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Conference Abstract

YI 1.7 Transmural Quantification of Murine Vascular Smooth Muscle Cell Density Distribution from 3D Microscopy Images

Koen W.F. van der Laan[1,2,*], Koen D. Reesink[1,2], Myrthe M. van der Bruggen[1,2], Armand M.G. Jaminon[1,3], Remco T.A. Megens[1,2,4], Leon J. Schurgers[1,3], Tammo Delhaas[1,2], Bart Spronck[1,2,5]

[1]*CARIM School for Cardiovascular Diseases, Maastricht University*
[2]*Department of Biomedical Engineering, Maastricht Unieristy*
[3]*Department of Biochemistry, Maastricht University*
[4]*Institute for Cardiovascular Prevention, Ludwig Maximilians University (LMU)*
[5]*Department of Biomedical Engineering, School of Engineering & Applied Science, Yale University*

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ABSTRACT

Purpose: Investigating the biomechanical role of smooth muscle cells (SMCs) in arteries requires knowledge of their structural distributions. Compared to histology, 3D microscopy offers non-destructive *ex vivo* imaging under realistic conditions [1]. Robust 3D segmentation of SMCs, however, is challenging. We propose a method for automatic SMC quantification, and assessed its potential using a murine SMC apoptosis model.

Methods: After euthanasia, carotid arteries (control and with induced SMC apoptosis: SM22α-hDTR [2]) were excised and mounted between micropipettes (Figure A). Nuclei were stained with SYTO41. Arteries were imaged using two-photon microscopy [1], while stretched to *in vivo* length and pressurised to 100 mmHg (Figure B). Image stacks were processed as follows: 1) deconvolution; 2) nuclei segmentation using vesselness filtering [3,4] (Figure C); 3) cylindrical coordinate system identification; 4) splitting of coincident nuclei, based on cores defined from groups of neighbouring voxels with similar orientations [3] (Figure D and E); 5) cylindrical coordinate system re-identification; and 6) cell density-distribution quantification (Figure F). Segmentation performance was assessed by comparing with manual cell counts.

Results: Figure E demonstrates the method's ability to split undersegmented coinciding nuclei. Cell counts were lower in SM22α-hDTR than in control; algorithm-derived counts were comparable to manual (Figure F). The control sample showed multiple SMC layers, while the SM22α-hDTR sample showed a single SMC layer (Figure F), which was confirmed visually.

Conclusion: We developed a precise tool to quantify SMC distributions in *ex vivo* murine arteries, to facilitate quantitative modelling of SMC biomechanics. We intend to expand the current approach to address cell orientation, shape, and size.

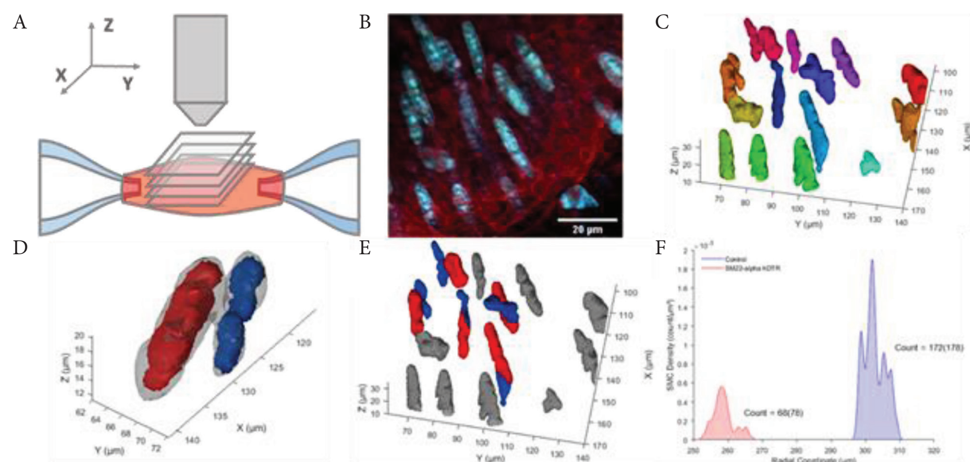

Figure | (A) Imaging set-up illustrating acquisition of z-stack of slices. (B) Example slice of 3D stack; cell nuclei are shown in blue while elastin fibres are shown in red. (C) Segmentation results from vesselness filtering of example image stack, colours indicate separated nuclei (step 2, Methods). (D) Coinciding nuclei, corresponding with the left orange nuclei in C, shown in grey, with cell cores shown in red and blue (step 4, Methods). (E) Coinciding nuclei splitting results of nuclei shown in C. Non-split nuclei are shown in grey, while split nuclei are shown in red and blue. (F) Transmural SMC densities and cell counts for one control and one SMC apoptosis sample; manual cell counts are given between parentheses.

*Corresponding author. Email: k.vanderlaan@maastrichtuniversity.nl

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