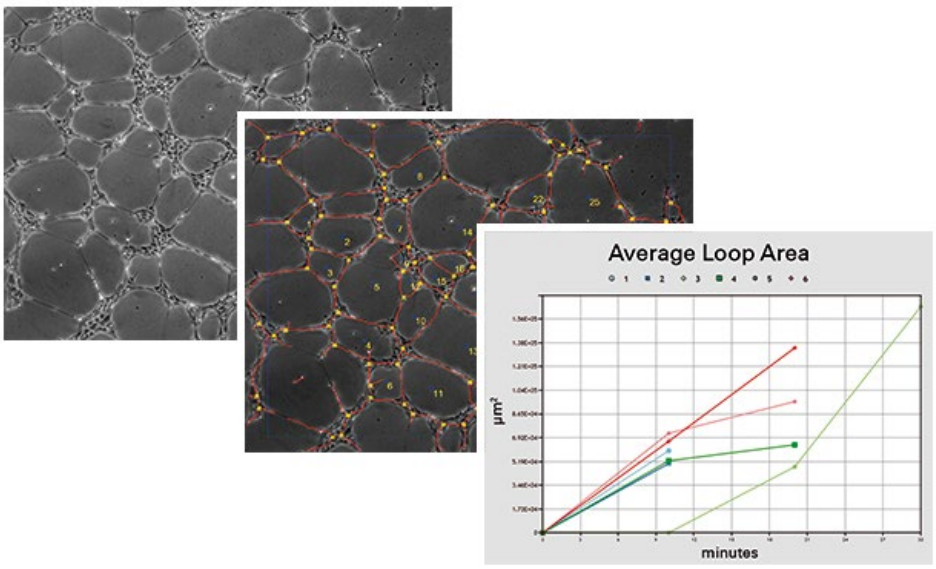
Data Analysis of Tube Formation Assays

The tube formation assay is a simple but powerful *in vitro* tool for screening substances for anti- or pro-angiogenic effects. These effects can be measured by different parameters, such as tube length or the number of loops formed on the gel surface.

Creating powerful analyses from tube formation assays requires consideration of several aspects before, during, and after the experiment to ensure correct and reproducible results.

This Application Note provides guidelines for practical analysis after gathering data from tube formation assays and focuses on analyzing and interpreting this data. To optimize your assay, please read Application Note 27: Optimizing Tube Formation Assays.



ibidi offers various solutions for tube formation assays:

- µ-Slide 15 Well 3D
- µ-Slide 15 Well 3D Glass Bottom
- µ-Plate 96 Well 3D
- µ-Plate 96 Well 3D Glass Bottom

Related Documents:

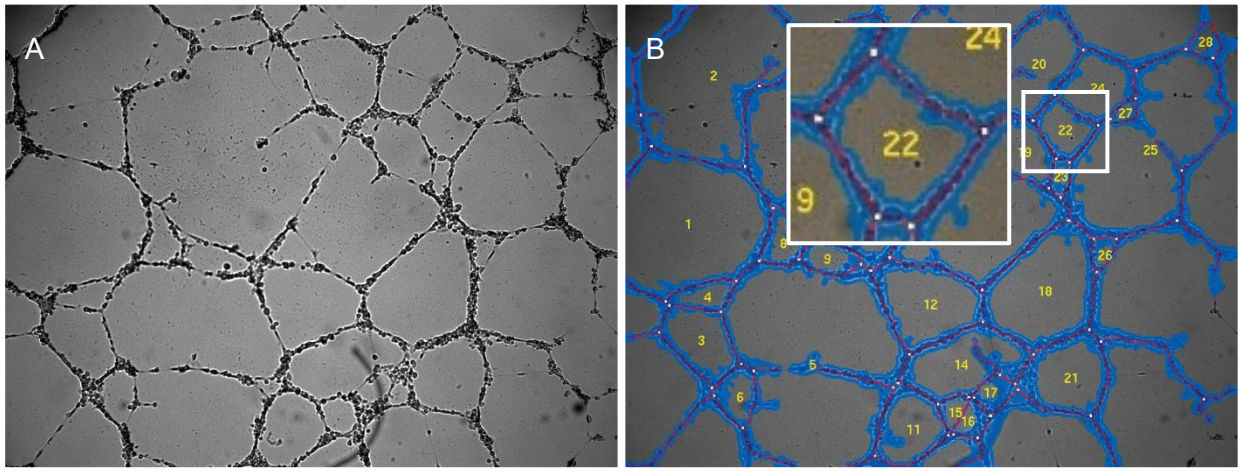
- Application Note 05: Tube Formation Assay in the µ-Plate 96 Well 3D (PDF)
- Application Note 19: Tube Formation Assay in the µ-Slide 15 Well 3D (PDF)
- Application Note 26: Preparation of Collagen I Gels (PDF)
- Application Note 27: Optimizing Tube Formation Assays (PDF)
- Application Note 66: Tube Formation Assay With Laminin-Collagen I Gel in the µ-Slide 15 Well 3D (PDF)

1 Output Parameters of Tube Formation and Angiogenesis Assays

The expression “tubes” describes the cords of cells that are visible in a 2D network. It does not mean, specifically, that the cords have a lumen. In tube formation assays, four key parameters can be determined from phase contrast images, as shown in the figure below.

