An All-Optical Approach for Probing Microscopic Flows in Living Embryos

Willy Supatto, Scott E. Fraser, and Julien Vermot

DATA S1: SUPPLEMENTAL MATERIALS

A. SUPPLEMENTAL METHODS

Embryo preparation, imaging, laser ablation, and image processing

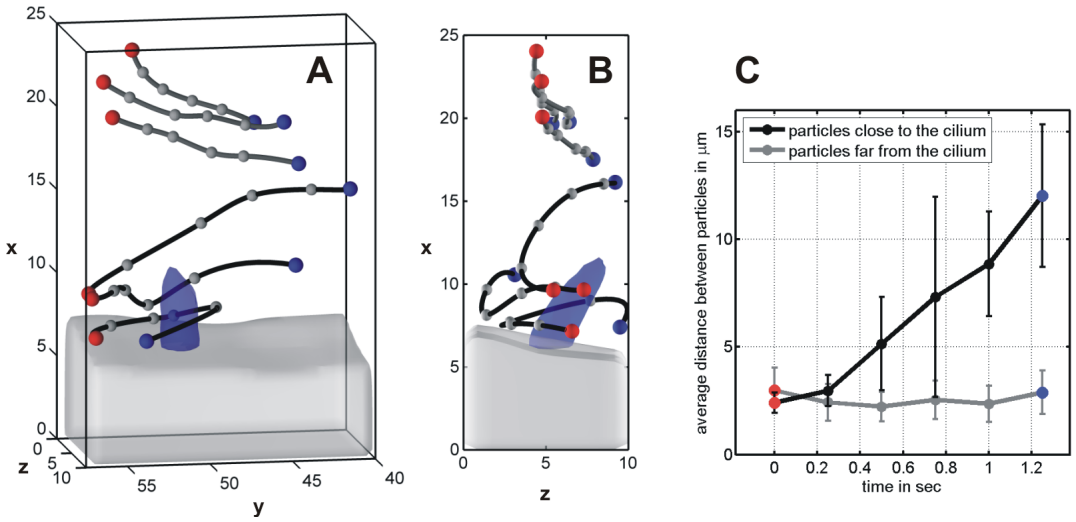
Zebrafish embryos were loaded with Bodipy TR dye (Molecular Probe) for 30 minutes and mounted in agarose 0.7% in Danieau solution (Bodipy TR labeling in red and gray in Fig. 1D-E and 1F, respectively). 2-photon excited fluorescence (2PEF) imaging (Fig. 1D-E and Movie S1) and femtosecond laser ablation were performed at 820nm using a Chameleon Ultra laser and a Zeiss LSM510 microscope on 6 somites stage embryos. The typical conditions for deep tissue ablation were: 5µm linescan, 200µm/s scan speed (512 pixel line with 50 µs pixel dwell time), 300fs pulses, 80MHz repetition rate, 820nm wavelength, using an average of 250 to 350mW power after the objective. Fast confocal images (Fig 1F, Movies S2, S4 and S5) were collected on a Perkin-Elmer Spinning-disk confocal (SDC) microscope. The acquisition parameters were: 44 frames per second, 11 z-planes every 1µm, 10µm in depth, 0.4µm per pixel in x and y (corresponding to 3D stacks at 4Hz). Experiments were done using a 40x/NA 1.1 objective lens (Zeiss). 3D-particle tracking was performed automatically with Imaris (Bitplane) and corrected manually. Considering the spatial sampling in the z direction, the signal of each particle extends over several z-planes within each z-stack. We did not observe significant motion of the particle signal in between z-planes at 44Hz, thereby confirming the absence of aliasing artifacts. 3D reconstructions and renderings were obtained either with Imaris (Fig. 2A and Movies S3-5) or with Matlab (Fig. 2B and 3, Suppl. Fig. 1 and Movie S6). The velocity field analysis was done with custom Matlab scripts. The vesicle surface (gray in Fig. 2-3, Suppl. Fig. 1 and Movies S3-6) and the midline volume (green in Fig 2-3 and Movie S3) were manually segmented from experimental data.

