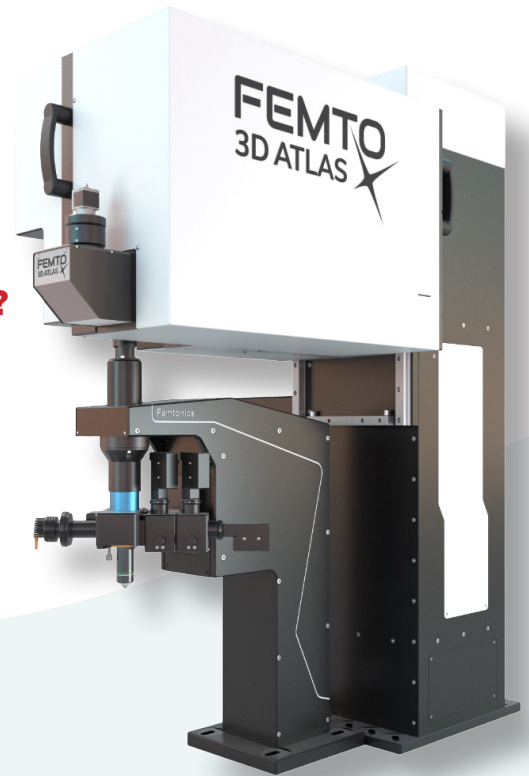


BLOOD FLOW TRACKING AND ANALYSIS

FEMTO 3D ATLAS



✦ How to accomplish blood flow imaging with Atlas?

- **Compatibility** with blood flow indicators
- Two pairs of acousto-optic crystals for XY and Z **rapid 3D focusing**
- 4D Beam Conditioning Unit for **stabilization of laser beams**
- Two **different wavelength** two-photon laser sources
- **Fast switching** between two lasers in case of quasi-simultaneous dual excitation
- **Simultaneous calcium and blood flow imaging** is possible

✦ Tracking complex 3D blood flow?

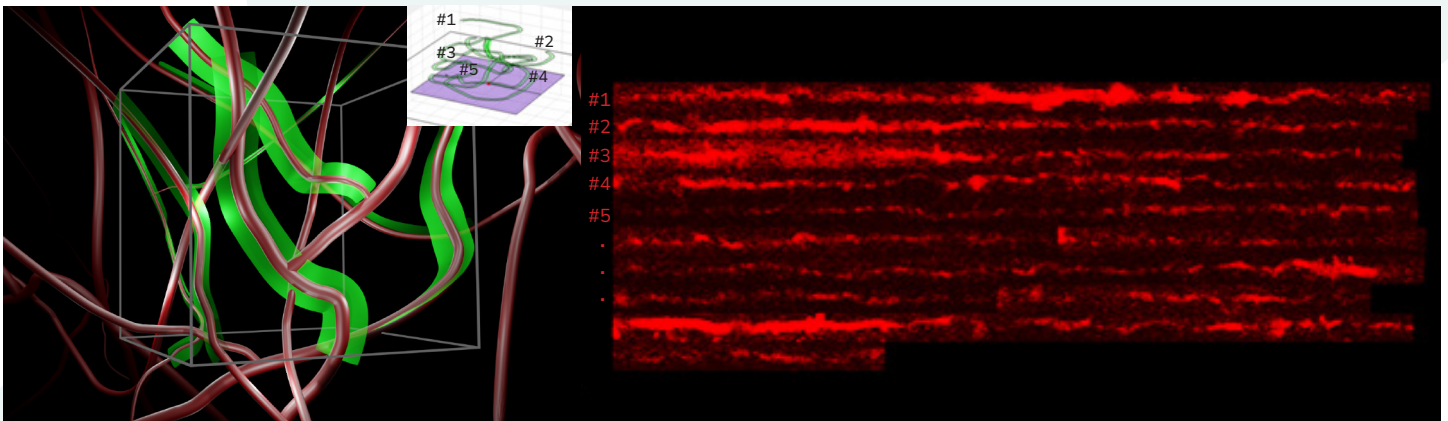


Figure 1: Maintaining a proper scanning speed for 3D blood stream measurement, the FEMTO3D Atlas is able to scan an up to 5 mm long ribbon network. A. 3D reconstruction of the ribbon pattern laid down to measure blood flow in the mouse cortex. B. Blood stream visualization of a 4,78 mm long 3D ribbon-network; scanning speed: 7 Hz.

