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Quantification of tube topography on micrographs of fluorescent staining of multi-layers cell culture.

PLOS One

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ABSTRACT

The induction or inhibition of angiogenesis can be assessed in vitro by the differentiation of endothelial cells. Indeed, these cells are capable of forming structures resembling a capillary network in culture. This approach is known as Endothelial Tube Formation Assay (ETFA).

This type of analysis is traditionally performed on a low confluence monolayer of cells to assay the effect of soluble compounds on the formation of tubes. However, the modulation of tube formation by cell-cell interactions requires the co-culture of cells that leads to the creation of a multi-layer micro-tissue. Additionally, the identification of cells in the micro-tissue requires fluorescent staining resulting in discontinuities in the signal.

The present protocol allows to analyze the topography of tubes on micrographs of fluorescent staining of multi-layers cell culture.

The limit of this method resides in the fact that the 3D organization of the micro-tissue is not reconstructed and, thus, a part of the tube network is not analyzed.

OPEN ACCESS

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Protocol status: Working
We use this protocol in our workspace and it is working.

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PROTOCOL integer ID:
38466

Keywords: co-culture,
fluorescent staining, tube
topography, quantification

Software

1 Install Icy (requires JDK 8+).

Software

Icy	NAME
Windows/MacOS/Linux	OS
BioImage Analysis Lab (Institut Pasteur)	DEVELOPER
http://icy.bioimageanalysis.org	SOURCE LINK

1.1 Download the Icy script "ImageJ background subtraction." (file name: protocolfile-imagej-background-subtraction1)

See: <http://icy.bioimageanalysis.org/protocol/imagej-background-subtraction/>

1.2 Install the Icy plugin "Membrane Filter."

See: <http://icy.bioimageanalysis.org/plugin/membrane-filter/>

1.3 Install the ImageJ plugin "Luts Macros and Tools Updater."

See: <http://image.bio.methods.free.fr/ImageJ/?Luts-Macros-and-Tools-Updater>

- 1.4** Install the ImageJ plugin "Angiogenesis Analyzer for ImageJ."
See: <http://image.bio.methods.free.fr/ImageJ/?Angiogenesis-Analyzer-for-ImageJ>

Preprocessing

- 2** In Icy, open the image to be analyzed.
- 2.1** Extract the channel of interest (Channel -> Extract -> Channel x).
Save the new image as TIFF.
- 2.2** Subtract the background (Tools -> Protocols -> Load: protocolfile-imagej-background-subtraction1).
Settings: Rolling=20.
Save the new image as TIFF.
- 2.3** Reduce the discontinuities and noise on membranes (Plugin -> Membrane Filter).
Settings: default parameters.
Save the new image as TIFF.

Definition of ROI

- 3** In ImageJ (in Icy), open the image obtained after preprocessing.
- 3.1** Enhance the contrast (Process -> Enhance Contrast).
Settings: Saturated pixels: 0.0%; Normalize; Equalize histogram.

- 3.2** Subtract the background (Process -> Subtract Background).
Settings: Rolling ball radius: 5.0 pixels; Sliding paraboloid.
Save the new image as TIFF.

Analysis of tube topography

- 4** In ImageJ (in Icy), open the image obtained after the definition of ROI.
- 4.1** Start Angiogenesis Analyzer.
Settings: select all options except "Suppress isolated elements."
Threshold values: Minimum object size: 10 pixels; Minimum branch size: 25 pixels; Artifactual loop size: 850 pixels; Isolated element size threshold: 50 pixels; Master segment size threshold: 30 pixels; Iteration number (advised 2 to 5): 3 iterations; Show iteration (for single analysis): 3 iterations.
- 4.2** Apply the Blurred Mask Tool (Angiogenesis Analyzer -> Blurred Mask Tool) to the whole surface of the image.
- 4.3** Change the color encoding of the image to RGB color (Image -> Type -> RGB color).
- 4.4** Run the analysis (Angiogenesis Analyzer -> Analyze HUVEC Phase contrast).

Save and analyze

- 5** Save the result files (Angiogenesis Analyzer -> Save Current Analysis). Measurements are saved as a tab-separated values file (with "xls" extension) and can be further analyzed in a spreadsheet.