Quantitative Perfusion Estimates from Two Photon Fluorescence Microscopy Maps

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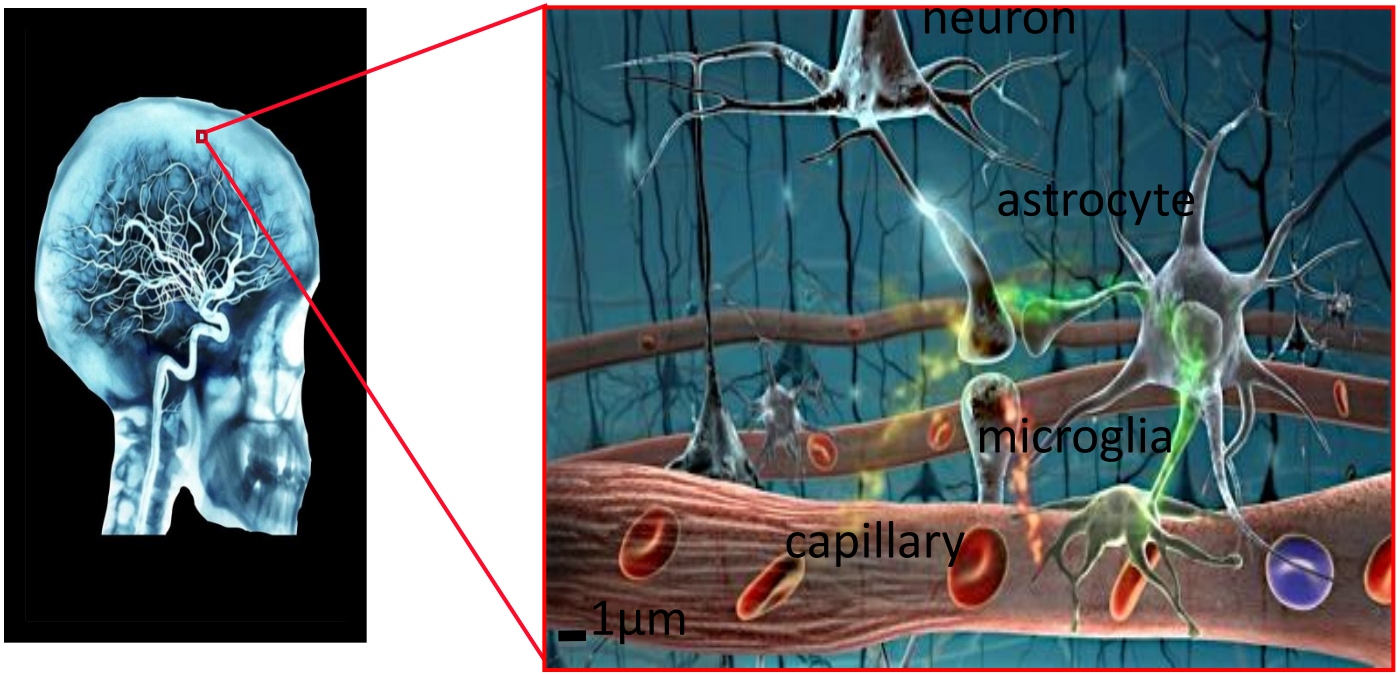
The Brain



2% total body weight
Consumes 20% O_2 and 25% of glucose available to the body

Neurovascular Coupling:
Changes in neuronal activity are tightly coupled to changes in blood flow and oxygenation

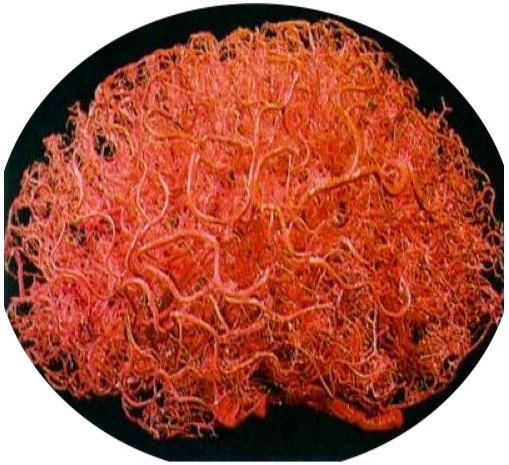
Neurovascular coupling

Brain Tissue

increased neuronal activity

Increased glial signaling

Increased metabolism demand



Cerebral Vasculature

adaptation in flow, volume, and oxygenation of the vascular bed

Mediators

NO, GABA, 5HT, NE, DA, Ach, NPY, K+

Why neurovascular coupling is important?

- Many models of neurovascular coupling have been proposed.
  - relating BOLD fMRI to neural activity seen on the millimeter scale

- Mechanisms underlying neurovascular coupling are not fully understood
  - application of BOLD fMRI to the studies of stroke and brain diseases has had limited scope
  - the basic question is “what is the BOLD fMRI signal is measuring ?” or the lack of detailed understanding of the neurovascular coupling at the micron level.

- No model exists at the micron level that can help in understanding the link between neuronal and vascular 3D network state.

- Understanding neurovascular coupling at the micron scale will support:
  - bottom-up modeling of BOLD fMRI signal
  - platform for characterization of alterations in brain hemodynamics in disease

The Challenge

How do you quantitatively characterize neurovascular coupling on the micron scale in vivo ?

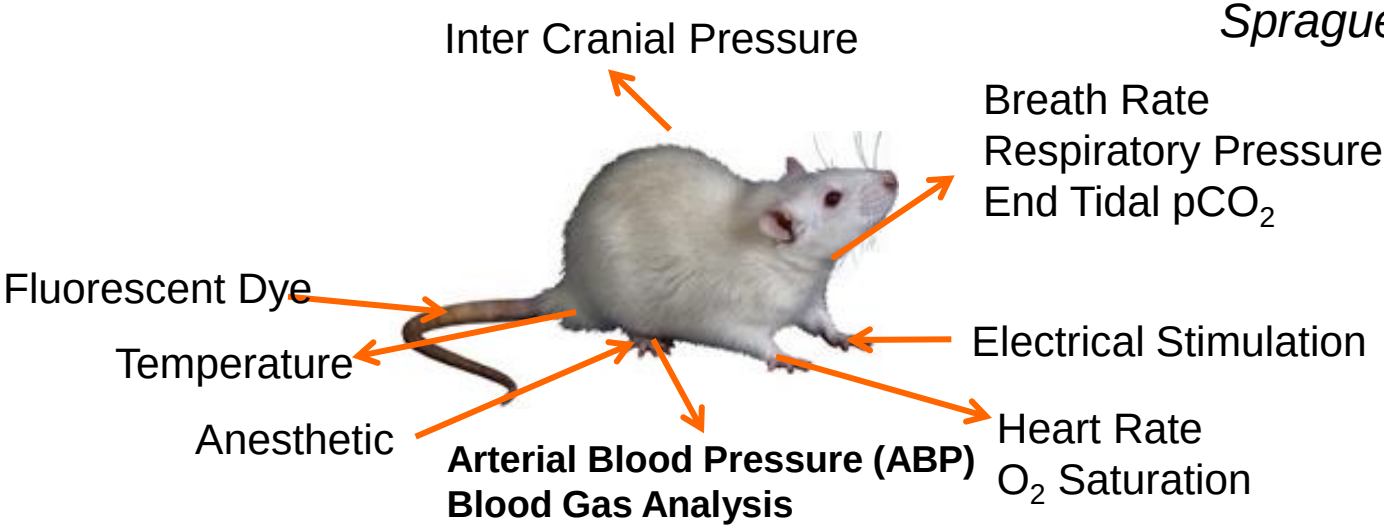
Goal:

Quantitative estimation of cerebral hemodynamics at the micron scale

- Cerebral blood flow (CBF) refers to volume per minute moving through the vessels (nL/min)
- Perfusion refers to nutrient supply by the blood through the capillary bed in the brain tissue (mL/g/min)

Animal preparation

Sprague-Dawley rats (120-150g)

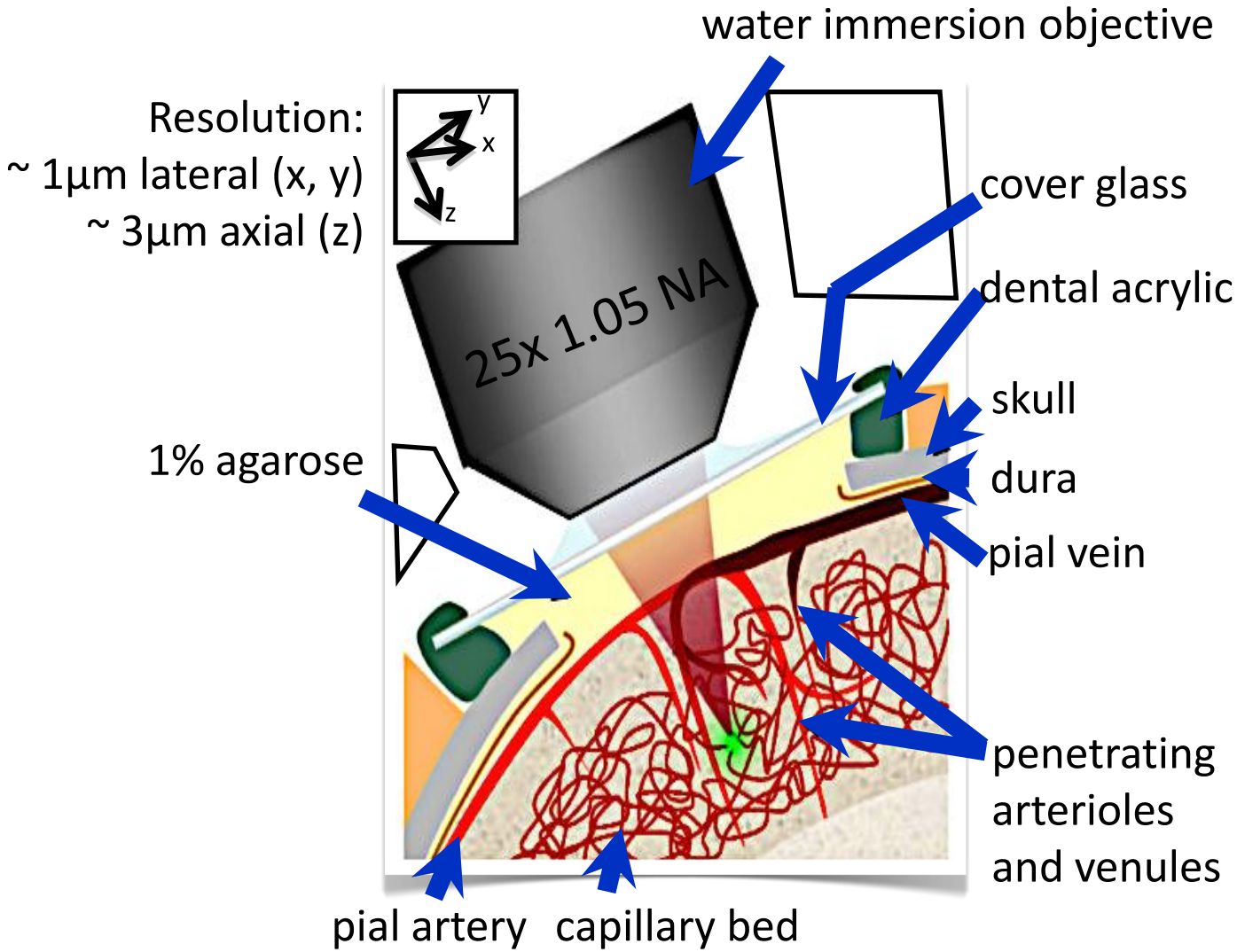


1. Surgery under iso-flourane
2. Tracheotomy + mechanical ventilation
3. Cannulation of tail vein, femoral artery and vein
4. ICP recording via transducer placed inside subarachnoid space of the spine (lumbar region)
5. Craniotomy over S1FL
6. Imaging under alpha-chloralose
7. IV administration of fluorescent dextran (Texas Red)

