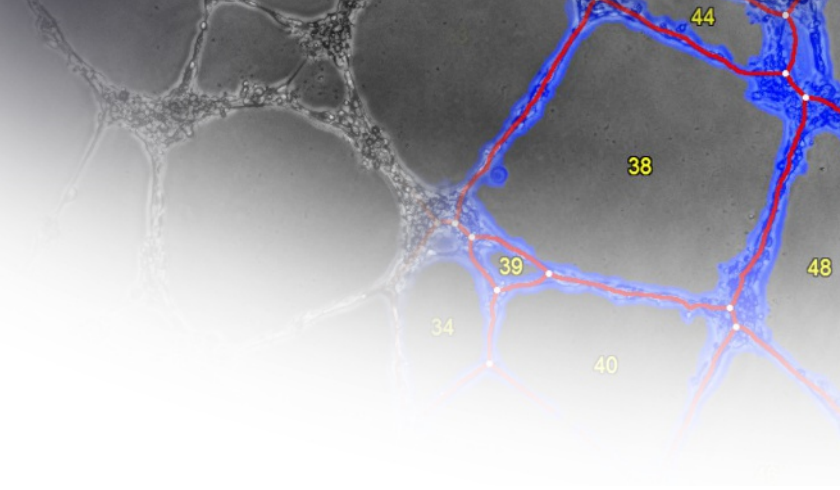
Wimasis
Image Analysis



WimTube

Application note

The formation of new blood vessels is essential for the growth and development of biological systems. The research focus of many drug screening and cell signaling studies aims at the interaction of initial and continuous growth and sustentation of solid tumors and its complex physiological processes due to angiogenic activity.

The in vitro Tube Formation Assay is based on the differentiation of endothelial cells and the formation of tube-like structures on an extracellular matrix such as Matrigel® (BD). This assay can be conducted to screen compounds for angiogenic activity and is able to deliver good, reproducible results.

The major advantage of this assay is that it offers the opportunity to monitor angiogenic behavior over time and therefore enables the estimation of time dependent effects on neovascularization.

Objective, reliable, and precise quantification of your Tube Formation images is ensured by our WimTube solution.

Components recquired:

- Wimasis Wimasis account (you can get one for free here).
- Endothelial cells or other experimental cell lines.
- Cell Growth medium.
- Matrigel ® (BD).
- A slide suitable for angiogenesis.
- An inverted microscope, preferably with automated image acquisition system and stage top incubator for live cell imaging.

Experimental endpoints:

- Inhibition of angiogenesis.
- Substance mediated enhancement of growth and development of new blood vessels.

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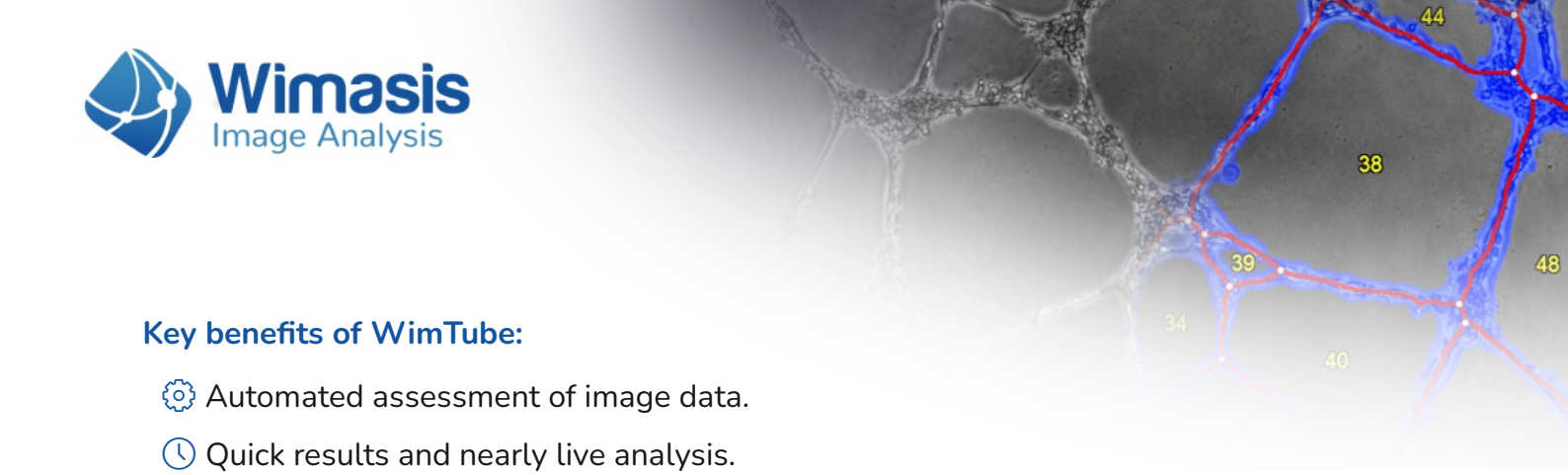

Key benefits of WimTube:

- Automated assessment of image data.
- Quick results and nearly live analysis.
- Objective and reliable measurements.
- Worldwide accessibility via Wimasis.com.
- Optimized assay-specific algorithms to enhance data quality.

Step Process of the Assay:

1. Gel preparation and cell seeding.
2. Acquisition of microscopy images.
3. Quantitative image analysis.
4. Data analysis and evaluation.

Step 1: Gel Preparation And Cell Seeding

- Place Matrigel ® 24 hours prior the experiment at 4°C on ice and in the refrigerator. Slow thawing over night enables gentle degassing of the gel.
- Apply the gel to the slide with precooled pipet tips (4°C) and place the slide for 30 min in the incubator for polymerization.
- Prepare cell suspension as usual and define the expected final cell number per well. Depending on your specific cell type and the dimensions of the slide design, the application of 2 x 10 5 cells/ml might vary.
- Apply cell suspension into each well, let the slide rest for a few minutes, and avoid shaking. This way all cells sunk to the bottom of the slide and an homogeneous cell distribution is achieved.
- Place the slide in the incubation stage of the microscope.

Step 2: Acquisition Of Microscopy Images

- Move the dish until you have the center of the dish aligned with the center of your observation view. When taking the time-lapse images, please use the same position in order to reduce data variation in the read out results.
- Start the observation process by taking images several times throughout the following hours. Typically, the timepoints at which images are taken are at 1, 4, 6, and 24 hours in the experiment. Specific timepoints might vary based on the type of cells and the respective migration speed.

Step 3: Quantitative Image Analysis

- **Register** and **log in** on Wimasis.
- **Upload** your images to WimTube.
- After successful transfer of the data, you will be notified via e-mail about the status of your order. Detailed results will become available in the **results** section of Wimasis.

Step 4: Data Analysis And Evaluation

- After having been processed, you will be able to download the overlay images and CSV data files with the tube formation metrics of your image set.
- The analysis readout (CSV files) will include the data outlined in the "WimTube: Analysis results in detail" document that you can download **here**.