BLOOD FLOW TRACKING ANALYSIS

FEMTO 3D ATLAS DICHRO

BLOOD FLOW TRACKING ANALYSIS USING FEMTO3D ATLAS DICHRO SYSTEM

To precisely measure blood flow velocity in 3D in the living tissue, possibly together with other imaged parameters (ie. cellular activity) may be very important in different biological disciplines and applications. Two-photon microscopy, especially the real 3D acousto-optical two-photon microscopy provides a flexible and precise tool to do that, and can do so in the field of the Neurosciences, Immunology, Dermatology, Oncology etc.

MICROSCOPE EQUIPMENTS

- two pairs of acousto-optic crystals for XY and Z rapid focusing (3D imaging)
- 4D Beam Conditioning Unit for stabilization of laser beams
- two different wavelength two-photon laser sources
- Dichro system for fast switching between two lasers in case of quasi-simultaneous dual excitation

TECHNICAL PROCEDURE FOR ACQUISITION AND ANALYSIS

Here we injected a red dextrane intravenously that makes blood vessels appearing in red. The passing red blood cells are shown as shadows in the red stream **(Figure 1A).** It is quite proper to measure speed of blood stream regarding distance is taken by a red blood cell in time-dependent manner **(Figure 1B).**

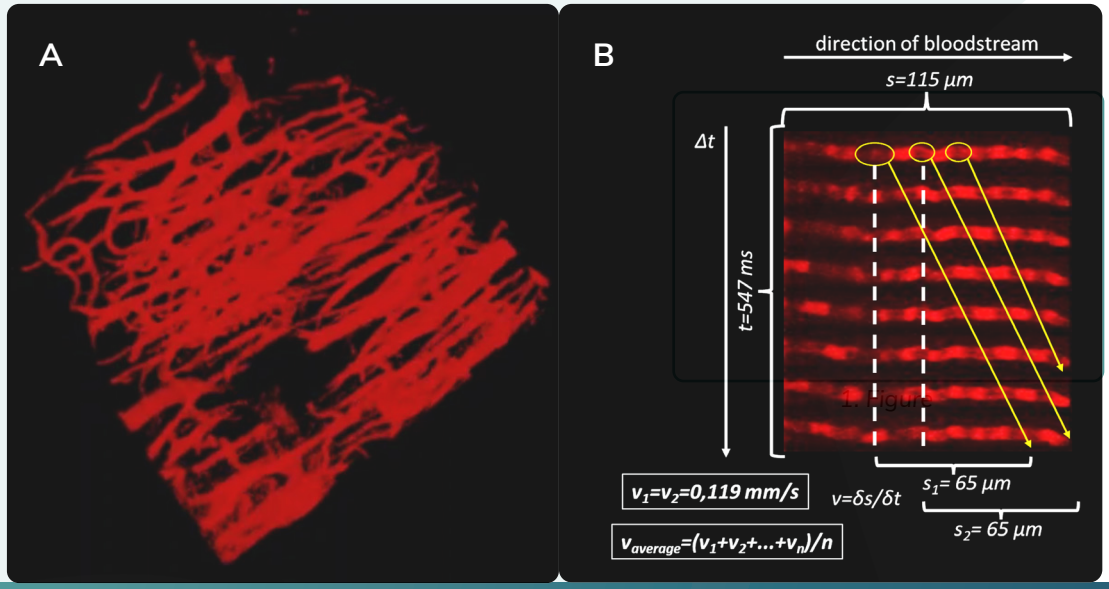

Figure 1. How to measure blood flow velocity using 3D acousto-optical two-photon imaging.
A An example image and the blood stream visualisation from one 3D ribbon in ~200 um depth. **B** A montage image showing the stream of individual red blood cells (yellow cicle and arrows). The average speed in a certain part of capillaries or vessels is measurable from values of red blood cell speeds (v1, v2...vn).

Rapid ROI imaging by FEMTO3D Atlas AO system enables 3D ribbon scanning following vessels through more layers of the cortex. The total length of the ribbons can reach up to **~5 mm**, while maintaining a resolution and speed sufficient to measure the velocity of blood flow **(Figure 2).**