Imaging during:

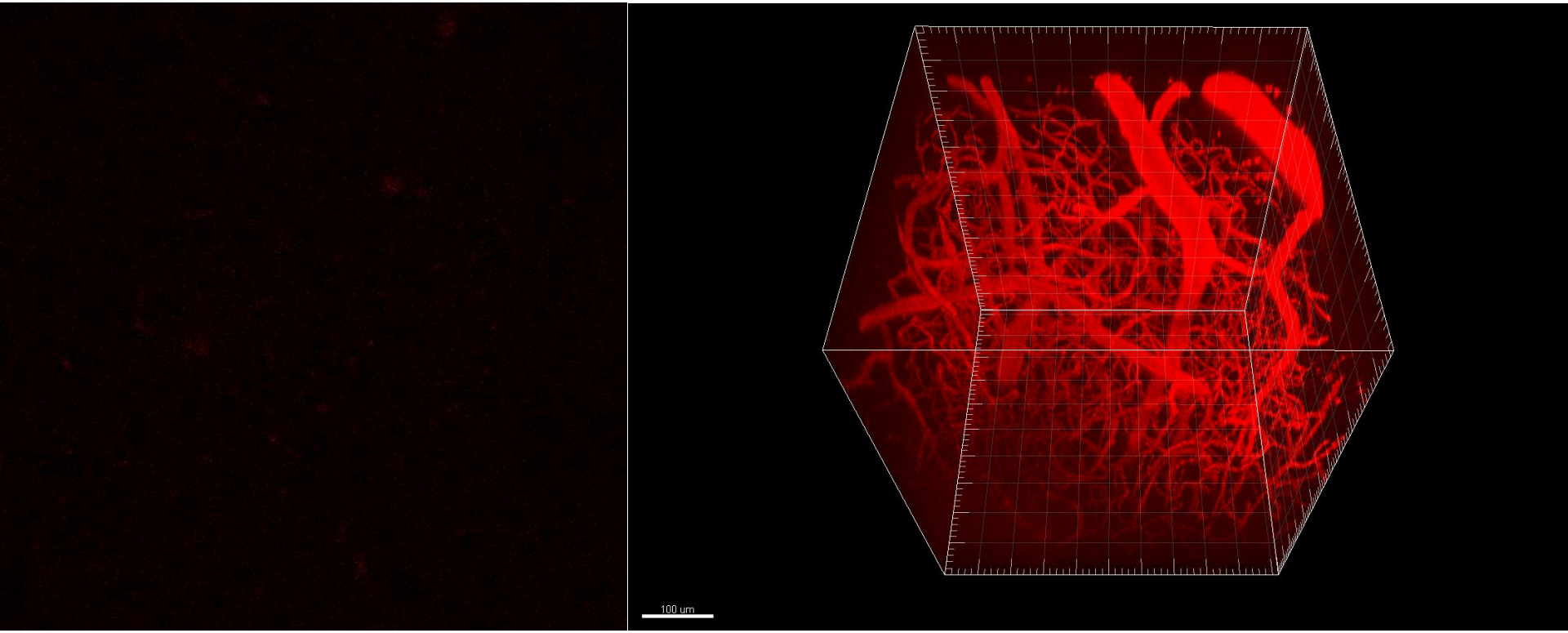
a. Anatomical 3D image following 33 mg/kg bolus Texas red dextran
a. 2D time series of bolus injection

2PFM Set-up



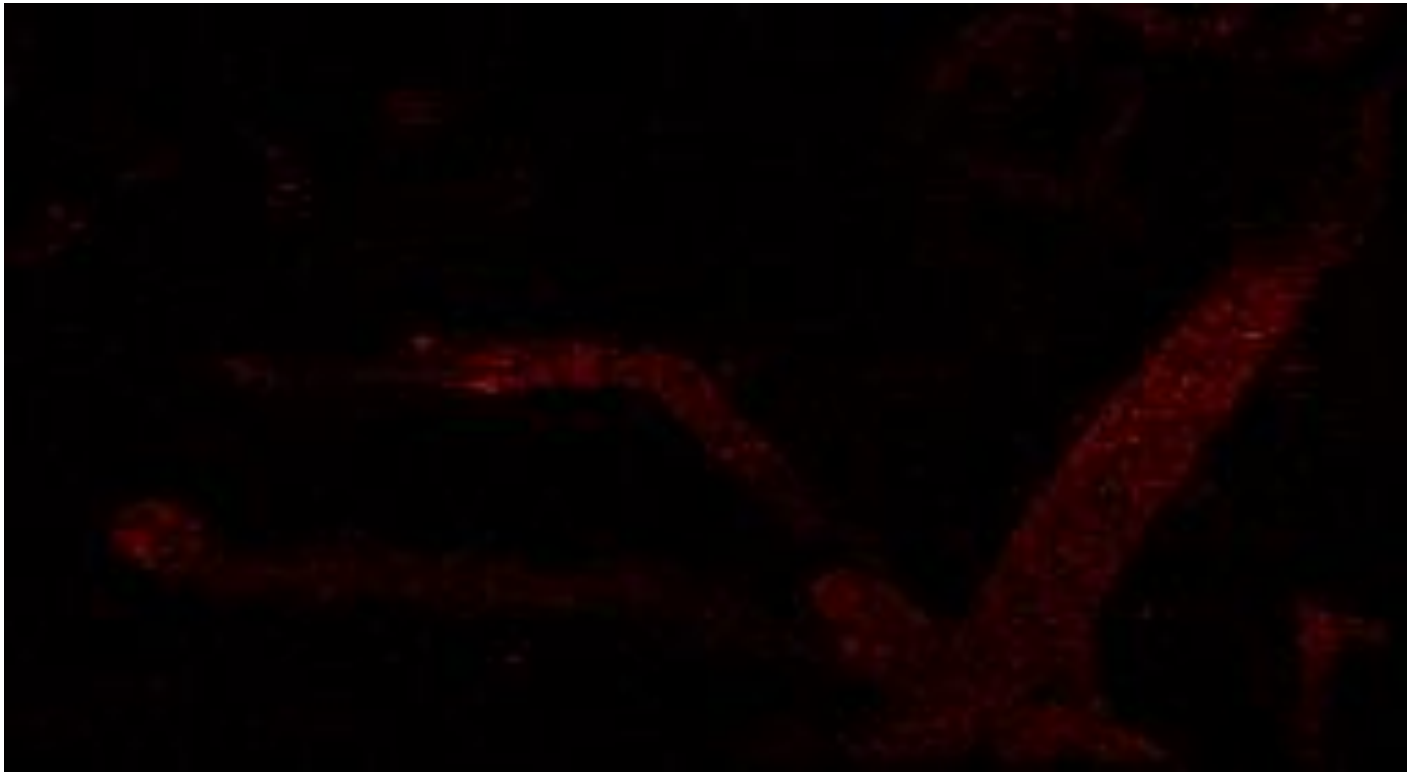
Adapted from - Kherlopian *et al. BMC Systems Biology* 2008 **2**:74

3D anatomical stacks acquisition



- Microvasculature clearly visible up to 600µm.
- Single 2D imaging plane is ~ 512 x 512 µm
- Lateral resolution 1µm and axial resolution 3 µm

2D bolus time series



- Single 2D imaging plane is ~ 250 x 250 μm
- ~ 50 μm below the cortical surface at 0.31 ± 0.07 fps
- Spatial resolution 1.59 μm/pixel

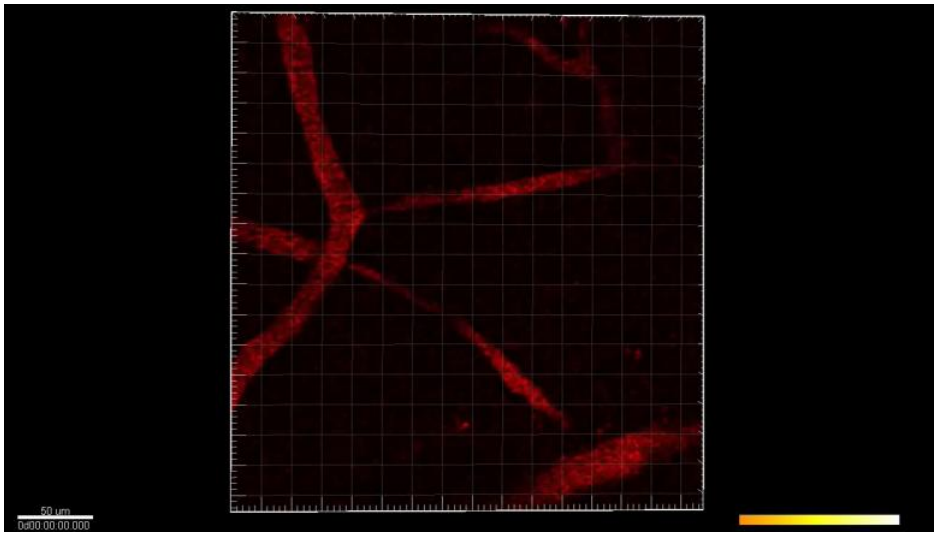
Analysis of perfusion estimation

- Estimation of transit time (TT) from the bolus time series.
- Identification of closed paths between vessels in the FOV of the bolus tracking plane.
- Estimation of transit time in the individual segments (multiple paths).
- Estimation of cerebral blood flow (CBF) and tissue volume irrigated.

