Measuring Vessel Diameter and Vascular Permeability (Pv) in Volumetric T-series

Galvo-galvo Z-stacks of the dextran dye (FITC) were acquired at 512x512 pixels (0.3 μm/pixel) using a Nikon 16X objective (NA 0.8) with 6X zoom, employing lambda=800nm illumination at a power output of 50-60mW. Z-stacks were captured at 5 μm step intervals, covering a depth ranging from approximately 200-300 μm. A time series (T) of 10 second duration was acquired at each Z-interval, comprising 10 frames (1Hz).

Prior to analysis, acquired image stacks (16-bit TIF files) underwent preprocessing to mitigate PMT shot noise using a 3D median filter (2 pixels, FIJI/ImageJ). Line scan areas measuring 14-50x6 μm were manually selected throughout the Z-stack by an experimenter blinded to the experimental history, with a focus on targeting smaller diameter vessels (3-10 μm).

Following selection and storage of line scan regions, TIF-image stacks were batch-processed, and intensity profiles were collected for each TIF, encompassing each group of 10 frames/every 5 μm-Z-step. Custom-written Matlab and Python scripts were employed to determine the full-width half maximum (FWHM) for each line-profile, allowing estimation of vessel diameter (*will work towards making code public access as appropriate via GitHub in the future). In addition,as shown in Figure 1, values for Fv, Fe, and Fi were calculated, where Fv is the mean fluorescence from the interior voxels, Fi is the total fluorescence from the vessel wall voxels, and Fe is the total fluorescence from all exterior points including those on the vessel wall (Fi). Vessel wall permeability (Pv) was calculated as the mean of Pv = Fe/Fv over 10sec (10 T-frames was subsequently calculated by dividing Fe (2x10 voxels) by Fv (9 voxels).