Analysis of Vascular Diameter and Velocity of Blood Flow using Two-Photon Laser Scanning Microscopy (TPLSM) Data

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Introduction

• We present a methodology to estimate the change in diameter and velocity in two-photon laser scanning microscopy (TPLSM) data.
• For diameter estimation, a contrast is needed between the vessel (wall) and the surrounding tissue; this is achieved by intravenous injection of a contrast agent (e.g. fluorescein-conjugated dextran).
• For velocity estimation, we need a contrast between blood cells and plasma.
• Analysis can estimate baseline diameter/velocity, spontaneous fluctuations (not related to stimulus), and stimulus-evoked changes (e.g. in response to a somatosensory or visual stimulus)
• Limits for diameter resolution: Ultimately the resolution is limited by the number of pixels. However, our method achieves sub-pixel resolution based on calculation of reference intensity profiles and polynomial fitting of the computed mean squared error (see below).

TPLSM data

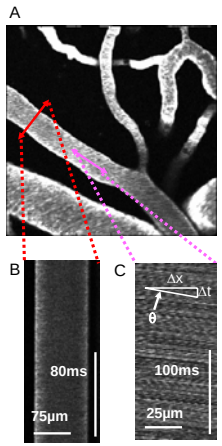

Figure 1,
A – Typical view of surface vessels, acquired using TPLSM
B – Line scan data across the vessel.
C – Line scan data along the axis.

$$velocity = \frac{\Delta x}{\Delta t}$$

$$\tan\theta = \frac{\Delta t}{\Delta x}$$

$$Therefore,$$

$$velocity = \frac{1}{\tan\theta}$$

- Diameter estimation – To estimate the diameter change, we obtain a reference profile. By comparing this reference to subsequent data, we can estimate how much the vessel edges has shifted and using this information, we can calculate the diameter change. This method provides better resolution than by counting the number of pixels above an intensity threshold (Chaigneau et al. and Hutchinson et al.) to derive the diameter. The method can be used to analyze data obtained from line scans perform across the vessel (Figure 1B), as well as data from freedraw line scans. The only requirement is that the line must pass through both edges of the vessel.
- Velocity estimation – Line scans are performed along the axis of the vessel (Figure 1C). When the lines are displayed together, they form a space-time image. The dark streaks are formed by the movement of the non-fluorescent red blood cells (RBCs). The velocity can be estimated from estimating the slope of the dark streaks. The method is a modification of an algorithm described in detail by Schaffer et al. However instead of rotating (which can lead to cropping) to find the slope of the streaks, we resample the data using a sampling grid with different resampling angles. This can be viewed as applying a shear force to a rectangular sampling grid.

References

•Two-photon imaging of cortical surface microvessels reveals a robust redistribution in blood flow after vascular occlusion. C. B. Schaffer, B. Friedman, N. Nishimura, L. F. Schroeder, P. S. Tsai, F. F. Ebner, P. D. Lyden and D. Kleinfeld. Public Library of Science: Biology (2006) 4: 258-270
•The relationship between blood flow and neuronal activity in the rodent olfactory bulb. E Chaigneau, P Tiret, J Lecoq, M Ducros, T Knopfel, S Charpak. Journal of Neuroscience (2007) 27 (24): 6452-6460
•Spatial flow-volume dissociation of the cerebral microcirculatory response to mild hypercapnia. EB Hutchinson, B Stefanovic, AP Koretsky and AC Silva. Neuroimage (2006) 32 (2): 520-530

Diameter Estimation Procedure

- Obtain a reference intensity profile of the vessel by averaging (over time) the initial segment of data (Figure 2A). Enough lines are averaged to obtain a good representation of the intensity profile (Figure 2B) of the vessel. However, averaging too many lines will result in the smearing of the edge due to movement or vessel dilation.
- The raw data was low pass filtered in time to obtain more reliable estimates. Zero phase delay filtering was used to ensure no artificial delay is introduced. The filter used is a Parks-McClellan optimal equiripple FIR filter, with a transition band between 2 to 4 Hz, with ripples of 0.2 and 0.25 respectively for the passband and stopband.
- For each line in the filtered data (Figure 3A), it is compared to a shifted reference profile (Figure 3B) by computing the mean squared error (MSE). The displacement at which the MSE is at a minimum indicates the shift of the vessel edge (Figure 3C).
- Because the MSE is calculated at each integer shifts (limited by number of pixels), we can use polynomial fitting (2nd order) to find the location of the theoretical minimum of MSE (Figure 4).
- Two separate cost functions are calculated for each edge, and the relative shifts of the edges are used to calculate diameter change. Figure 5 shows the raw data with the estimated diameter.