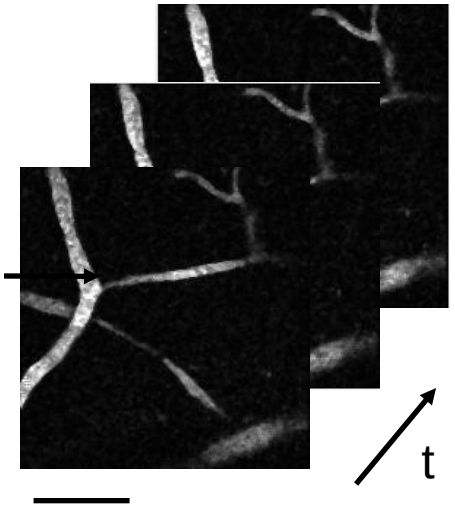
Transit time estimation from bolus time series

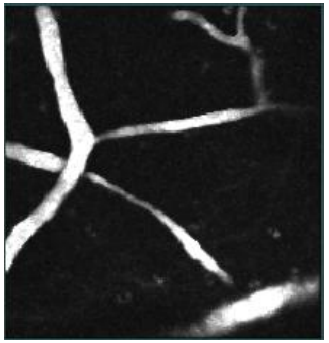
Pre-processing of bolus time passage

bolus time series

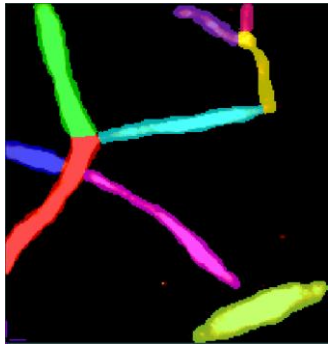


2D spatial median filtering





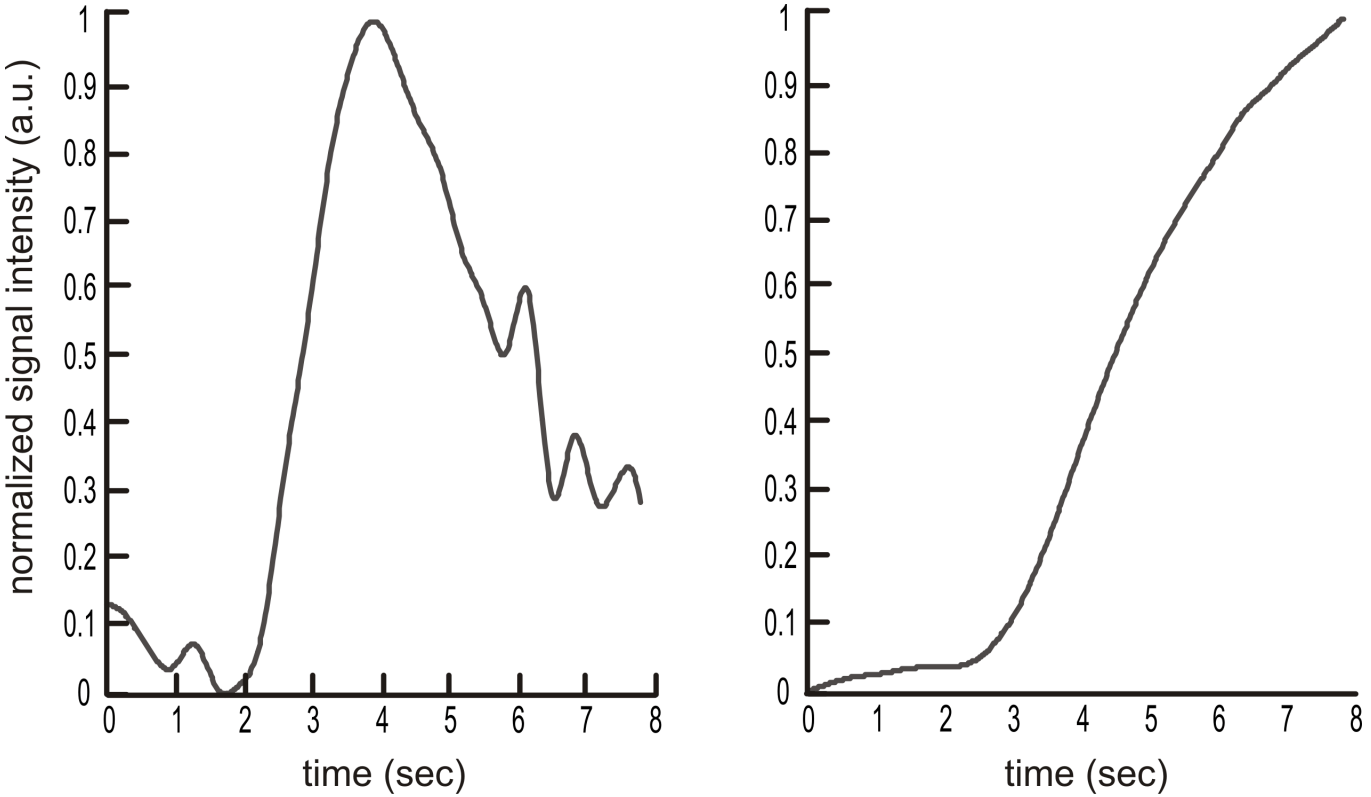
MIP



vessels in FOV labeled

Transit time estimation

- The signal intensity curves from bolus passage are normalized and integrated over time.



- We model the bolus passage as a linear dynamical process.

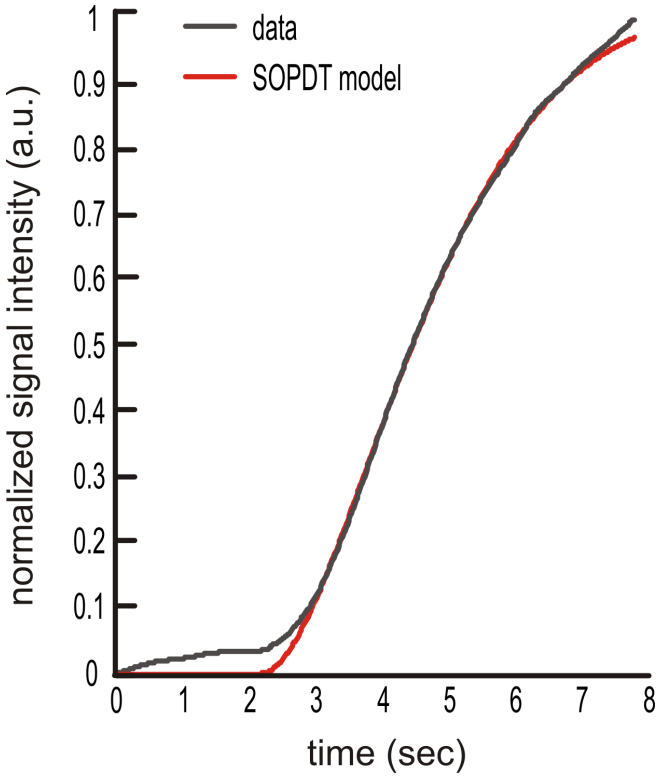
Second-order plus dead time model (SOPDT)

- SOPDT model function (Rangiah et al. 2006) was used to estimate damping ratio (ξ), natural frequency (ω) and dead time (θ).

- Laplace domain transfer functions were then calculated:

$$G(s) = \frac{e^{-\theta s}}{s^2 + 2\xi\omega_n + \omega_n^2}$$

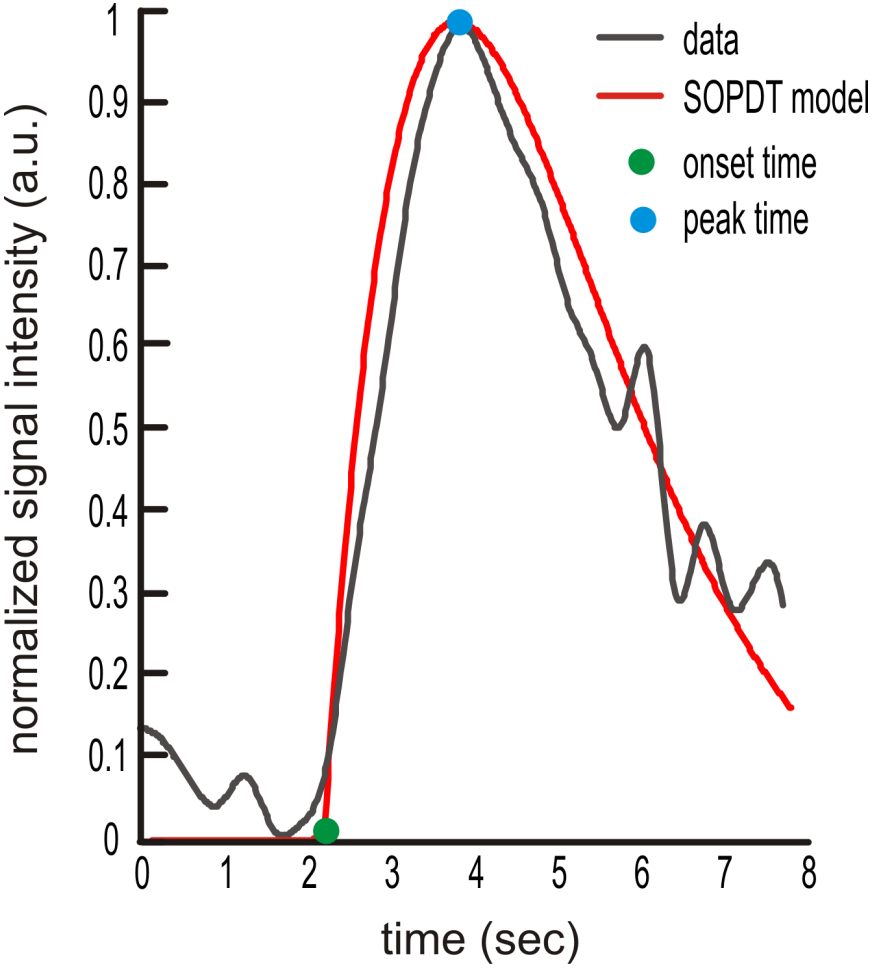
$$G_6(s) = \frac{e^{-2.2s}}{s^2 + 0.0177s + 0.0001}$$



Second-order plus dead time model (SOPDT)

- Impulse response of the transfer functions was used to calculate the onset time and peak time.