- Cell-covered area [%] (blue area)
- Tube length [px] (red lines)
- Number of branching points (white dots)
- Number of loops (yellow number)

These key parameters show a consistent relationship to each other during the experiment. Therefore, it can be sufficient to determine only one feature: in this Application Note, only the length of the tube will be used as a representative value for tube formation.

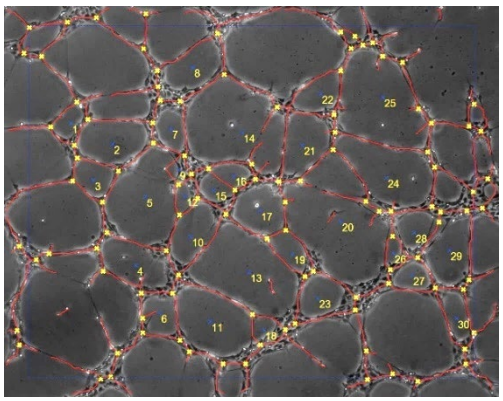


Phase contrast image (A) and analyzed image (B) shows the key features of tube formation analysis: cell-covered area (blue), tubes (red), loops (yellow), and branching points (white).

Further values, such as mean tube length, total tube length, and mean area of loops, can be calculated from these parameters.

Calculating the Total Tube Length

Image analysis can be performed using various software dedicated to tube formation assay analysis.



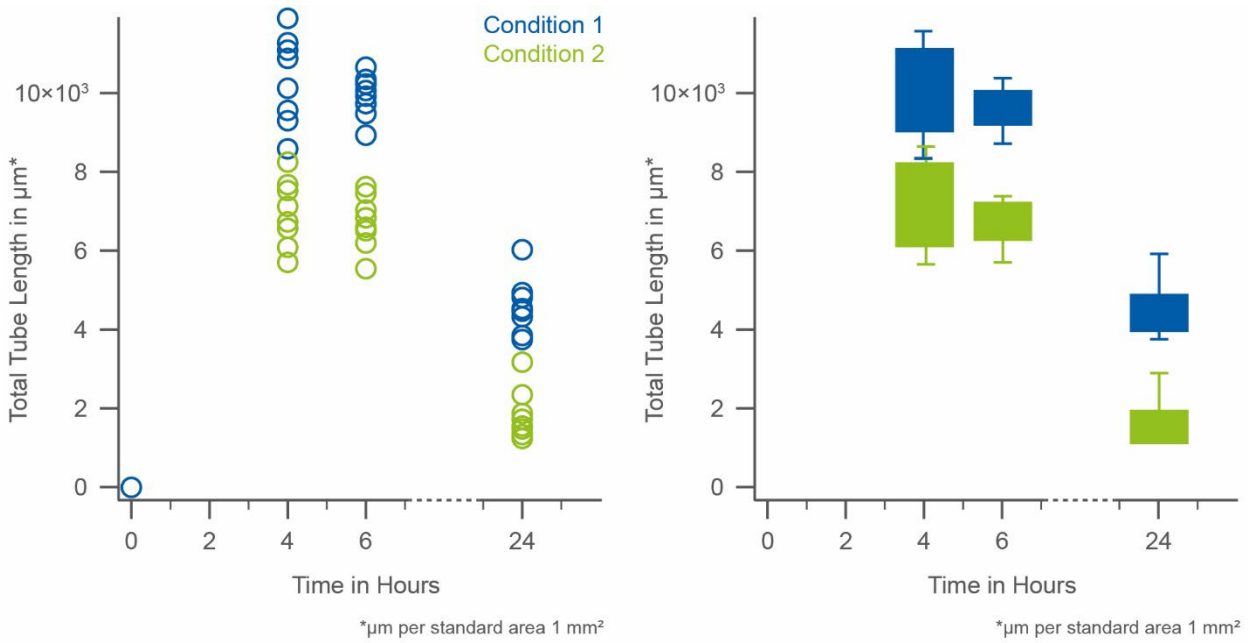
Exemplary output examples of automated tube formation analysis.

2 Statistical Evaluation

Tube formation is a complex process involving a wide variety of biochemical reactions and pathways. Therefore, it is recommended to have a minimum of three biological replicates per treatment condition be used for statistical analysis to obtain representative values.

For a reliable analysis and to obtain average values, we recommend conducting a multiwell analysis with replicates for each condition. For instance, each well analyzed yields a tube length value. These values should be averaged for each condition and calculated with a standard deviation. The standard deviation between data points collected from the same slide under the same conditions should be less than 10%. If the well-to-well variation is too high, the experimental conditions should be optimized, following Application Note 27: Optimizing Tube Formation Assays.

The following diagrams show exemplary the total tube length of HUVEC cells grown on Matrigel® in two different conditions (Condition 1 and 2), imaged using an ibidi Stage Top Incubator mounted on a phase contrast microscope. For each condition, eight independent wells at four distinct time points (0 h, 4 h, 6 h, and 24 h) were imaged.



Exemplary data analysis of the total tube length of eight individual wells of two biological conditions at four different time points (0 h, 4 h, 6 h, and 24 h). Individual data are shown as dot plots (left) and box plots (right).

The single data points are then aggregated and displayed in a box plot. Statistical analysis can be done by e.g., an ANOVA or a **Student's t-test**, in which the data points of the different conditions are tested against each other. The Student's t-test, for example, gives evidence on whether the two separate data sets with t-distributed values show a statistically significant difference between each other.