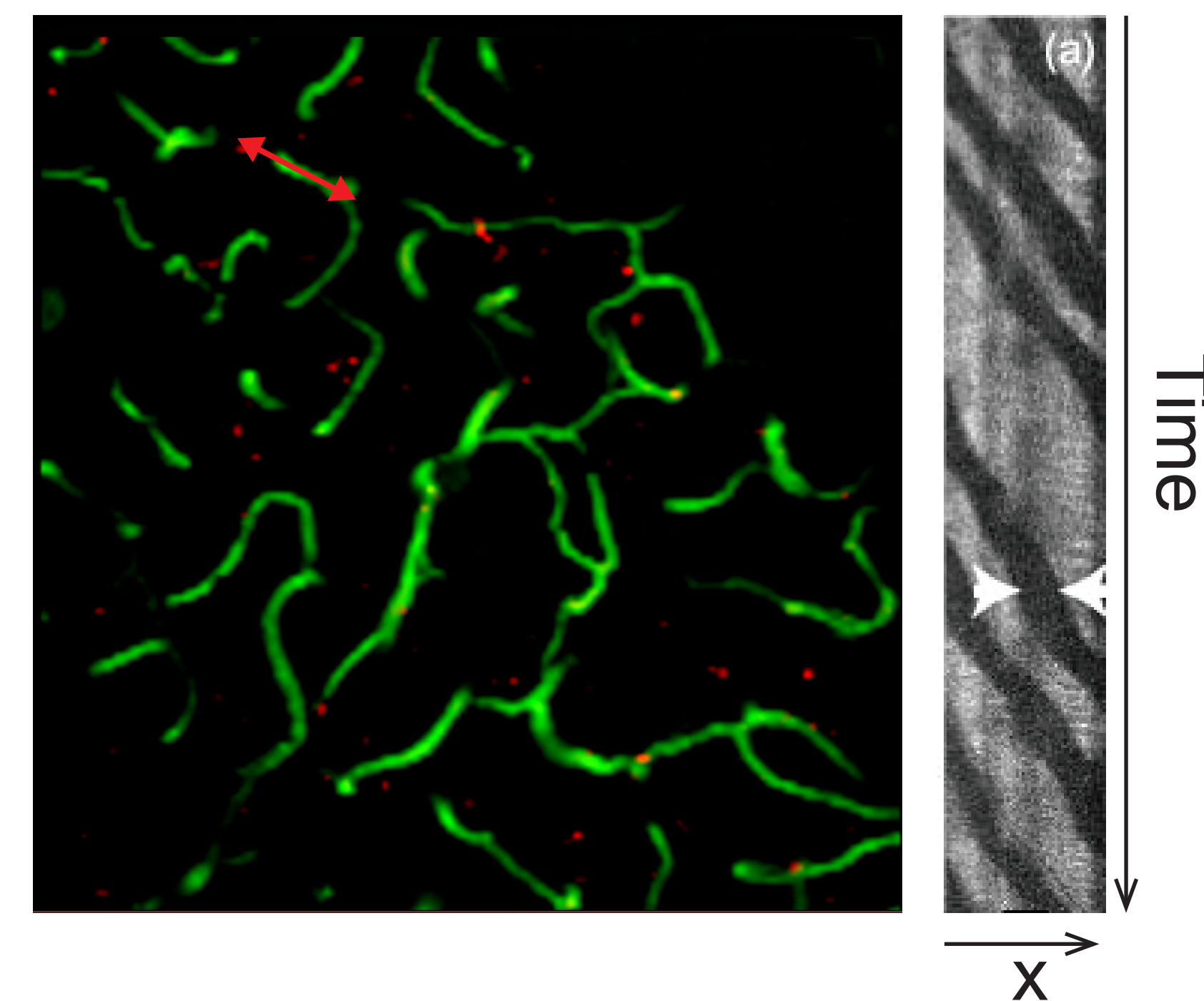




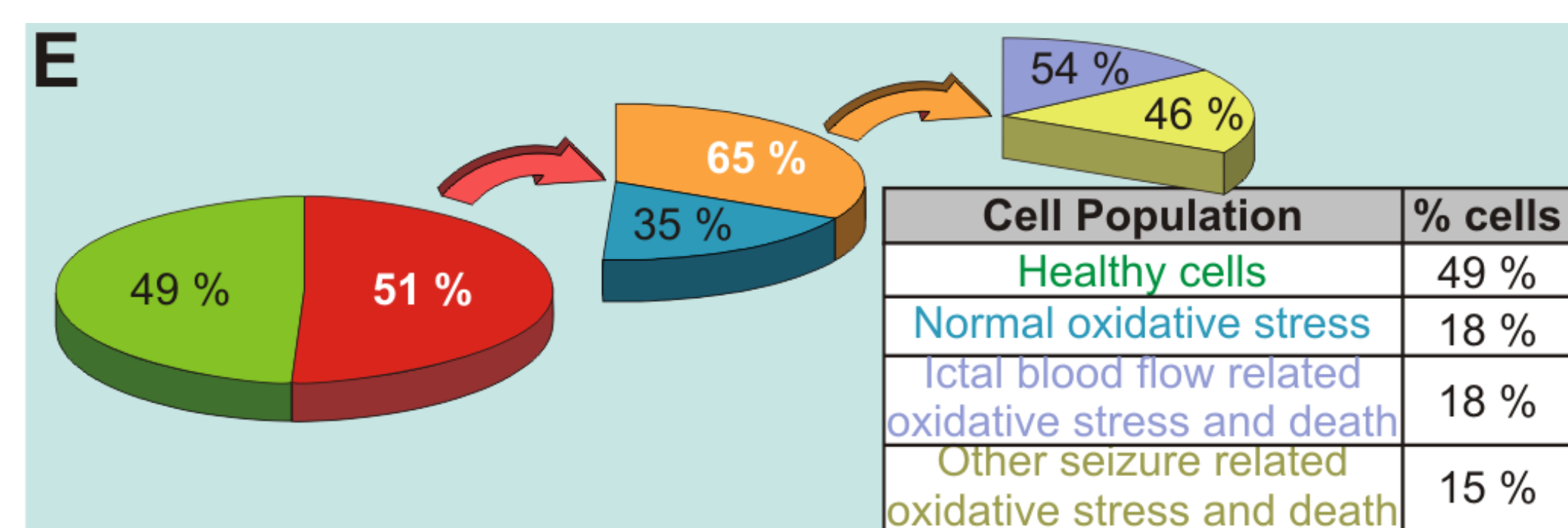
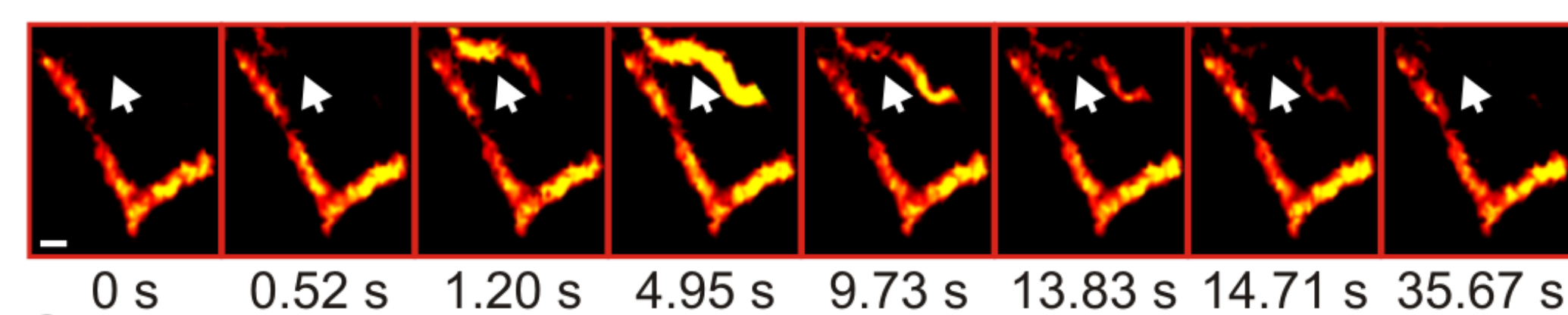
Motivation

Research of functional control of microvasculature uses scans of small capillaries to study the delivery of nutrients and O₂ to brain cells, using of fluorescent dyes which label blood serum.



Can it be that a vessel is not there to begin with?

Idea comes from hippocampus data in epilepsy models: vessel occlusions (vasospasms) would release and the vessels would suddenly “appear” in the recording. We also showed that this occlusions and releases are relevant to cell death and neural degeneration, so they may have functional effects too.