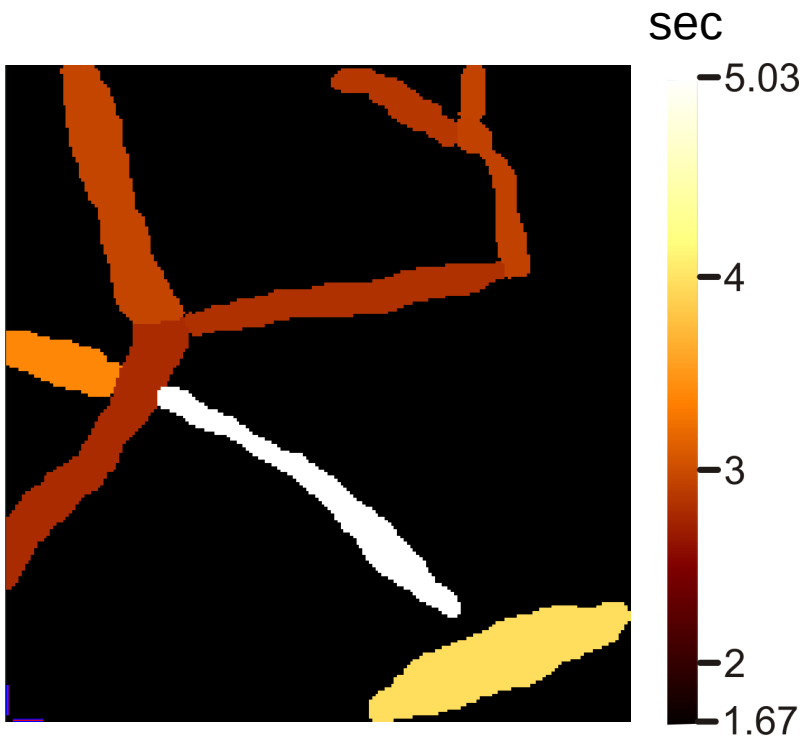
$$t_o = \theta \; ; \; t_p = \frac{\tan^{-1}\left(\sqrt{1-\xi^2}\,/\,\xi\right)}{\omega_n \sqrt{1-\xi^2}}$$

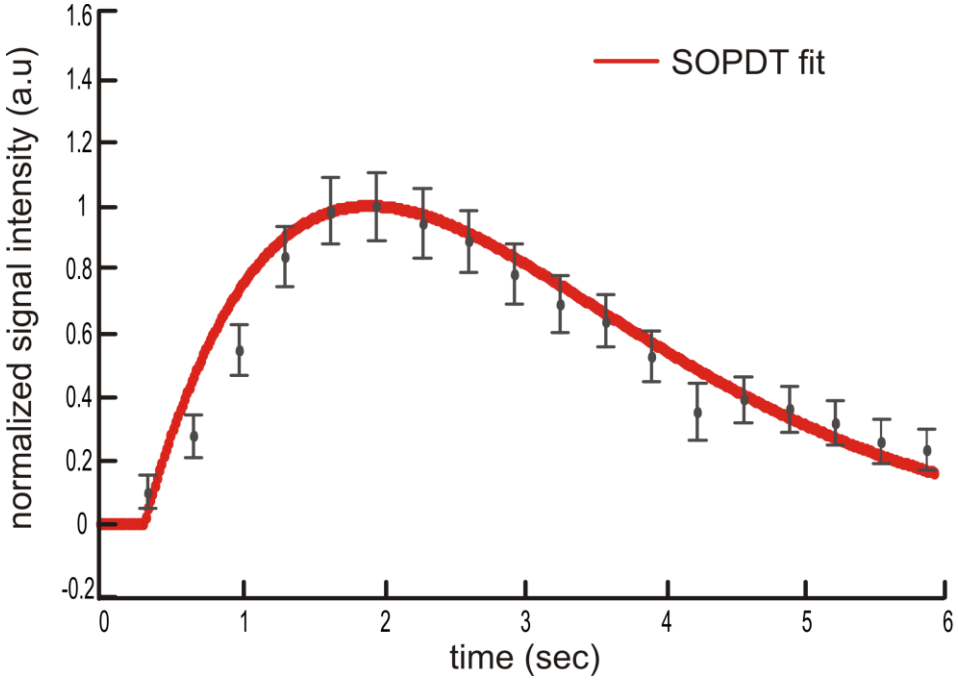


Transit time estimation

- Transit time (normalized to earliest onset time) is computed as

$$tt = (t_o + t_p) - \min(t_o)$$

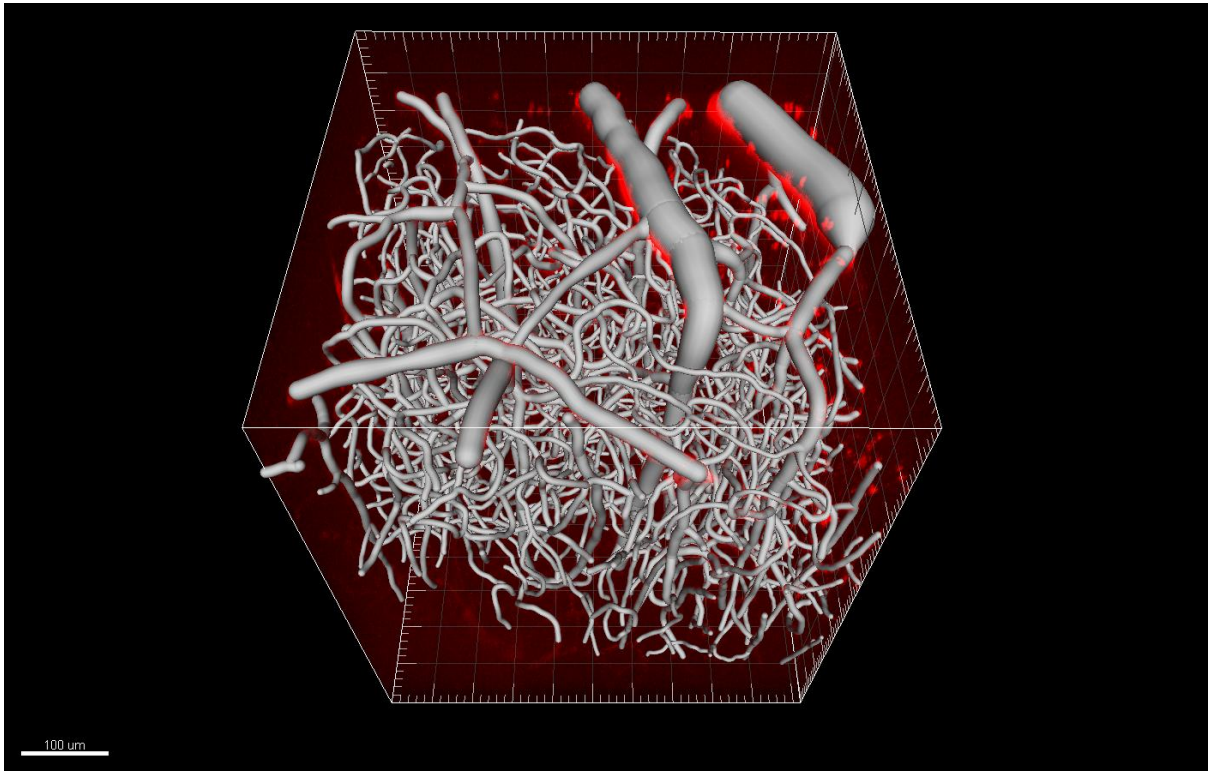




Identification of closed paths and estimation of TT in the individual segments

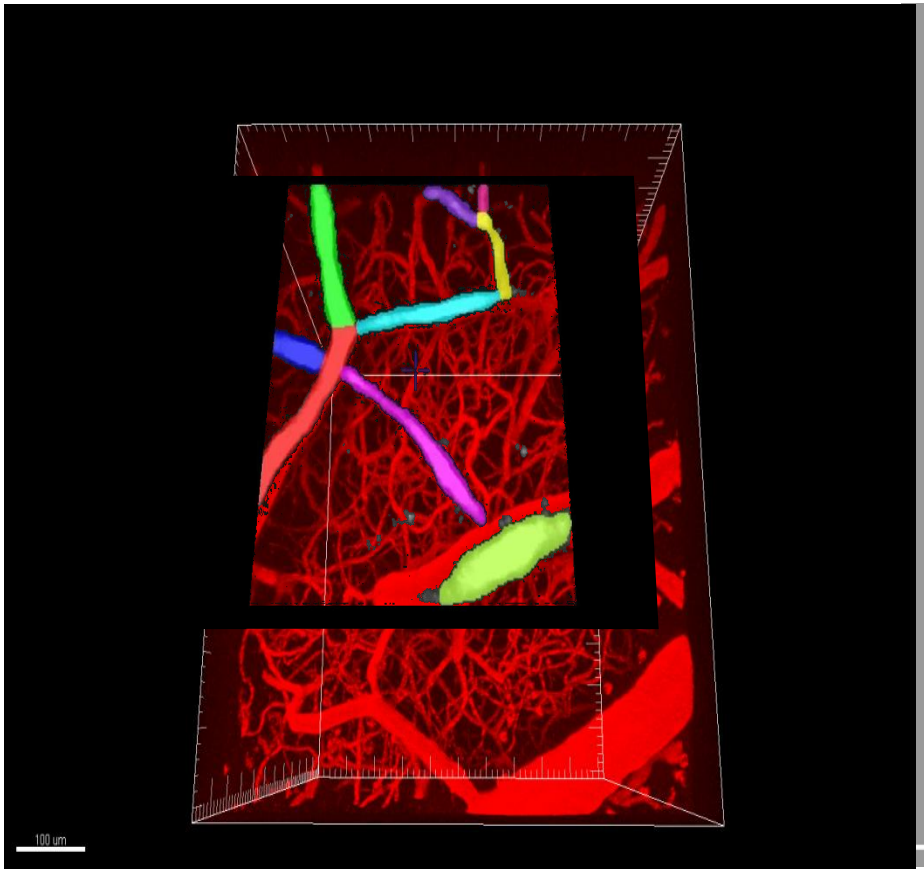
Segmentation of the 3D vascular stacks

- Imaris (Bitplane Scientific Software) was used for semi-automated segmentation of the 3D vascular network.