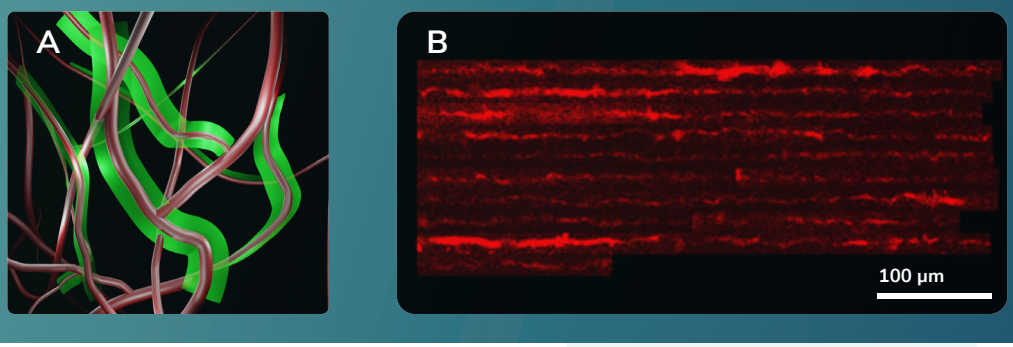

Figure 2. Holding a proper scanning speed for 3D blood stream measurement, FEMTO3D Atlas is able to reach up to **5 mm long ribbon network**. **A** 3D reconstruction of laid ribbons for measuring blood flow in mouse cortex. **B** Blood stream visualisation of **4.78 mm long 3D ribbon-network;** scanning speed: 7 Hz.

SIMULTANEOUS IMAGING OF DIFFERENT STUCTURES

Research of cardiac diseases may be supported by a combined method for simultaneously imaging Ca^{2+} activity and the blood flow. It is a new opportunity getting local information about neuronal and cardiac status. Using FEMTO3D Atlas Dichro system with two laser-source on two different wavelength, blood cell movement and neuronal Ca^{2+} activity are simultaneously measurable. Using two different lasers, fast switching between two different laser sources enables a quasi-simultaneous imaging on two different emission wavelengths. This may provide a nice opportunity for studying detrimental consequences of stroke regarding vascular conditions, neuronal damages, and even regenerations processes **(Figure 3).**

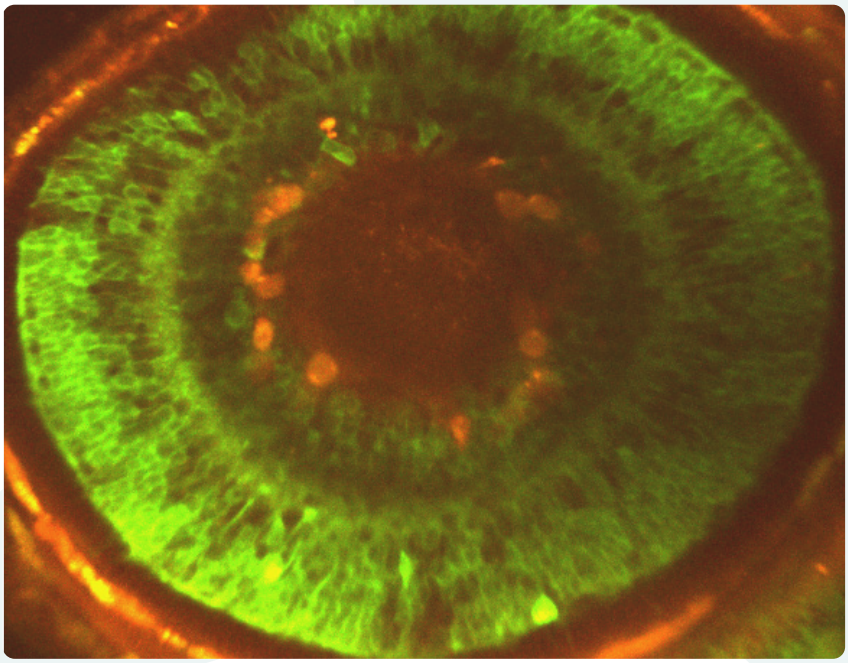

Figure 3. *In vivo* imaging of blood flow in zebrafish eye. Color red indicates tdTomato labelled blood cells, color green marks GCaMP6 labelled neurons.

SUMMARY

This whitepaper provides a simple desciption of how to acquisite and analyse blood flow velocity in 3D, through more layers of living brain cortex. The scope endowed with our state-of-the-art acousto-optic microscopy technology, the FEMTO3D Atlas Dichro enables fast 3D ROI scanning in a larger volume, as well as, a quasi-simultaneous detection of Ca^{2+} activity and blood flow using two different laser sources. Our own-developed C++ and MATLAB-base sofware package provides built-in ROI and curve analysis options meeting all needs.

The world-class multiphoton devices of Femtonics can answer all your experimental needs. Seize the opportunity and discuss, or try them with our microscopy experts.

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