Objective

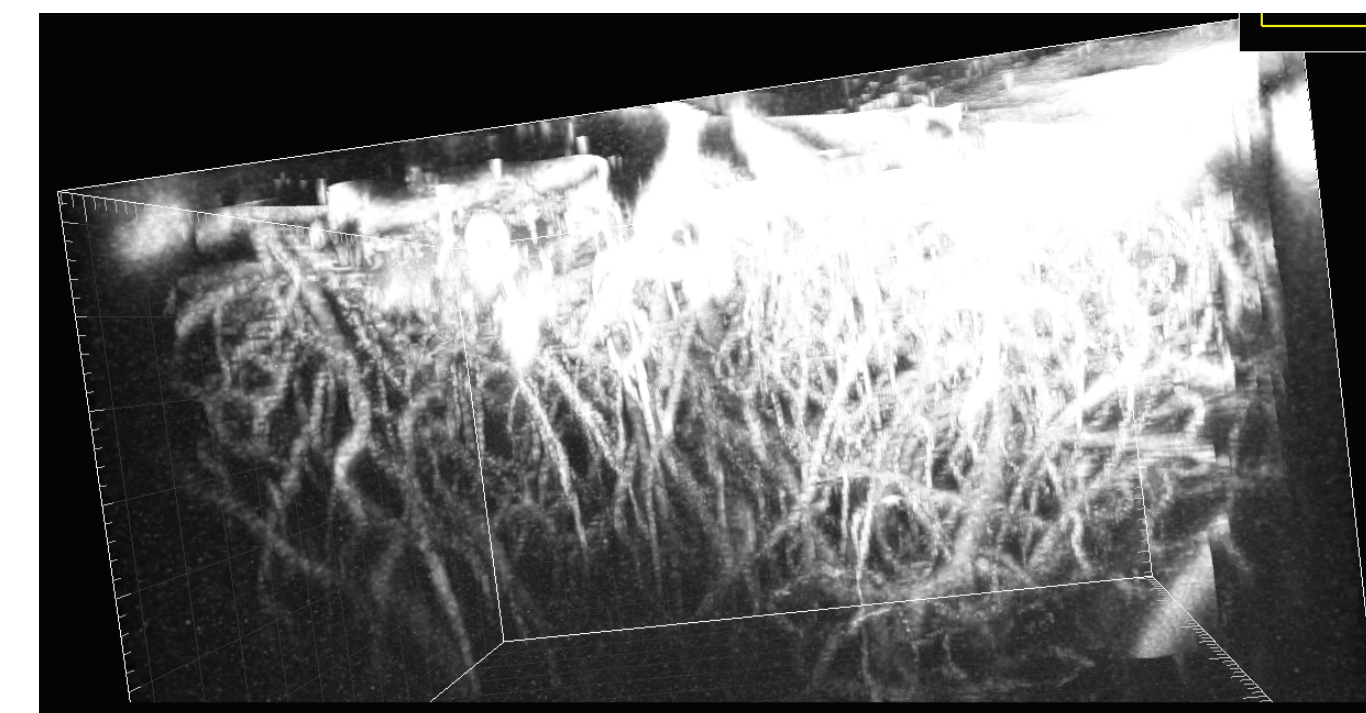
To test for the presence of a “reserve” of blood vessels, we need to compare two-photon recordings of microvasculature with immunohistochemical staining on the same tissue:

- Two-photon images blood serum (in vivo)
- Immunohistochemistry labels the vessel walls