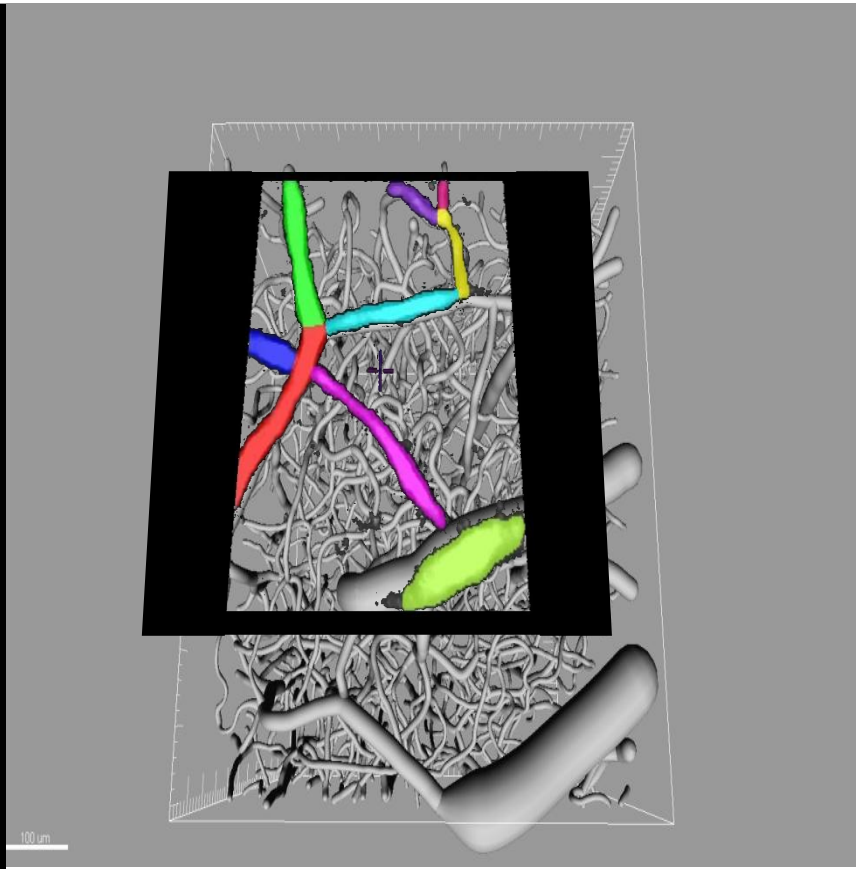


- Vertex-wise radii and x,y,z coordinates

Registration of bolus plane to the 3D network



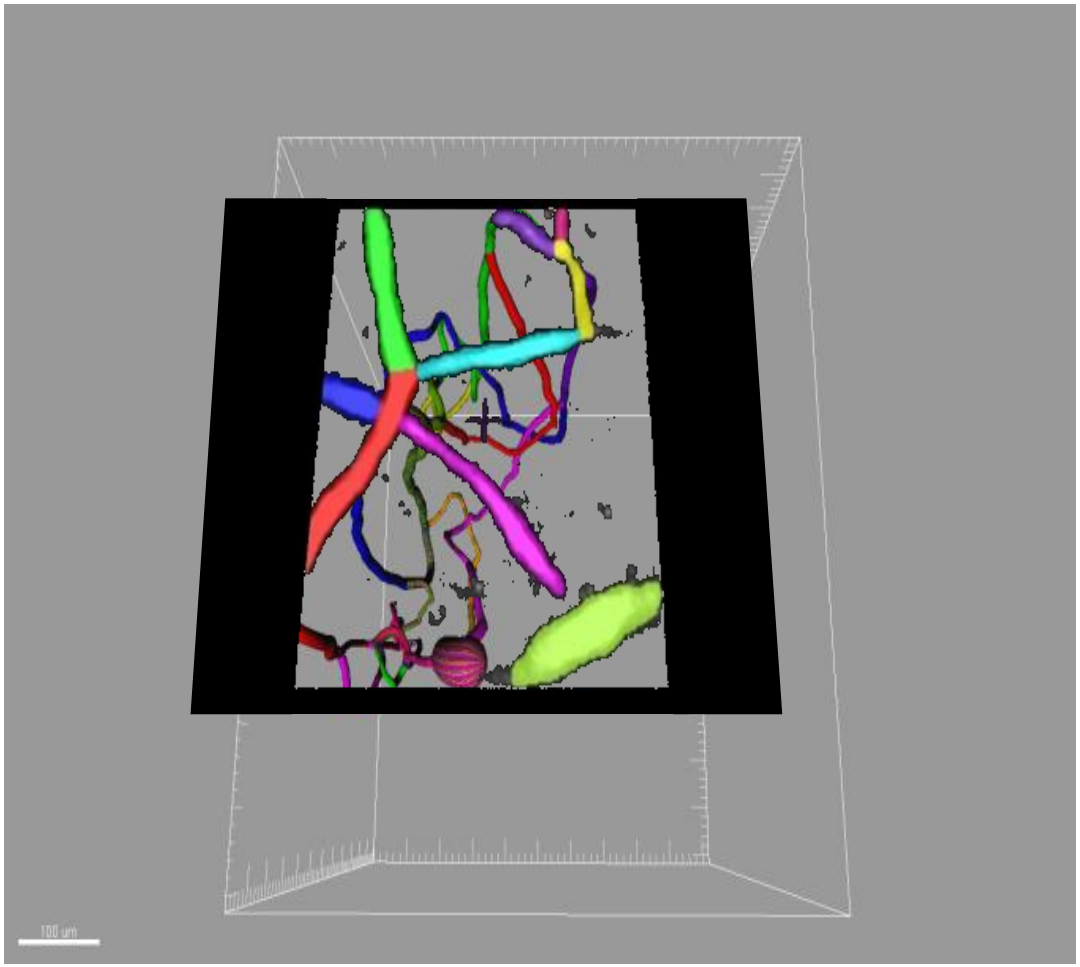
2D image plane from bolus tracking on the 3D image



2D image plane from bolus tracking on the 3D segmented image

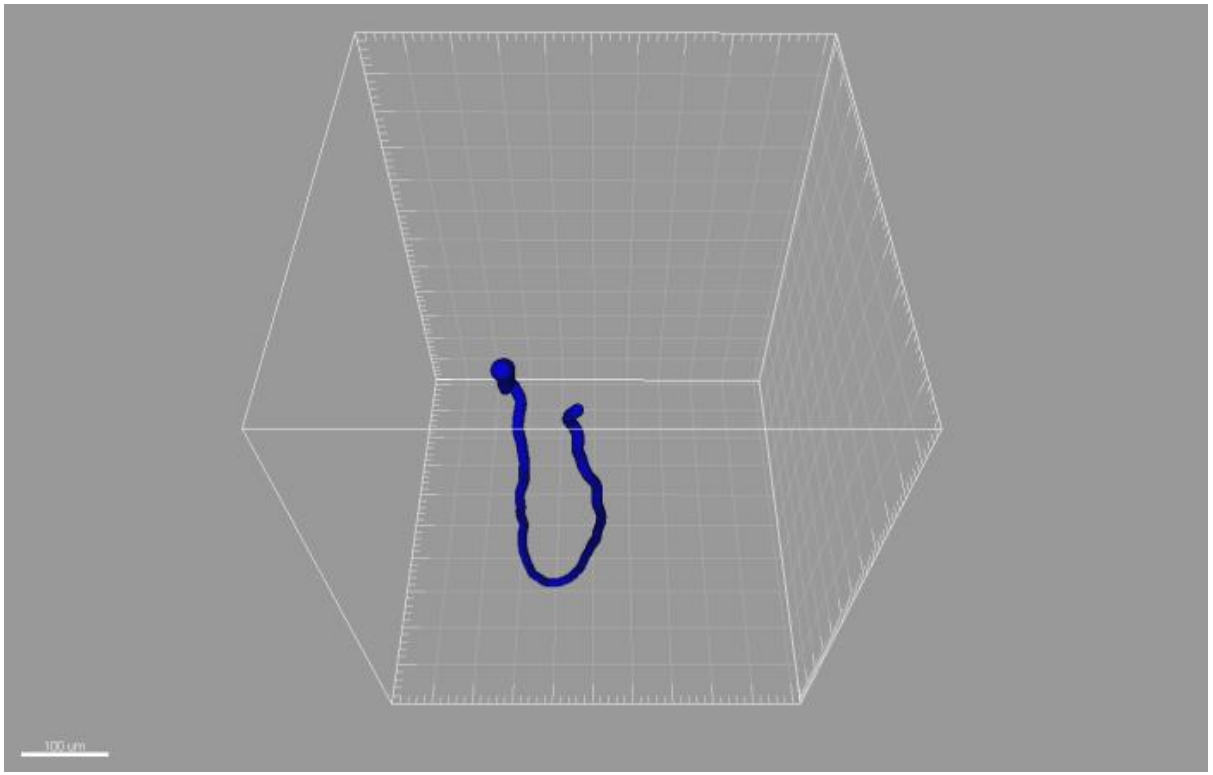
Closed path identification

- Closed path identification between any two vessels of the bolus tracking plane by tracing through the 3D network.



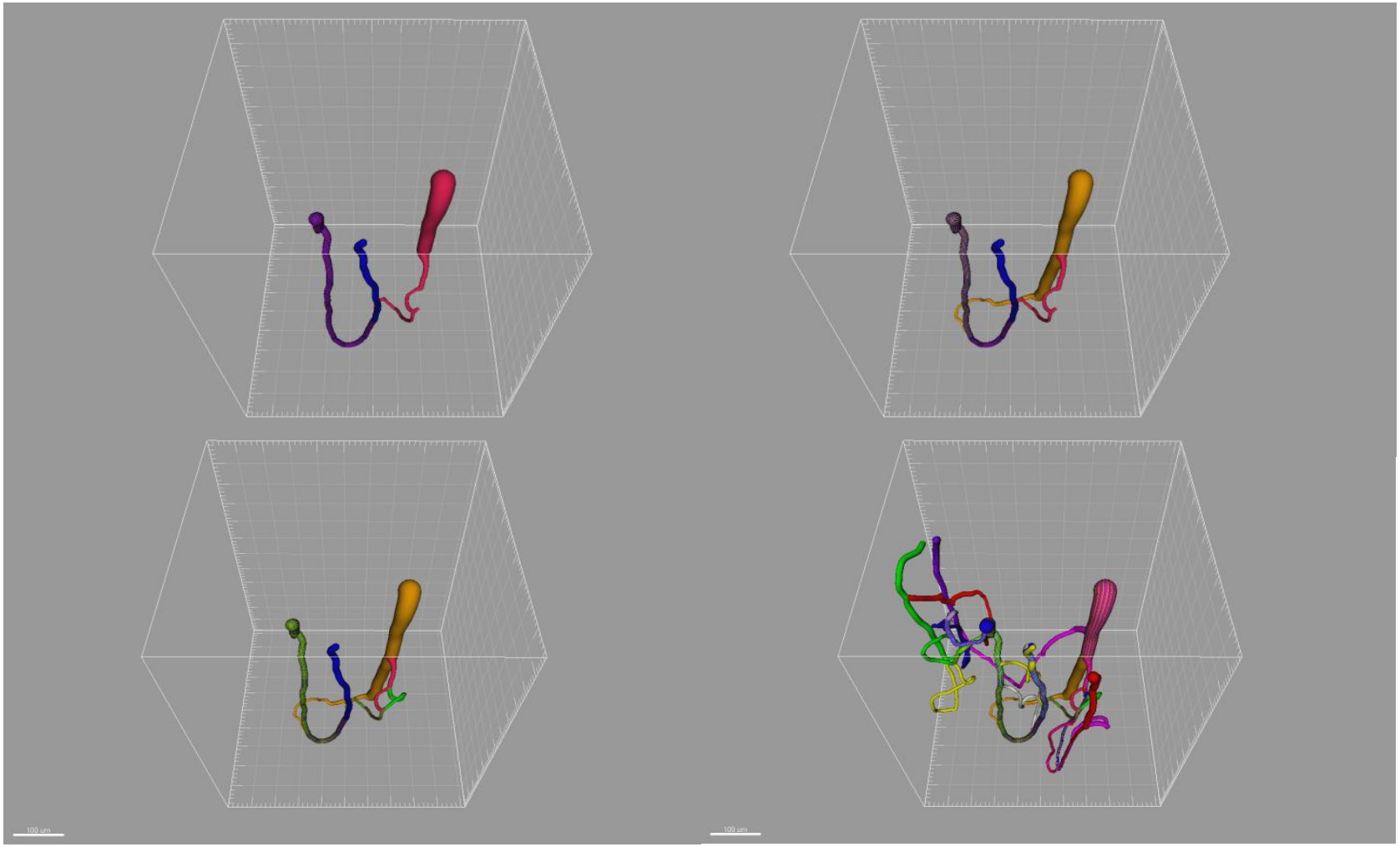
Perfusion estimation – closed paths

- For direct closed paths, perfusion and CBF estimation is can be computed easily
- CBF = CBV/TT (from the central volume principle)
- Perfusion = CBF/tissue volume irrigated