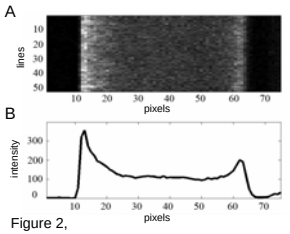

Figure 2,
A – First 50 lines (45ms) of raw line scan data.
B – Reference intensity profile

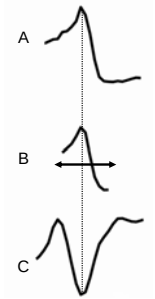

Figure 3,
A – Line scan data
B – Reference profile
C – MSE for different shifts of the reference profile

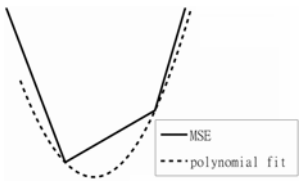

Figure 4, graph showing the MSE in solid lines and the fitted polynomial function in dashed line.

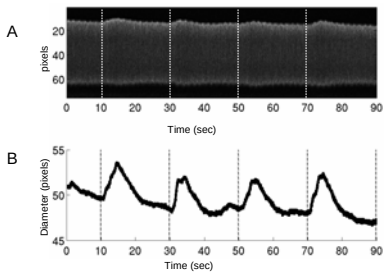

Figure 5,
A – Raw line scan data
B – Estimated diameter, dotted lines indicate time of stimulus presentation

Velocity Estimation Procedure

To estimate the slope of the dark streaks, we resample the data at different angles. At a particular resampling angle, the resampled data will have a slope of zero. And so the resampling angle can be used to calculate the velocity.

- Extract a segment of data (approx. 45ms) for processing
- Decide the range of angles to examine.
- For each angle, resample the data. An example of the sample grid is shown in Figure 6.
- After resampling, perform singular value decomposition (SVD) on the interpolated data, and calculate its separability.

$$separabili\,ty = \frac{\lambda_1^2}{\sum_{n=1}^{N}\lambda_n^2}$$

- To save computation time, the search for the maximum is performed in 2 stages. The first stage scans through the angle range very coarsely with a sampling interval of 5 degrees. Then a sampling interval of 1 degree is used to provide a more precise estimate of the slope.
- Use polynomial fitting to find the angle at which the separability is at a maximum. The angle is used to calculate the velocity. The result is shown in Figure 8.

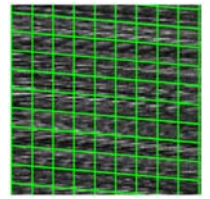

Figure 6 – Sampling grid (fewer samples shown for clarity) at an angle of 5 degrees.

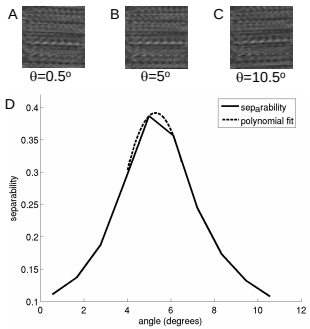

Figure 7,
A – Resampled data at 0.5 degrees
B – Resampled data at 5 degrees
C – Resampled data at 10.5 degrees
D – Separability versus angle function

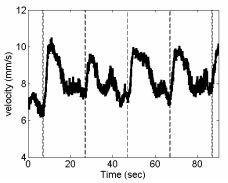

Figure 8, velocity change due to stimulus presentation

Conclusions and Future Developments

• We have presented a method for estimating diameter change of blood vessels imaged using TPLSM. The method can also be applied for data acquired using freedraw line scans. In the future, we will develop the method so that we will be able to estimate the diameter of diving vessels in a 2-D plane.

• Velocity estimation is very computationally intensive, but we can save time if we apply a more effective search strategy for the maximum separability.

Acknowledgements

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