3D visualisation and analysis of capillaries in human muscles using confocal microscopy

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Capillaries supply muscle tissue with oxygen and nutrients and remove waste metabolic substances. Different physiological, pathological and experimental conditions trigger remodelling of the capillary network. In this study we present the methodology that can be applied for 3D visualisation and measurement of the capillary network.

Methods:

- Triple immunofluorescent staining, confocal microscopy: Human skeletal muscles samples were taken at biopsy or autopsy. After freezing in liquid nitrogen 100 μm thick transverse sections were fixed in 10% formalin. Capillaries and muscle fibre outlines were stained by a triple immunofluorescent method, applying antibodies against CD-31 visualized in green and laminin visualized in red, followed by the fluorescein labelled Ulex europaeus lectin (UEA I). Stacks of images from 100 μm thick tissue sections were captured with a Zeiss LSM 510 confocal microscope with a 40x Plan-Neofluar oil objective (NA 1.3). Green and red fluorescence were excited with an argon (488 nm) and He/Ne (543 nm) lasers. Emission signal was filtered using narrow band (505-530 nm) and a LP 560 nm filter. Stacks of successive images were captured 0.43 μm apart with x-y dimensions of 318.2 μm x 318.2 μm.
- Correction for tissue shrinkage: Apparent thickness of the 3D image of muscle sections was estimated by the full width at half maximum (FWHM) value of average intensity of images in the stack and linear correction in axial direction was applied to correct for the tissue shrinkage during the sample preparation.
- Manual tracing of capillaries: The capillaries were traced manually on stereoscopic volume rendered image with 3D cursor controlled by a haptic device (SensableOmni) with force proportional to the gradient of the image intensity, to obtain representation of capillaries as chains of line segments. The haptic feedback serves as a cue for depth perception and enables better placement of cursor in the capillaries. The stereoscopic rendering is accomplished either by a special graphic card with CRT monitor and LCD shutter glasses or by anaglyph visualization and color glasses. The haptic method of tracing with stereovision is faster and more convenient than tracing the capillaries using ordinary mouse and 2D display of three orthogonal sections through the image stack, which on the other hand is more sensitive.
- Automatic detection of capillaries: Green channel of the 3D image was preprocessed by Gaussian smoothing and background removal by subtracting Lipschitz lower envelope. The thresholded image was processeded by Palagyi 6 pass algorithm to obtain line skeleton. The lines in binary image were automatically traced and converted to chains of line segments.
- Capillary length measurement: The lengths of individual capillaries were estimated by summing the lengths of the linear segments in chains representing the capillaries. The

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total length of capillaries in the image was obtained by summing the lengths of individual capillaries.

- Tortuosity: Sum of angles between successive line segments in the chains representing capillaries was calculated. The tortuosity was calculated by dividing the sum of angles by the total length.
- Orientation of capillaries within the sample: Length weighted mean structural tensor of line segments orientations was calculated. Anisotropy was characterized by the ratio of the biggest eigenvalue to the geometric mean of the other two eigenvalues.
- Connectivity: The number of capillary branchings was counted manually using 3D visualization of capillaries.
- 3D visualisation: The surfaces of muscle fibres were modelled by triangulated surfaces spanning the outlines of the fibers extremal crossections. The capillaries and fibre surfaces were rendered using OpenGL.

Results:

Table 1: Manual vs automatic measurement of capillary length [μm], tortuosity [rad μm^{-1}], anisotropy and connectivity in deep and superficial layer in one autopsy sample of human vastus lateralis muscle.

	manual				automatic		
muscle	length	tortuosity	anisotropy	connectivity	length	tortuosity	anisotropy
deep	3910	0.029	2.08	11	4060	0.080	1.89
superficial	4190	0.055	1.56	9	4420	0.092	1.65

The tortuosity was higher in sample from superficial layer, containing meander-like capillaries. Automatic measurement yielded reliable results of length and anisotropy only.

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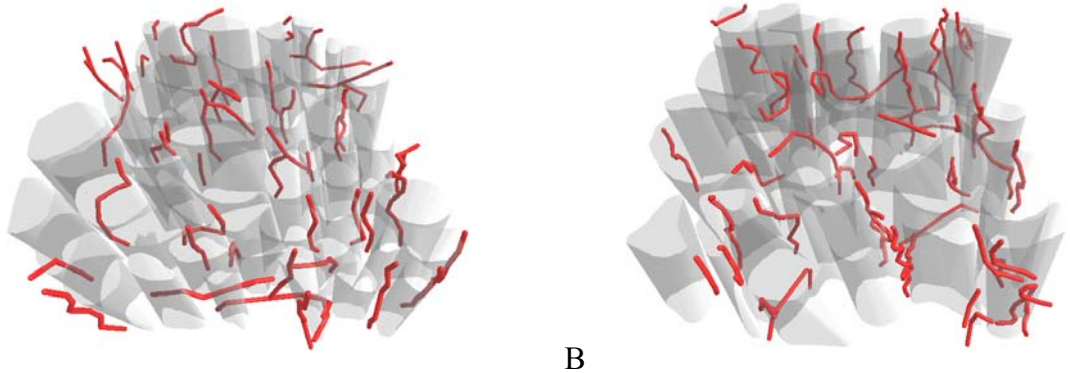

A B

Figure 1. 3D visualisation of capillaries and muscle fibres in deep (A) and superficial (B) human vastus lateralis muscle (results of manual tracing).