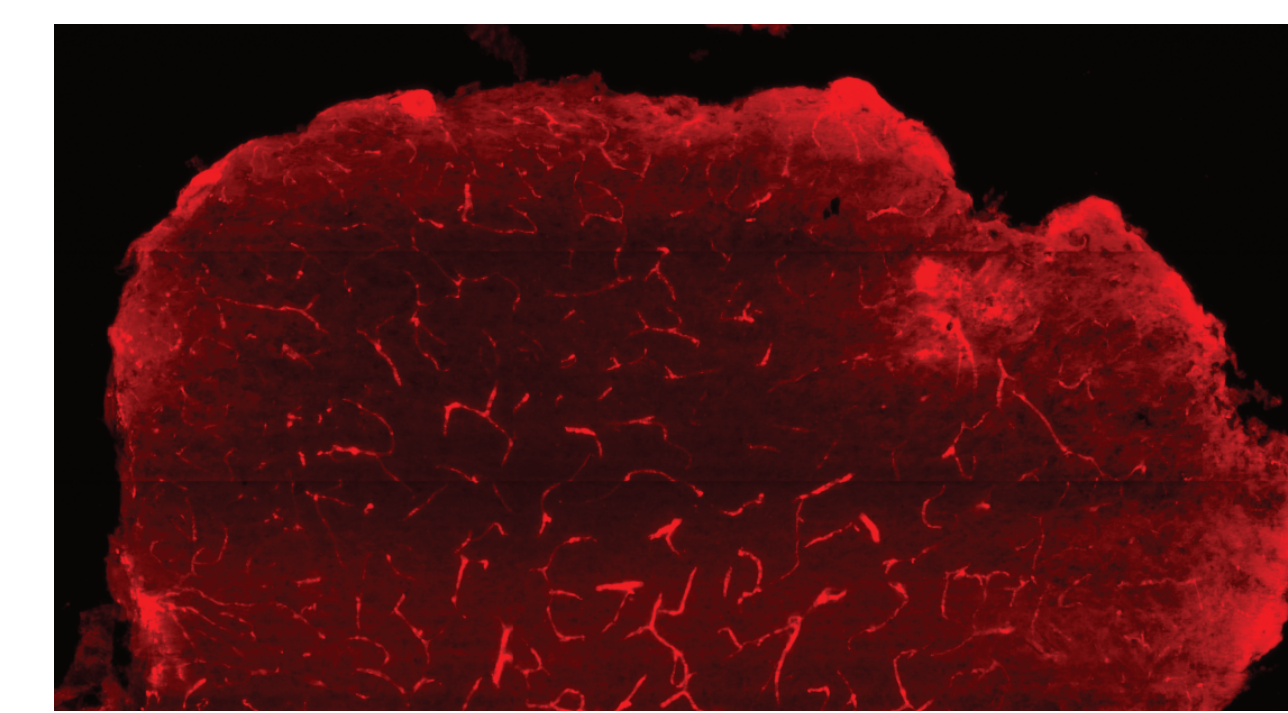
Therefore, the first step must be how to find a way to align this different three dimensional images of the same tissue.

Research Plan

Raw data



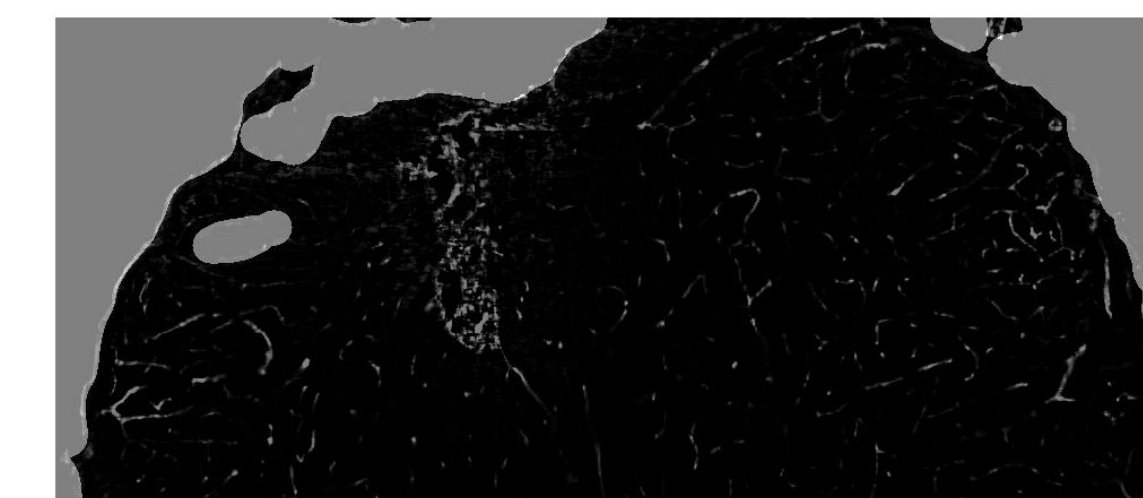
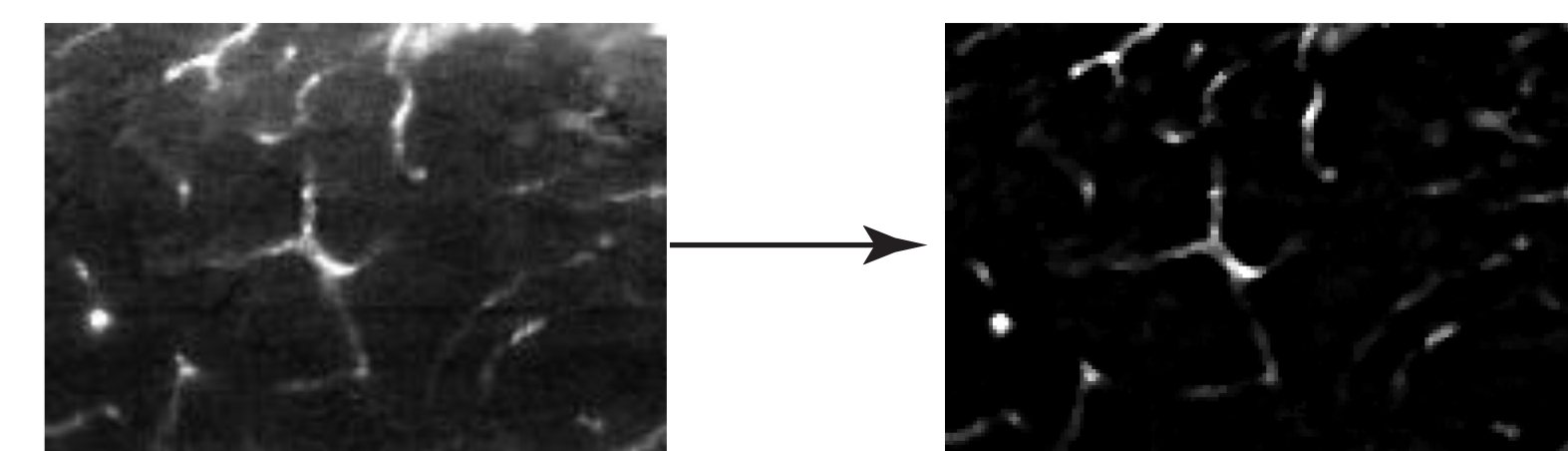
Multiphoton image