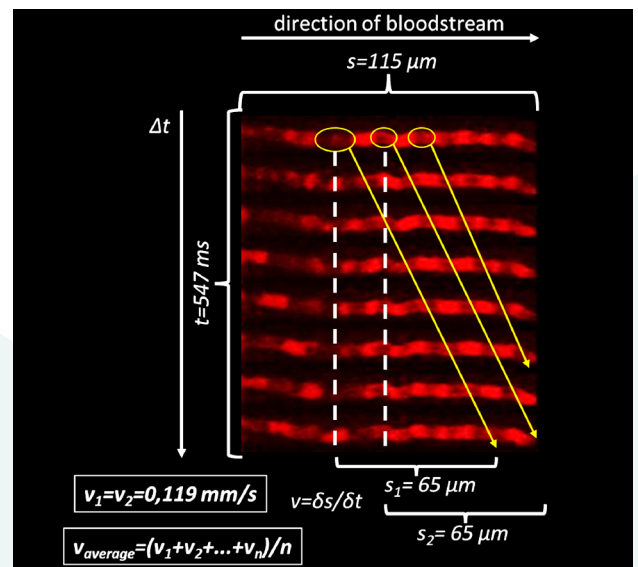
RIBBON SCANNING METHOD FOR BLOOD FLOW MONITORING

- **High Efficiency:** The ribbon scanning method offers fast, real-time blood flow monitoring by focusing on a ribbon-shaped area of interest (Fig 2A).
- **Reduced Artifacts:** The technique significantly minimizes motion artifacts, nearly eliminating them with real-time motion correction.
- **Detailed Visualization:** Provides clear and detailed images of blood vessels and flow patterns.
- **Dynamic Monitoring:** Ideal for capturing transient and dynamic changes in blood circulation.
- **Vascular Health Insight:** Enhances understanding of vascular health and disease through rapid imaging.

✦ Can you measure blood flow velocity?

METHOD FOR MEASURING BLOOD FLOW VELOCITY USING 3D TWO-PHOTON IMAGING

1. **Capture a 3D Image:** Obtain a 3D image of blood vessels at a specific depth (e.g., ~200 μm) and visualize the blood stream within a selected ribbon-shaped region.
2. **Identify Red Blood Cells:** Focus on individual red blood cells (RBCs) in the bloodstream, marking them in the image.
3. **Create a Montage Image:** Generate a montage image showing the movement of RBCs over time.
4. **Calculate Speed:** Measure the speed of each RBC (v_1, v_2, \dots, v_n) by analyzing their movement across the montage frames.
5. **Determine Average Velocity:** Calculate the average blood flow velocity in a specific vessel or capillary section by averaging the speeds of the RBCs.



✦ Is it possible to combine blood flow measurements with calcium measurements?

- Research of cardiac diseases may be supported by a combined method of **simultaneous Ca^{2+} activity and blood flow imaging**.
- Using the FEMTO3D Atlas Dichro with two laser sources of **different wavelengths**, blood cell movement and neuronal activity can be measured at the same time.
- **Fast switching** between the two laser sources makes it possible to image **quasi-simultaneously** on two different emission wavelengths.
- This method is useful for studying the detrimental consequences of stroke on vascular conditions, neuronal damage, regeneration processes, etc.

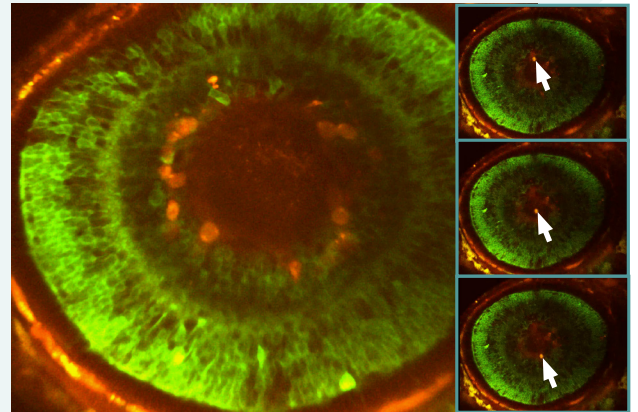


Figure 3: In vivo imaging of blood flow in zebrafish eye. RED: tdTomato sparse labelled blood cells. GREEN: GCaMP6 labelled neurons



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