in a well-researched field it is important to avoid unwarranted claims to novelty and unnecessary to overstate potential utility.

Yours sincerely,

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