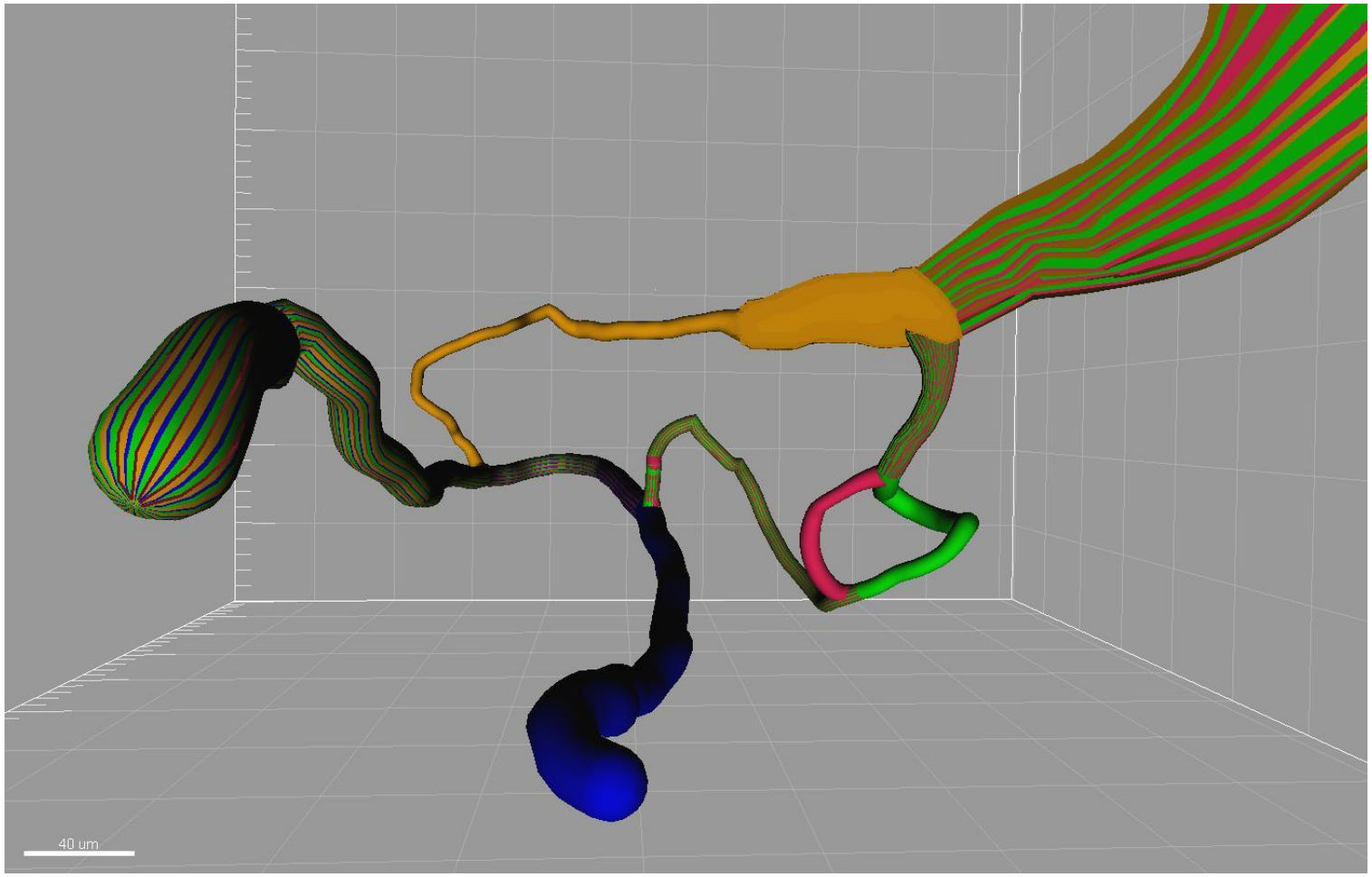


Perfusion estimation - multiple paths

- But, what if we have multiple connecting paths? How do you estimate perfusion in these individual segments?



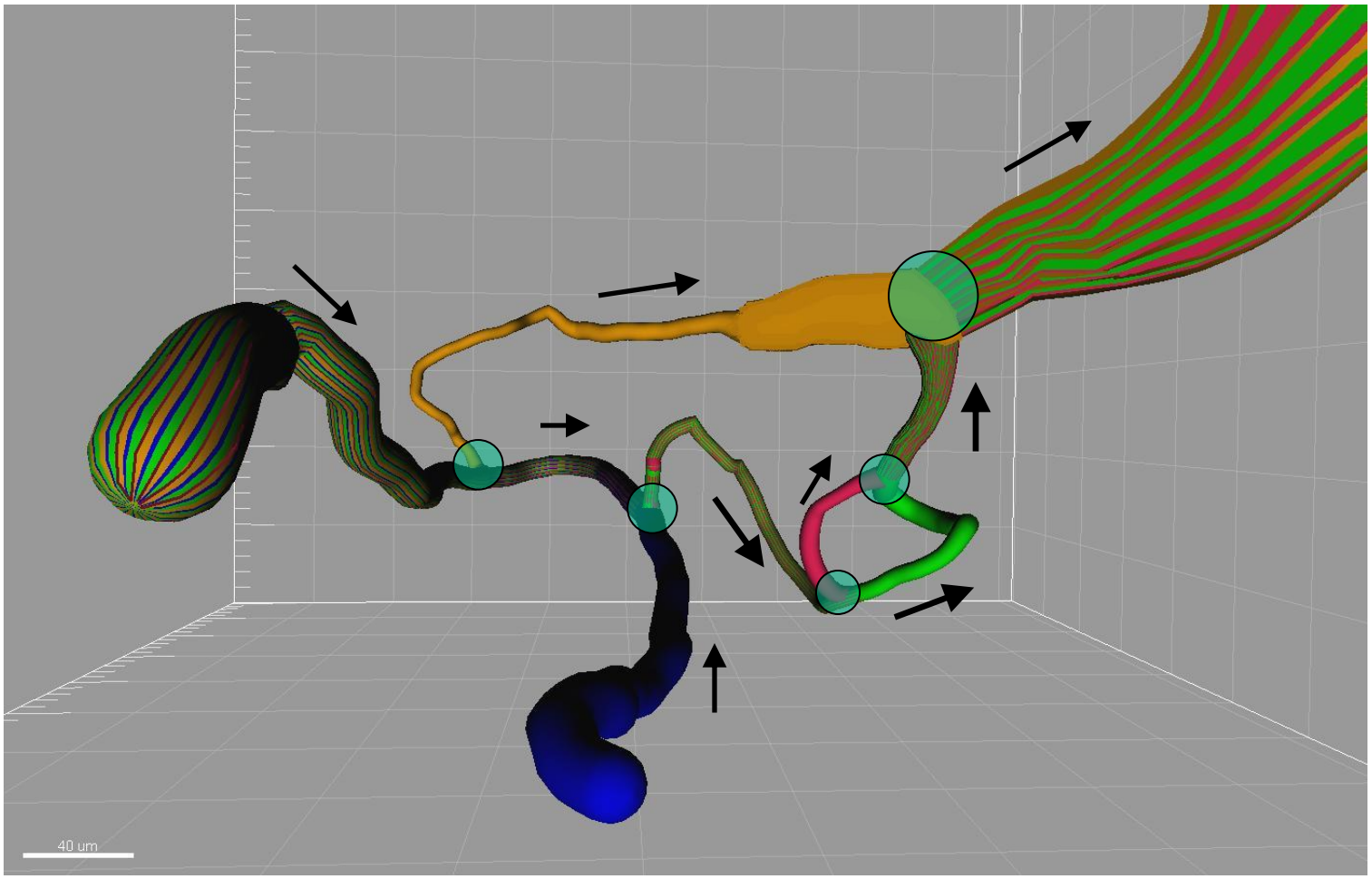
The problem in multiple paths



- We need to analyze the contributions of CBF in each of the segments

Modeling CBF in individual segments

- We approach the problem by modeling CBF as current flowing in a closed path.



