VIDEO LENSFREE MICROSCOPY FOR THE QUANTIFICATION OF ENDOTHELIAL CELLULAR NETWORK FORMATION

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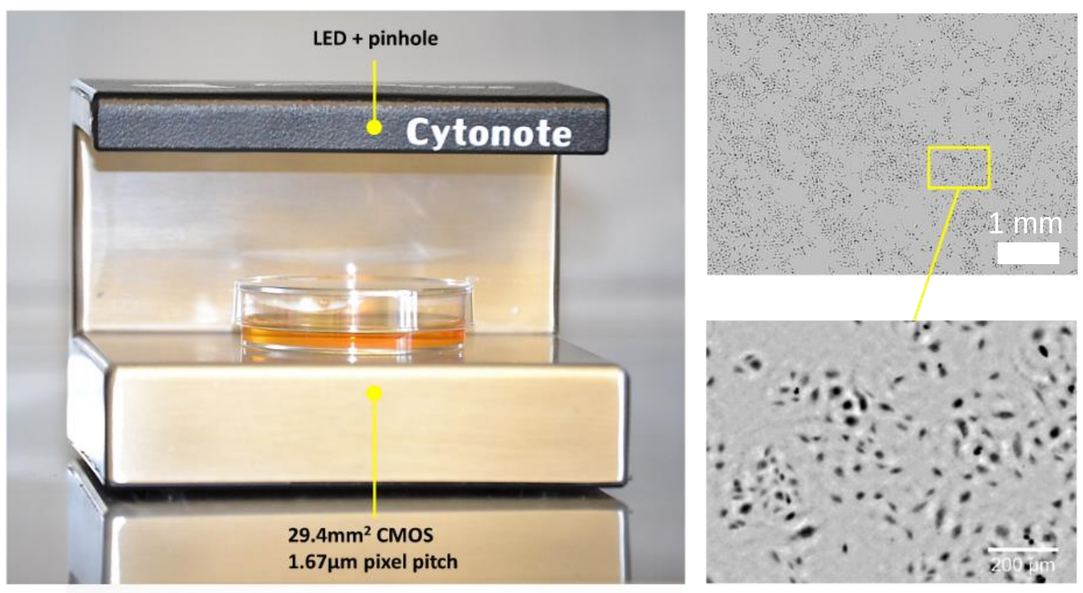
Introduction

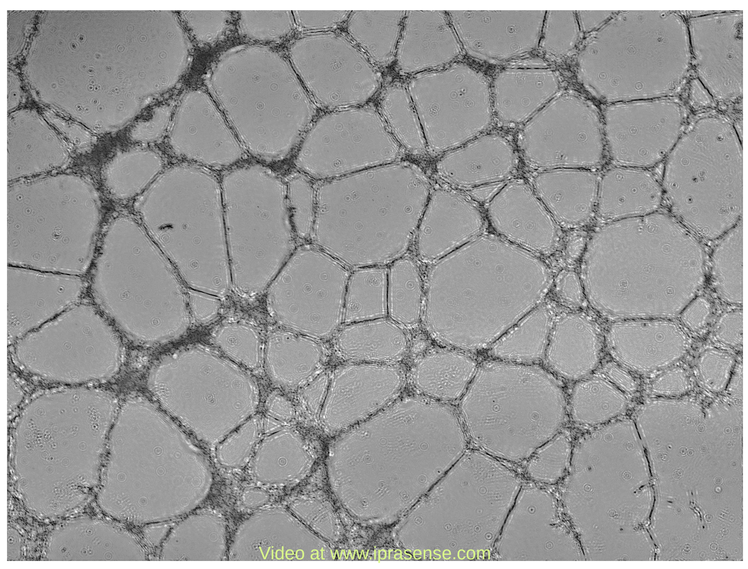
We assessed the use of the Iprasense Cytonote lensfree video microscope in combination with a customized version of the "Angiogenesis "Analyzer ImageJ plug-in. This turns into a unique platform to exhaustively and precisely study angiogenesis and cellular network formation over large field of view (29.4 mm2) and extended period of acquisition (days to weeks). As a first case study, we have analyzed and quantified the spontaneous network formation of HUVEC endothelial cells on three-dimensional (3-D) extracellular matrices.