Confocal image

Data preprocessing

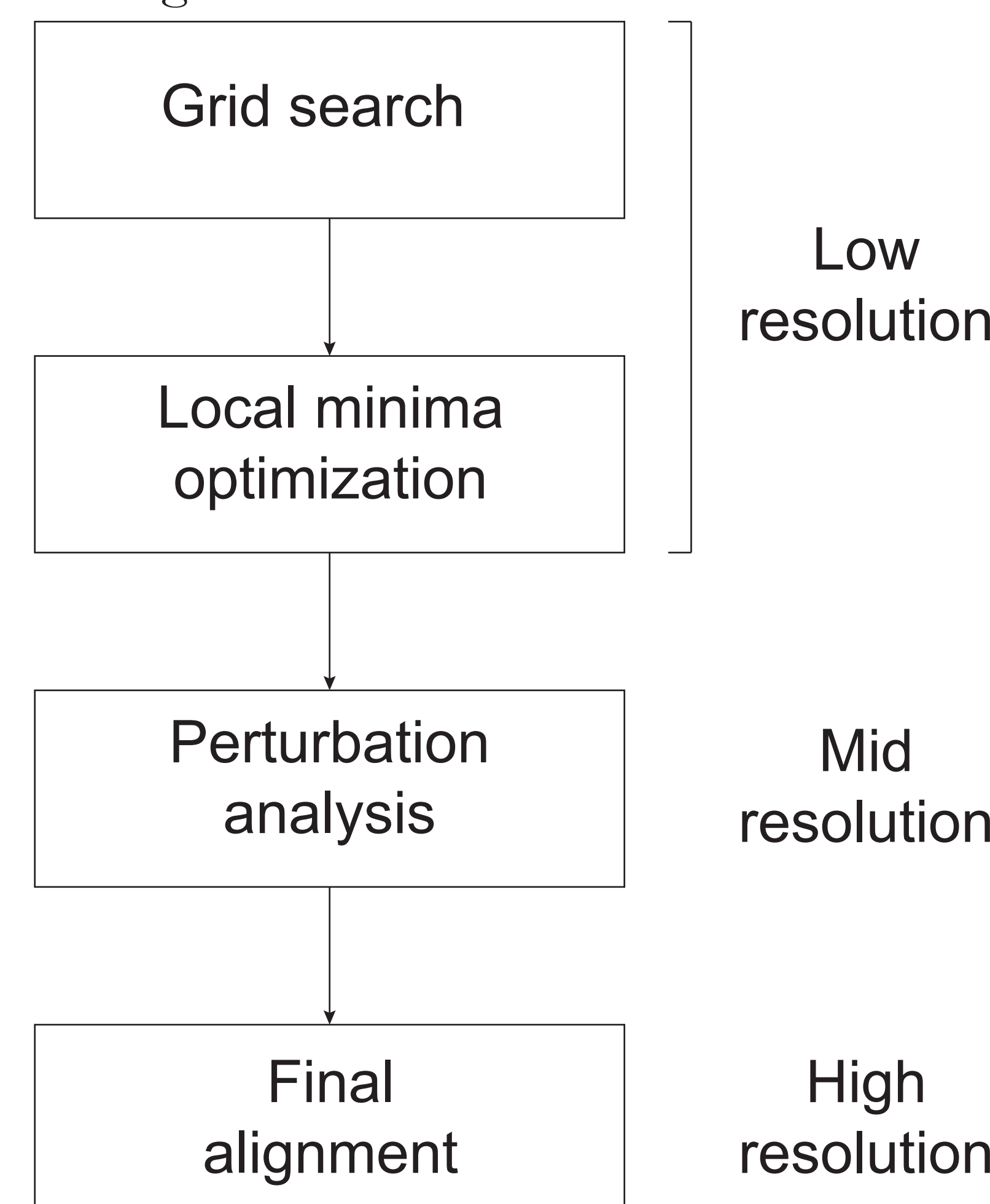
Two steps are required to clean up the confocal images: background removal and a mask selection that will tell the registration algorithm to ignore the edges of the tissue.