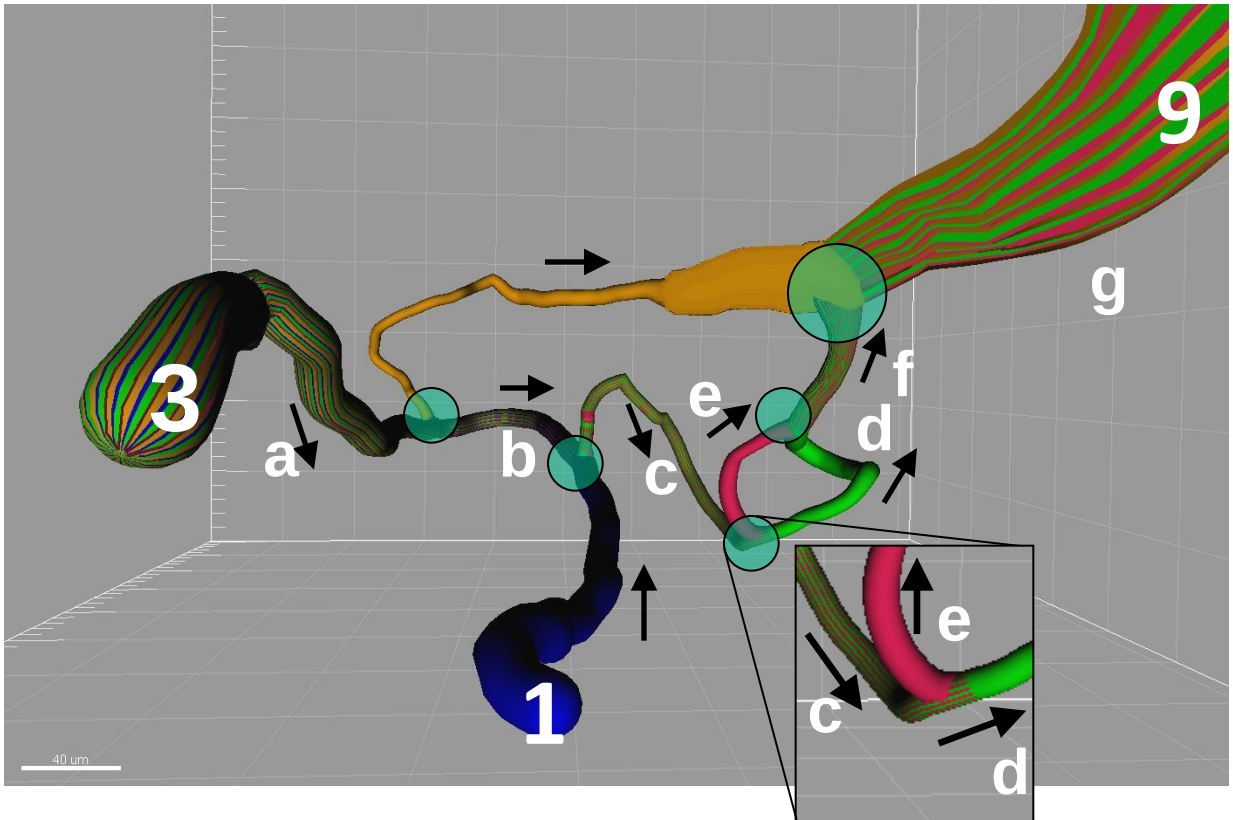
TT estimation in individual segments

- We solve for the unknown transit time in the individual segments based on equations of transit time and CBF.

$$tt_a + tt_b + tt_c + tt_d + tt_f + tt_g = tt_{3-9}$$

$$tt_a + tt_b + tt_c + tt_e + tt_f + tt_g = tt_{3-9}$$

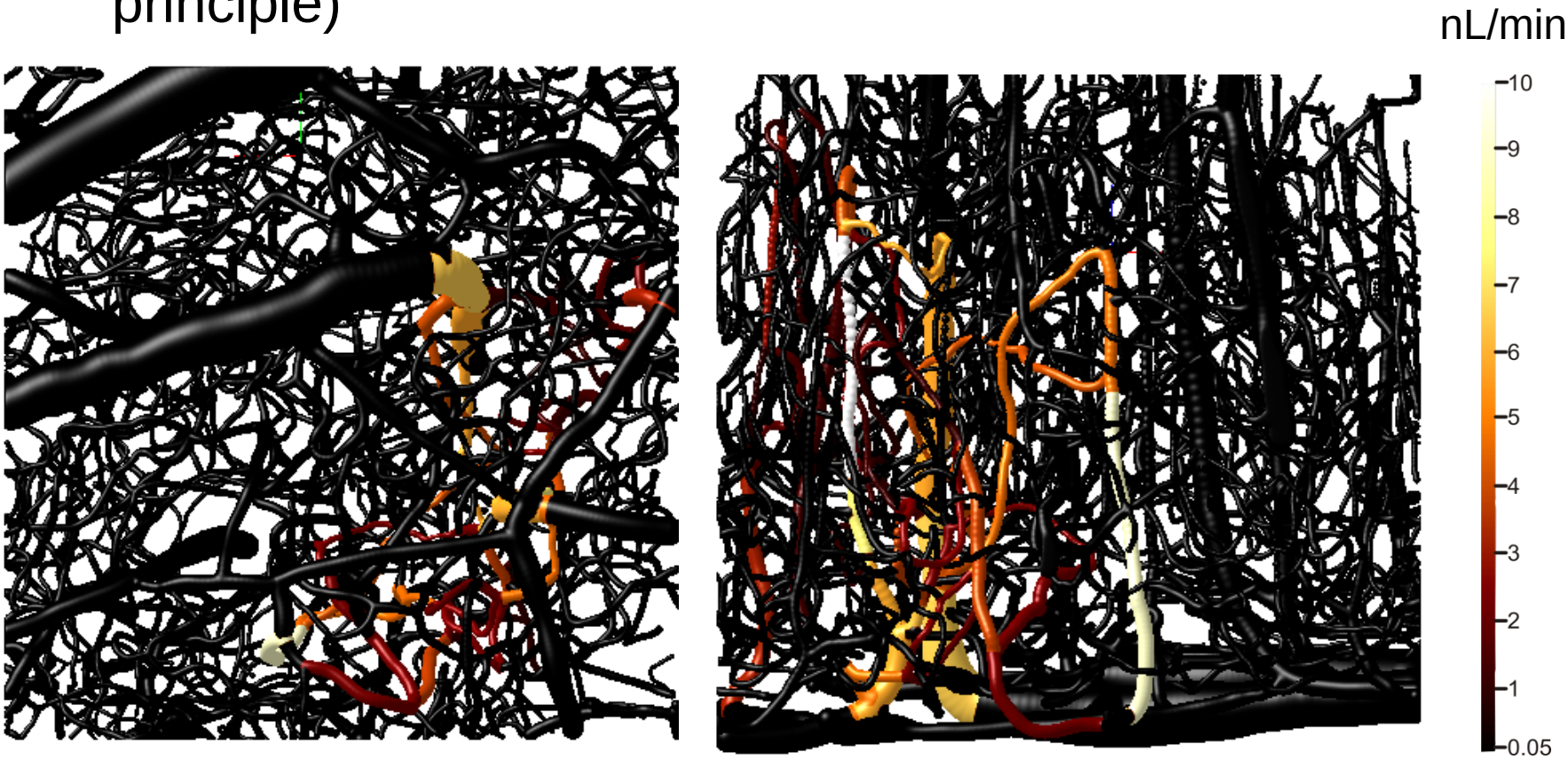
$$CBV_c / tt_c - CBV_e / tt_e - CBV_d / tt_d = 0$$



Estimation of CBF and tissue volume irrigated by the individual segments

CBF estimation in individual segments

- We can compute CBF=CBV/TT (central volume principle)



- CBF values coded in the individual segments

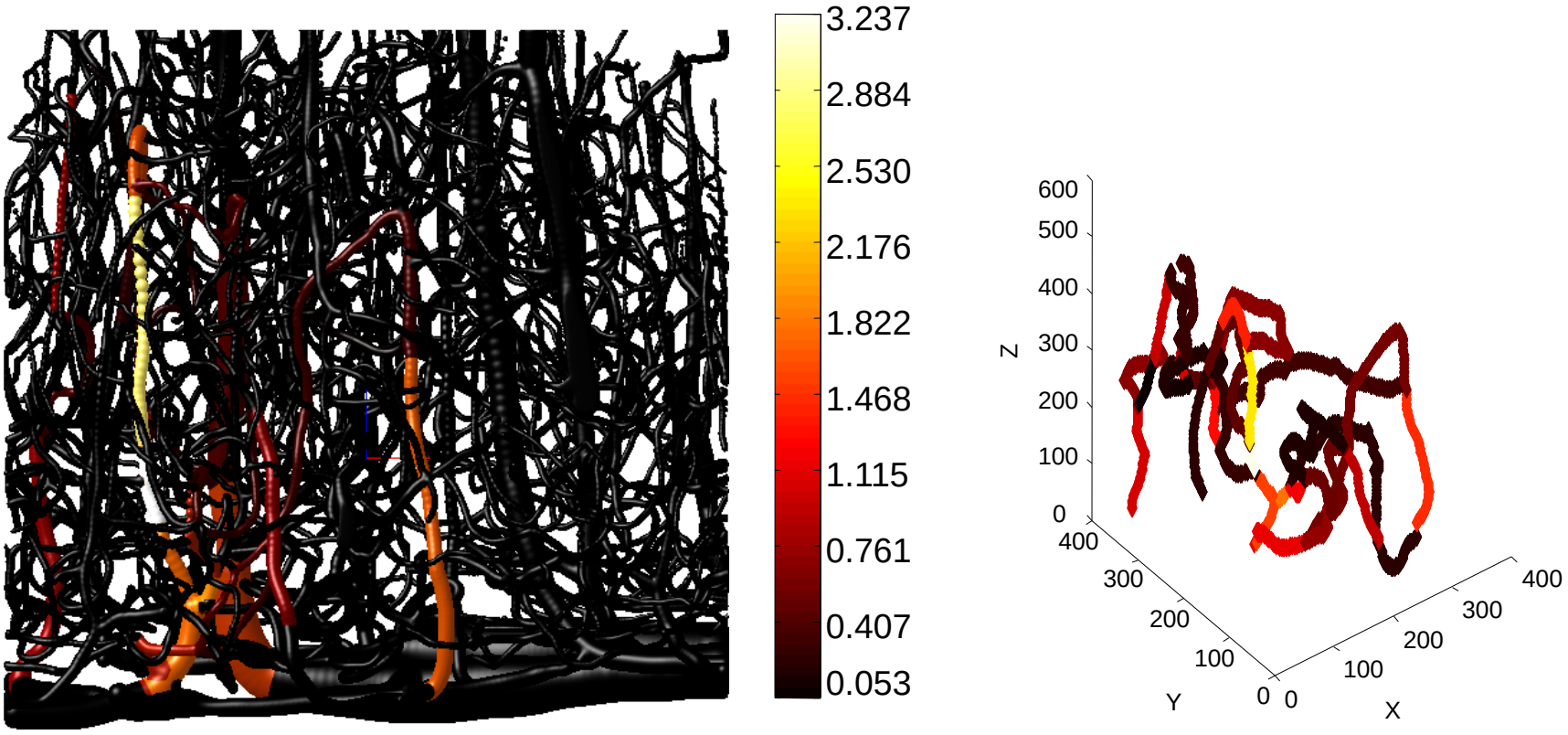
Estimation of tissue volume irrigated

- For perfusion, we need the tissue volume irrigated by the individual segments.

- Based upon the 2PFM literature, oxygen diffusion distance in the rat's somatosensory cortex is ~40-68μm (Masamoto et al. 2007).

