



# WimCAM Standard Tool

## Specifications for a successful analysis

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## 1 WimCAM tool

The chick chorioallantoic membrane (CAM) provides an *in vivo* model to study angiogenesis and evaluate several pro- and anti-angiogenic factors onto its surface, which are closely associated with, for example, solid tumour growth or metastasis. Therefore it is widely used in studies of cancer and other neovascular diseases, as well as in tissue engineering or biocompatibility issues.

WimCAM Assay is the Image Analysis Solution to quantitatively evaluate the growth and architecture of the new vasculature generated in CAM membranes, by means of the objective and reproducible measurements provided by this web-based automated solution. These measurements are obtained through an accurate detection of the blood vessels network using cutting-edge image processing techniques.

## 2 Specifications for the input files

### 2.1 General Description

WimCAM Assay takes as input not only isolated membranes images, but also top-view of the chorioallantoic membrane of a fertilized chicken egg images, so a window must be opened on the egg shell.

### 2.2 Valid Formats

WimCAM image analysis module accepts the following file formats: accepts the following file formats:

#### *Images*

jpg/jpeg

jp2

tif/tiff

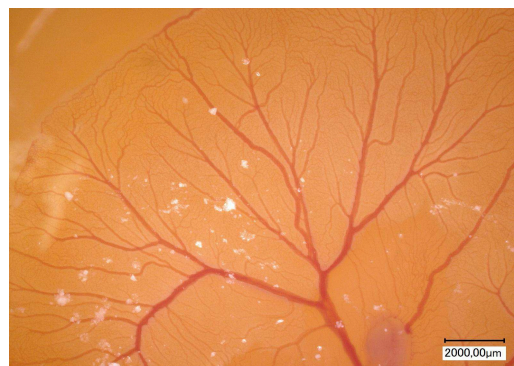
png

gif

bmp

### 2.3 Accepted Microscopy Techniques

WimCAM uses as input bright field microscopy images. No staining is needed



*Figure 1: CAM Assay brightfield image*

However, WimCAM is designed to adapt to all kinds of images and it accepts images of a wide range of microscopy techniques with the aim to provide every researcher with the best automated analysis.



## *2.4 Image Quality Characteristics*

In order to get the best results from the WimCAM solution the input images should have the following characteristics:

- No visible containers (e.g. wells, plates, chambers, etc.). They could be detected as false vessels.
- No additional information visible (e.g. scale bars, time stamp, etc.). Some microscope draw additional information on the image, like a scale bar. This should be avoided because these extra features distort the appearance of the image, resulting in a loss of information for the image analysis.
- Be focused. Images must be properly focused to enable sprouts to be viewed with the best contrast achievable in order to make the distinction between background and vessels as neat as possible.
- No artifacts, noise or debris: the background of the image should be clean and low on debris and artifacts. This will help the readout to be more precise and the data to be the most accurate.
- Homogeneous illumination. Achieving homogenous lighting conditions throughout the entire image will assure better results. Please, avoid images with bright spots in the center and dark spots in the corners.

We only guarantee accurate results, if the input images do meet these requirements. If that is not the case, we cannot assure reliable results of WimCAM standard tool, but we can make an adaptation of the standard tool to fit WimCAM to your images. To get an adaptation for WimCAM for free, contact us here:

[contact@wimasis.com](mailto:contact@wimasis.com) or +49 (0)89 452 44 66 0

### 3 Results descriptions

Results will be provided in a zip file that will have the following files within:

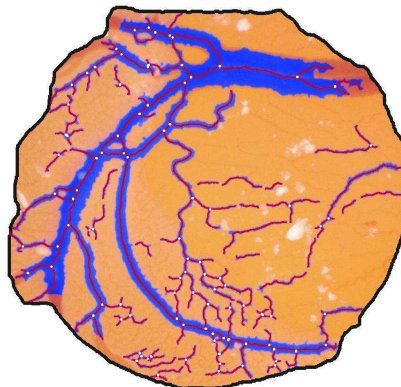
- Results image
- Results data
- Results data summary

Please, be aware that the parameters described in the following sections are the ones provided by the standard tool. Any other parameter could be added under request. Just contact us and tell us what you need:

[contact@wimasis.com](mailto:contact@wimasis.com) or +49 (0)89 452 44 66 0

#### 3.1 Results Image

Every input image will produce an output image, where the main aspects concerning the sprouting assay are enhanced as shown on the *Figure 2*



*Figure 2: example of a results image where the ROI perimeter(black line), vessels structure (blue), vessels skeleton (red), and branching points (white) are marked.*

This image will have the name of the input image followed by “\_Exp01” and will be saved in “jpg” format.



## 3.2 Results Data

Every input image will produce an output CSV (comma-separated value) format file with the following measurements:

### Global Metrics:

- **Vessels Density [%]:** Is the density of vessels in the ROI, calculated by dividing the number of pixels which belong to the vessels (blue regions) by the total number of pixels of the ROI (black lines)
- **Total Vessels Network Length [px]:** Is the length in pixels of the red skeleton.
- **Total Branching Points:** Branching points are parts of the skeleton where two or more segments converge on. They appear as isolated white areas on the vessels skeleton, so *Total Branching Points* corresponds to the number of them on the image.
- **Total Nets.** A net is an isolated region of vessels that contains, at least, one branching point. In other words, isolated segments are not considered as nets.

### Segments Characteristics:

- **Total Segments:** Is calculated from the vessels skeleton as its number of individual segments, discarding the branching points.
- **Mean Segment Length [px]:** Individual segments length are also calculated for all vessels represented as individual segments of the skeleton. The *Mean Segment Length* is the arithmetic mean of these length values.
- **Standard Deviation Segment Length [px]:** Is the standard deviation of the segments length, which values were already used for the calculation of the *Mean Segment Length*.

## 3.3 Results Data Summary

Every uploaded order produces a CSV file that summarizes the measurements of every of the individual CSV results data files. This file will have the name of the order number followed by “\_Summary”.