Lensfree microscopy for large field of view time lapse Imaging



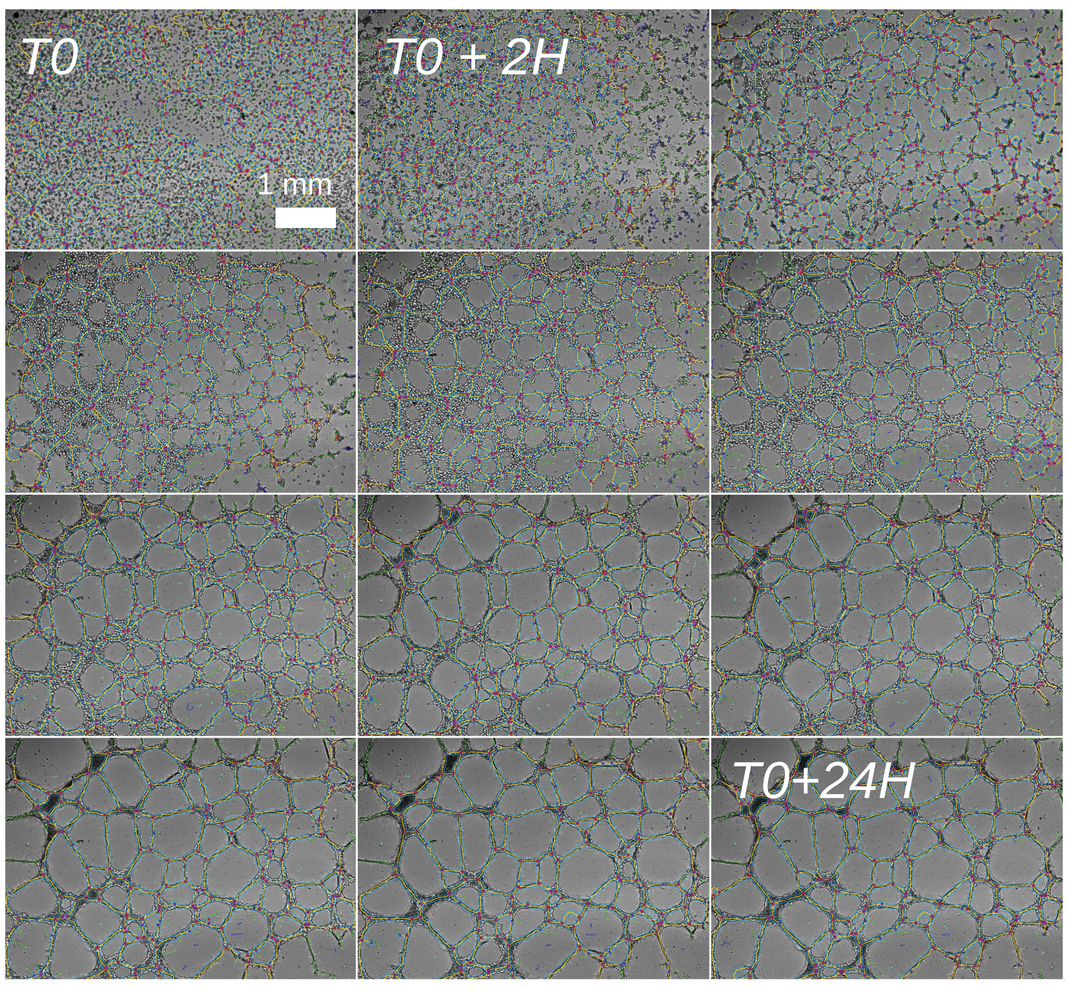
Experimental setup of the lensfree video microscope (2014 IPRASENSE Cytonote). (Left) A CMOS image sensor is used for image acquisition and an LED and a 150 µm pinhole for illumination (5 cm above the image sensor). (Right) Acquisition and phase reconstruction of a culture of primary human umbilical vein endothelial cells (HUVEC) (scale bar 1000 µm). The field of view is 29.4 mm2 and contains about 4000 cells.

Acquisition of the network formation of HUVEC endothelial cells

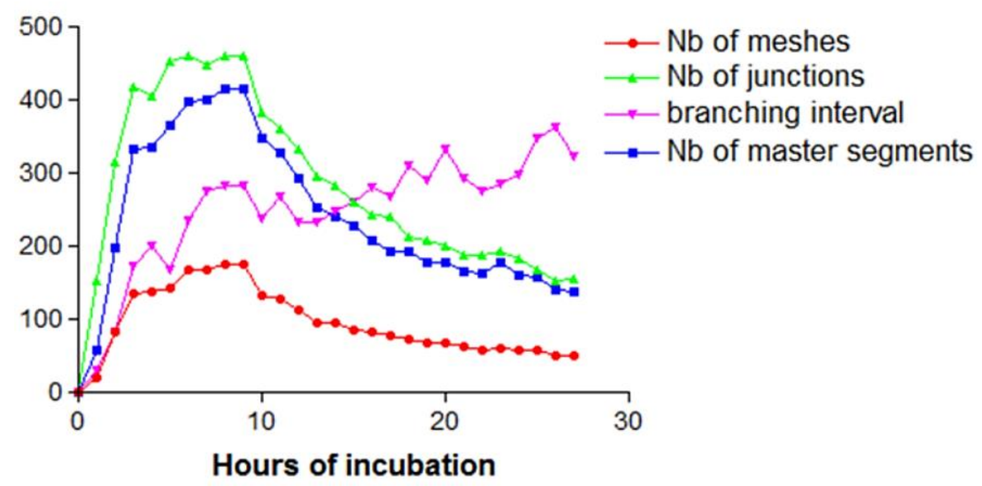


Network formation with HUVEC cells observed by means of lensfree video microscopy over 24 h. The cells are cultured on 75 µl of liquid Matrigel. 5.10^4 HUVEC cells were then seeded on Matrigel (BD Biosciences) and incubated at 37°C. Scale bar is 1 mm.

Automated analysis of the HUVEC network formation



Analysis is perfomed with a customized version of the "Angiogenesis "Analyzer ImageJ plug-in: Segments are in yellow, branches in green, red points are nodes, meshes (closed structures, also called tube-like-structures) are in cyan, and isolated structures are in blue. After 24h, the networks tend towards a stable architecture: total meshes area is around 9 mm², the number of meshes is approximatively 60, and their mean size is 0,15 mm².



Temporal analysis of the HUVEC network formation. The plot of the different devised metrics as a function of time clearly define three different steps for the network formation: initiation, a stabilization period and then the fusion of meshes. During the first 4 hours, the network is in formation: the number of meshes increases, and so do the number of segments and junctions (see figure 3d for the definitions). Following this, for 6 hours, the network remains stable. Towards the end, the meshes merge to form larger meshes. After 24 hours, the networks present the following architecture: total meshes area of 9mm², with 60 meshes, with an average size of 0.15 mm².

Conclusion

Lensfree video microscope in combination with the Angiogenesis Analyzer ImageJ plug-in provides **a unique mean to quantify angiogenesis and to screen ex vivo anti-angiogenic therapies.**

The methods is label-free, high-throughput, ease of use, working directly inside the incubator and relatively low-cost.

We expect that it will irreversibly change the quantification of cell culture, and angiogenesis quantification in particular.