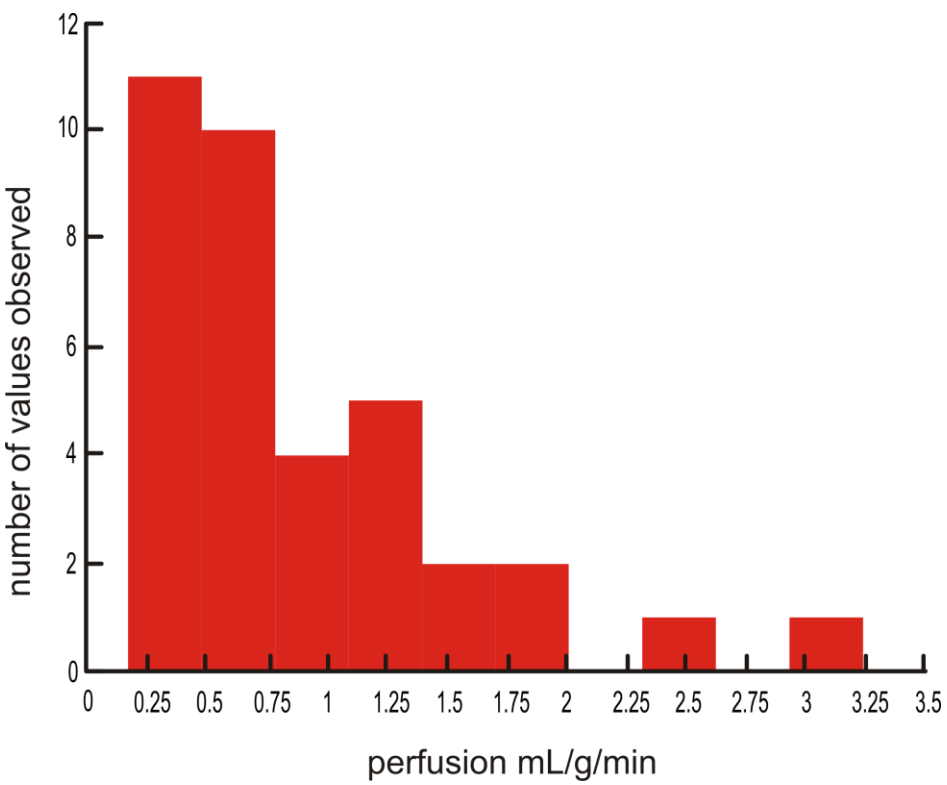
- Tissue volume irrigated can be estimated as a convolution of ~65 μm sphere and our vascular subtree centre lines.

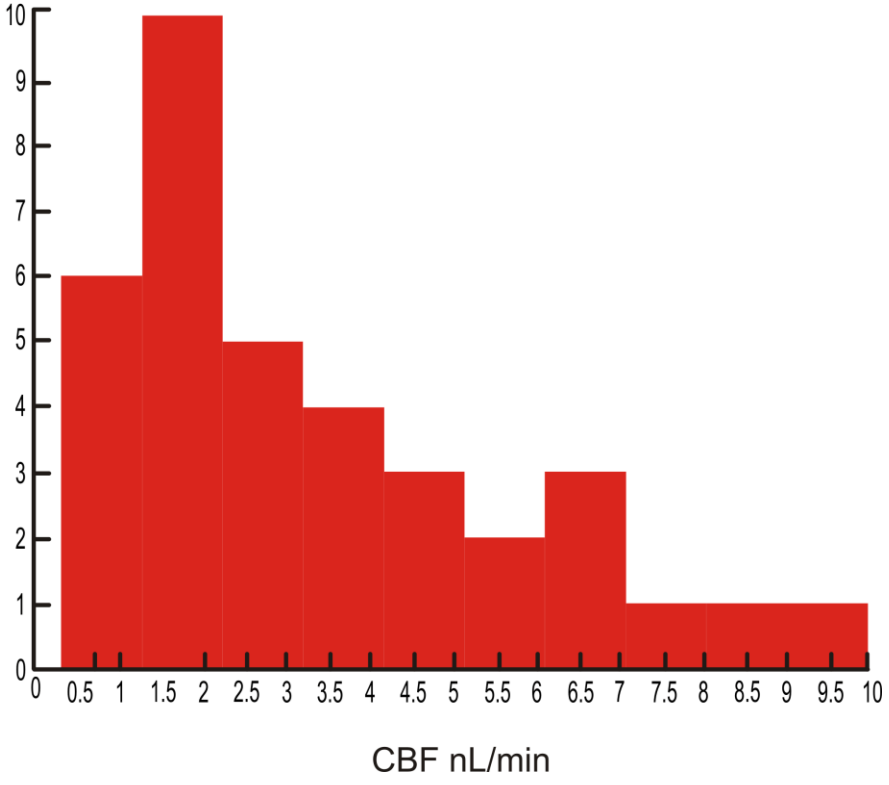
Perfusion estimation



- Perfusion values are coded in the individual segments.

Heterogeneity in perfusion and CBF





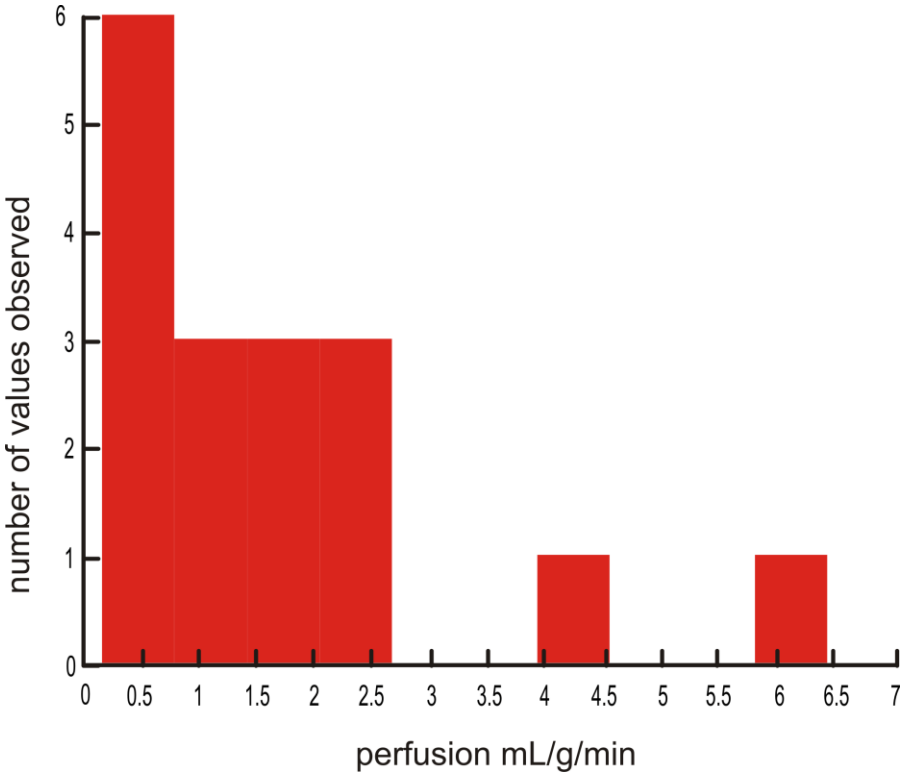
~16.67% low mean perfusion 0.20 ± 0.02 mL/g/min

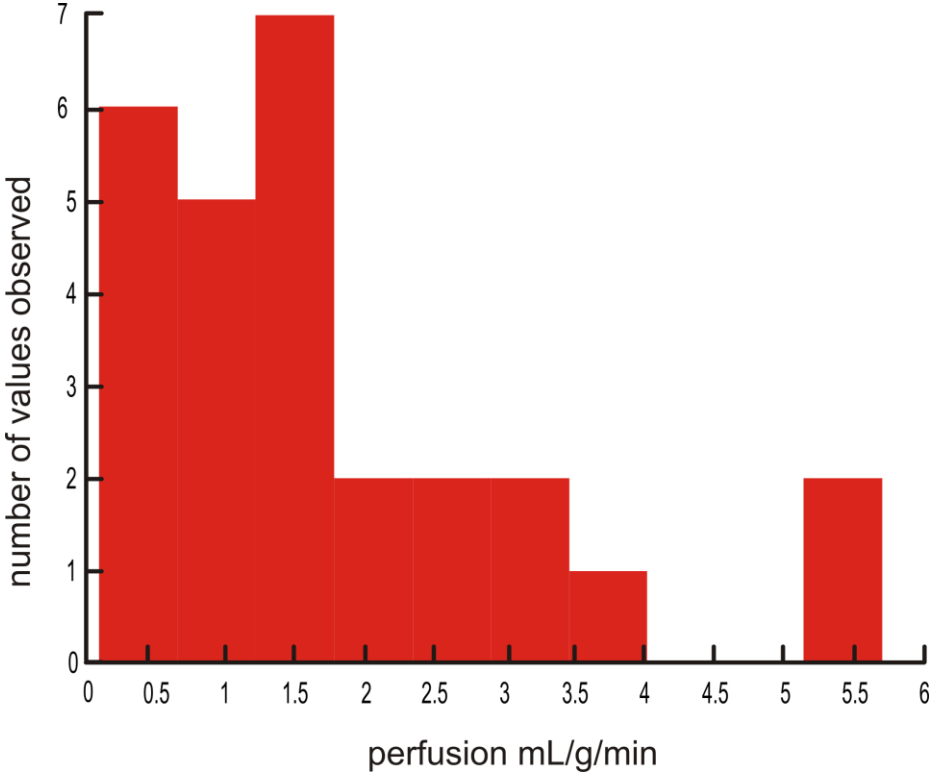
~61% physiological range 0.68 ± 0.29 mL/g/min

~19.44% mean perfusion 1.70 ± 0.38 mL/g/min

~2.74% perfusion value 3.23 mL/g/min

Heterogeneity in perfusion across rats





~7.4% low mean perfusion 0.16 ± 0.09 mL/g/min
~33.3% physiological range 0.64 ± 0.28 mL/g/min
~37.04% mean perfusion 1.72 ± 0.35 mL/g/min
~22.2% high mean perfusion 3.92 ± 1.24 mL/g/min

~11.76% low mean perfusion 0.18 ± 0.01 mL/g/min
~41.8% physiological range 0.68 ± 0.41 mL/g/min
~23.53% mean perfusion 1.77 ± 0.28 mL/g/min
~23.53% high mean perfusion 3.91 ± 1.83 mL/g/min

Perfusion estimation across modalities

- In the somatosensory cortex of rats under the same anesthesia protocol

  Optical Coherence Tomography (Boas 2010) ~0.51-0.68 mL/g/min

  Iodo[14C]antipyrine autoradiographic studies (Nakao 2001) ~0.6 mL/g/min

- Our results show *48.7%* of the segments within the physiological range with *median perfusion of 0.61 mL/g/min.*

Conclusion

- Results show evidence of heterogeneity in perfusion: we expect this heterogeneity to relate to local vascular density.

- A novel methodology to estimate perfusion at the micron level was developed: its application to a cohort of subjects may relate cortical microvascular topology and blood flow.

- Estimation of functional perfusion and CBF at the micron scale.

Acknowledgements

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