The sample preparation for confocal microscopy produces images with a strong background in the tissue area. The objects of interest in the image (blood capillaries) have widths ranging from 4 to 10 microns, so a solution that works well in the general case is to just subtract from the image a low-passed version of itself obtained by convolution with a gaussian kernel with standard deviation of 6 microns.

To obtain the image mask, we use to our advantage the strong background coming from the brain tissue to segment the sample using Huang’s method, after clipping the intensity to 90% of the maximum to remove outliers. Then the edges of the tissue are removed by using morphological erosion with a 15-micron radius disk as structuring element, followed by a closing with the same element to remove gaps.

Registration algorithm



The proposed algorithm is adapted from Jenkinson & Smith, who initially developed it for global registration of MRI images. It is a multiresolution registration, divided in three well-differentiated stages:

-In the initial alignment stage, a search is performed over the transformation space, and then an optimization is performed on the local minima of the search grid.

-The best three matches from the initial alignment stage are selected for a perturbation analysis, in which several perturbations of each transformation are optimized.

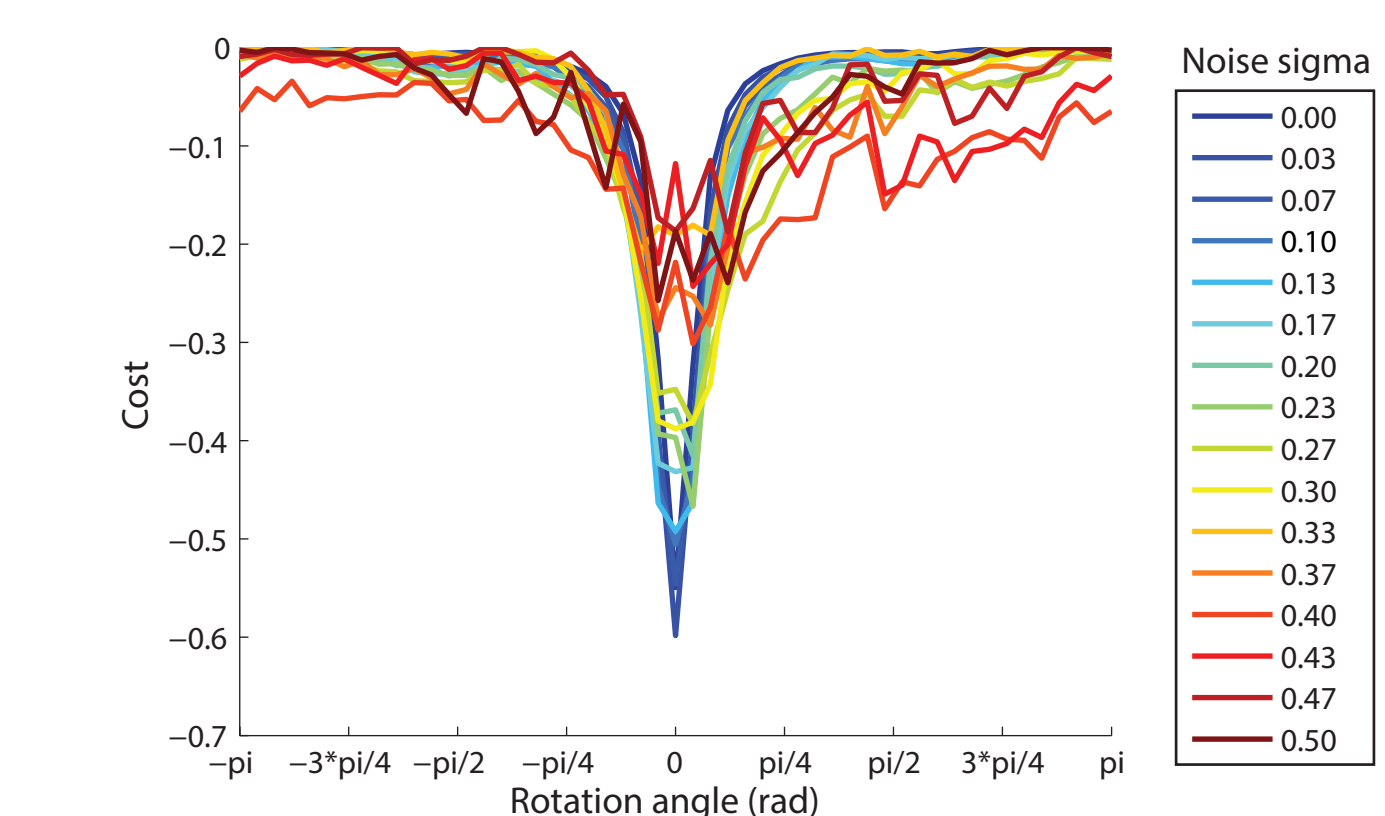
-The best candidate from the perturbation analysis is then chosen for a final alignment.