Assessment of tissue optical properties

In deep tissue nonlinear microscopy, it is useful to assess the optical properties of the tissue being imaged. For instance, the comparison of laser ablation conditions applied to two different tissues requires the knowledge of their optical properties. Experimentally, it is observed that the 2PEF signal detected in thick samples decreases exponentially with depth as $\exp(-z/l)$ for a constant incident power at the tissue surface, where z is the imaging depth. Under the assumptions that absorption can be neglected and that fluorescence collection efficiency is constant with depth, l characterizes the excitation light scattering and the optical aberrations due to the tissue. Furthermore, if optical aberrations can be neglected, l is half of the scattering length of the excitation light in the tissue (Oheim, *et al.* 2001).

M. Oheim, E. Beaurepaire, E. Chaigneau, J. Mertz and S. Charpak, "Two-photon microscopy in brain tissue: parameters influencing the imaging depth." J Neurosci Methods **111**, 29-37 (2001)

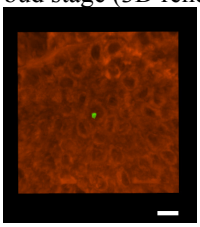
B. SUPPLEMENTAL FIGURE



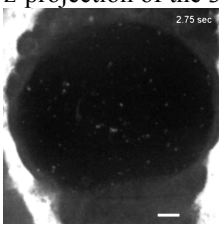
SUPPLEMENTAL FIGURE 1 Chaotic advection around the beating cilium. In order to confirm the chaotic nature of the flow surrounding the cilium, we presented on the same figure ((A), dorsal view and (B) posterior view) two groups of three tracks starting close (black lines) or far (gray lines) from the cilium (blue surface). The average distance between the three particles of each group is plotted over time in graph (C). The red and blue dots represent the particle position at the beginning (0 second in (C)) and at the end (1.25 seconds in (C)), respectively. The intermediate positions of the particle are plotted with gray dots at each experimental time points (every 0.25 second). At the beginning and in both groups, the 3 particles start from nearby positions (red dots, the average distance between particles is 2-3 μm). The average distance between the three particles remains constant over time for the group of particles far form the cilium (gray line in C), whereas this distance is 5 times higher after only 1.25 seconds for the group starting close to the cilium (black line in C). The quick divergence of the paths followed by particles starting nearby reveals the chaotic nature of the flow surrounding the cilium. This original feature in the context of Stokes flow at low Reynolds number has been predicted by the numerical simulation recently reported in (10). The axis scales in (A) and (B) are the same as in Fig. 2. The axis scales and positions in (A) and (B) are kept as in Fig. 2A. The error bars in (C) correspond to the standard deviation of the distances.

C. SUPPLEMENTAL MOVIES

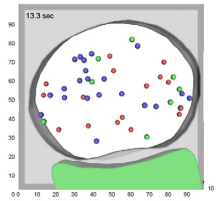
MOVIE S1 3D confinement of a femtosecond laser ablation 70µm deep inside a zebrafish embryo at tail bud stage (3D rendering of Figure 1D). Scale bar: 10µm



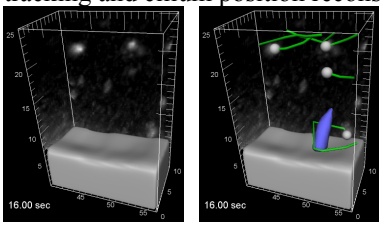
MOVIE S2 Fast confocal imaging of the seeded vesicle after laser ablation (time sequence of Figure 1F). z-projection of the 3D stacks recorded at 4 Hz. Scale bare: 20µm



MOVIE S3 Dynamic version of Figure 2A, exhibiting the track occurrence and the 3D particle motion in the vesicle. Scale and axes as in Figure 2A.



MOVIE S4 and S5 Moving particles around the beating cilium before (Movie S4) and after particle tracking and cilium position reconstruction (Movie S5). Scale and axes as in Figure 2A.



MOVIE S6 3D rotation of Supplemental Figure 1.