Optimizations are performed using a gradient descent algorithm, and the Mattes mutual information between images as cost function.

Results

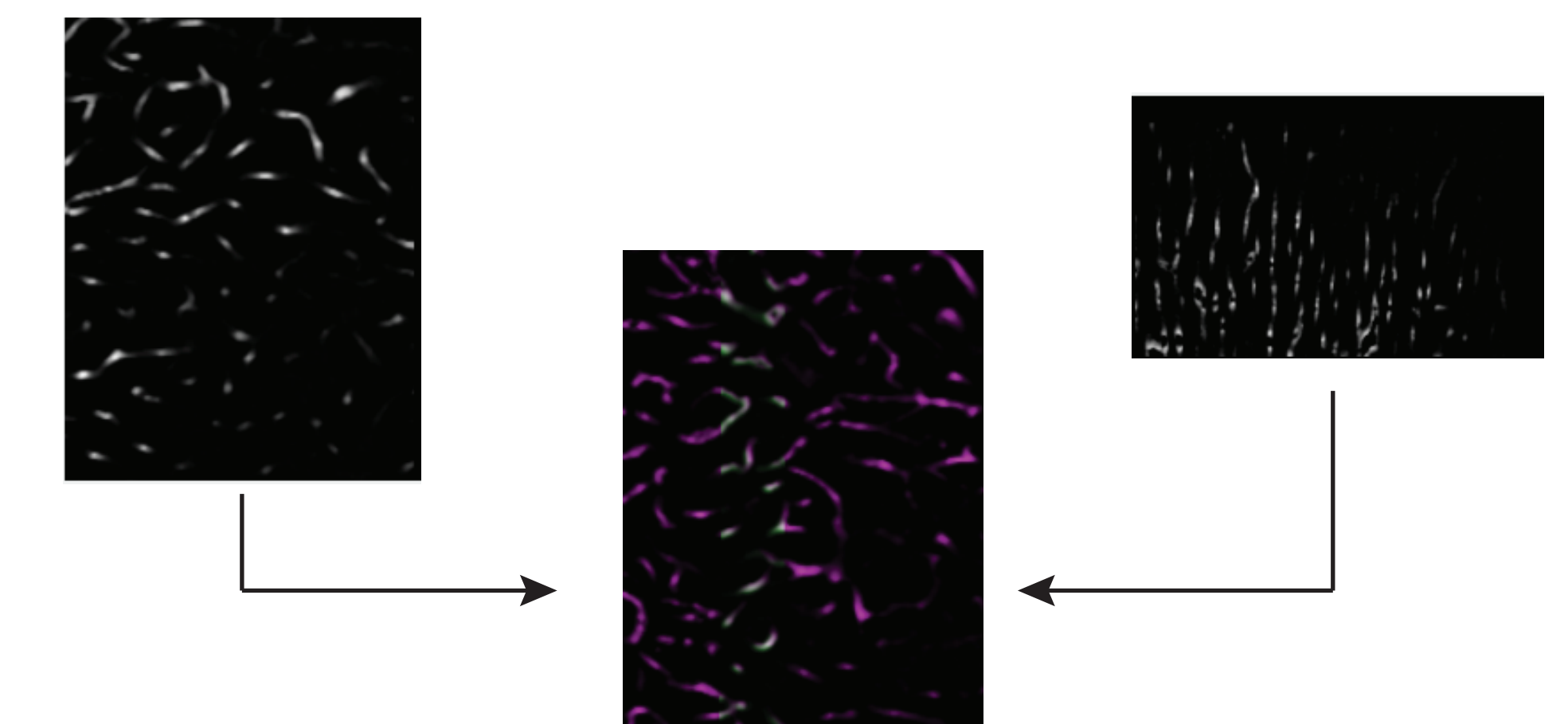
Synthetic data tests

Intended to test if the metric and algorithm are suitable for the characteristics of the data. This tests compare one image to one piece of itself that has been transformed, with some added noise too.



This figure shows, as an example, the good behavior of the tested metric to rotations on a single axis.

The following figure shows good results when aligning one image to one cut of itself that has been rotated and scaled.

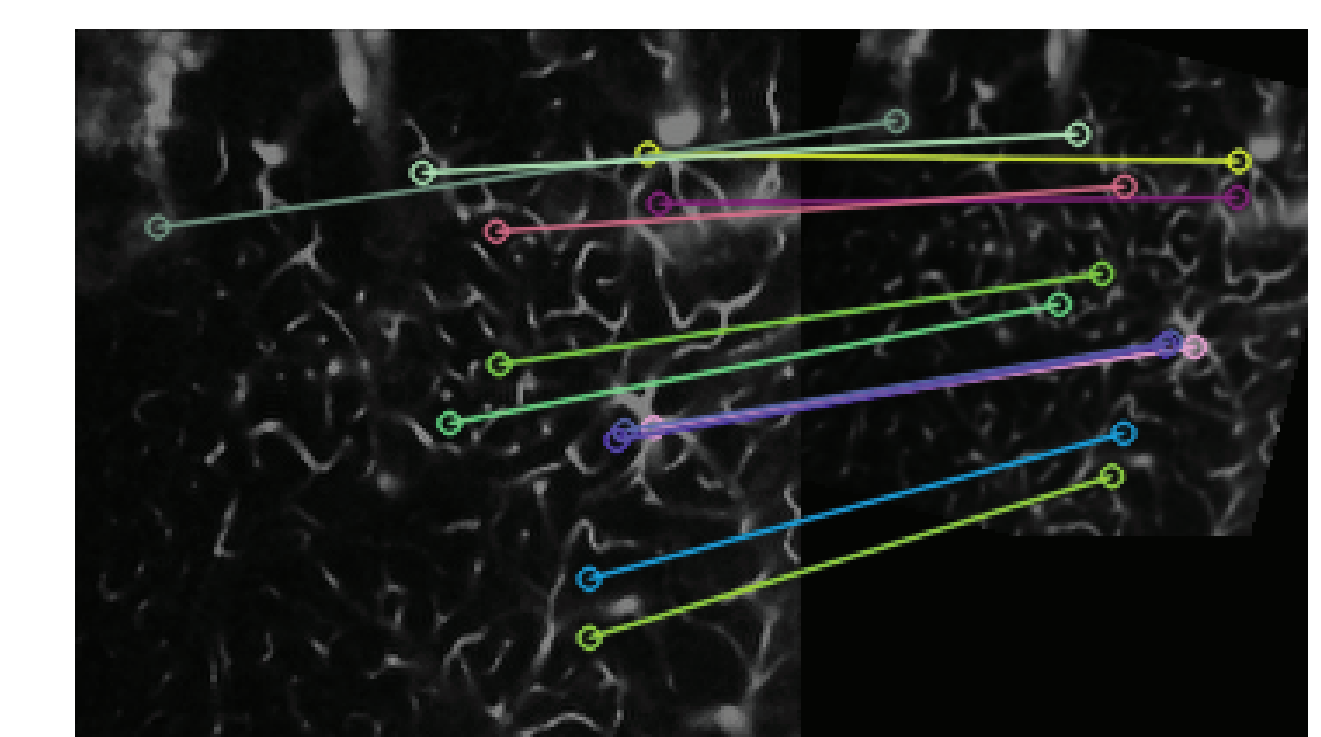


Next Year Planning

Research new and more appropriate methods for preprocessing the images, particularly anisotropic diffusion algorithms for background removal

Research into using algorithms such as SIFT for feature detection and matching, instead of the initial search stage.

SIFT works well in 2D synthetic tests. After the matching is done, is easy to estimate the transformation matrix using least squares or RANSAC.



Research methods for automatic segmentation and analysis of blood vessels, to automatically asses when a vessel is present in one image and not the other.