Supplementary information

Light sheet dynamic scattering imaging of microscopic blood flow

Kai Long,[a] Keertana Vinod Ram,[a] Shuhao Shen,[b] E Du,[c] Ziheng Ren,[d] Zhiyuan Gong,[d] Nanguang Chen[a,e,*]

[a]National University of Singapore, Department of Biomedical Engineering, 4 Engineering Drive 3, Singapore, 117583
[b]Xidian University, Guangzhou Institute of Technology, Guangzhou, China, 510555
[c]Shenzhen Institute of Information Technology, School of Microelectronics, Shenzhen, China, 518172
[d]National University of Singapore, Department of Biological Sciences, 16 Science Drive 4, Singapore, 117558
[e]NUS (Suzhou) Research Institute, No. 377 Linquan Street, Suzhou Industrial Park, Suzhou, Jiangsu, China

*****Correspondence to: Nanguang Chen, Email: biecng@nus.edu.sg

List of supplementary information

NOTE S1. Light sheet microscopy system characterization

The optical configuration of the light sheet microscope was adjustable for coping with different requirements including the spatial resolutions and depth of focus (DOF). Fluorescence beads 1 μm in diameter (15702-10, Fluoresbrite® YG Carboxylate Microspheres, Polysciences Inc.) were used to characterize the system in typically configurations. A laser at 473 nm was employed for fluorescence excitation, while a 525/39 nm BrightLine® single-band bandpass filter (FF01-525/39-25, Laser2000) was used for selective detection of the fluorescence emission. The beads were randomly dispersed in a transparent medium three-dimensionally and mounted between a cover slip and a glass slide as the imaging sample for system characterization.

The first typical configuration was related to Section 2.2, in which the DA and PCV with diameters around 30 μm in the zebrafish larva trunk region were selected for quantifying the blood flow velocities. In this case, the numerical aptertures were set to the highest possilbe values where the illumination objective NA was 0.1, and the detection objective NA was 0.25. Sample imaging results with this configuration were shown in Figs. S1(a) and S1(c). The othrogonal views of a single fluorescence bead were reproduced in Fig. S1(a), together with line profiles through the bead center. The lateral resolution was estimated at 1.81±0.08μm (red line in Fig. S1(a)), and the axial resolution was 5.15±0.14μm (blue line in Fig. S1(a)). Fig. S1(c) was the projection of the 3D volume along the y-axis. The DOF in this situation is assessed to be 19.43 ± 0.61μm by measuring the FWHM of the intensity profiles along the z-direction (depth) in the field of view. Considering the wavelength difference, the DOF would be around 23.69 μm in the dynamic scattering imaging mode where the laser wavelength was 640 nm. This was very close to the theoretical prediction of 24 μm.

In another experiment (Section 2.3), the vasculature in the zebrafish head region (around 300 μm thickness) was selected for 3D imaging. Relatively low numerical aptertures were set by reducing the aperture size of the irises at the back of two objective lenses, resulting in an illumination NA of 0.03 and a detection NA of 0.05. As shown in Fig. S1(b), the lateral resolution changed to 4.27±0.01μm (red line), while the axial resolution became 22.28±0.33μm (blue line). Compared to the previous case, the DOF was extended to about 300 μm (Fig. S1(d)). Generalizing the results to dynamice laser scattering imaging at 640 nm, the DOF would be around 365 μm, which was adequate to cover the enture larva and agreed reasonably well with the theoretical value (393 μm).

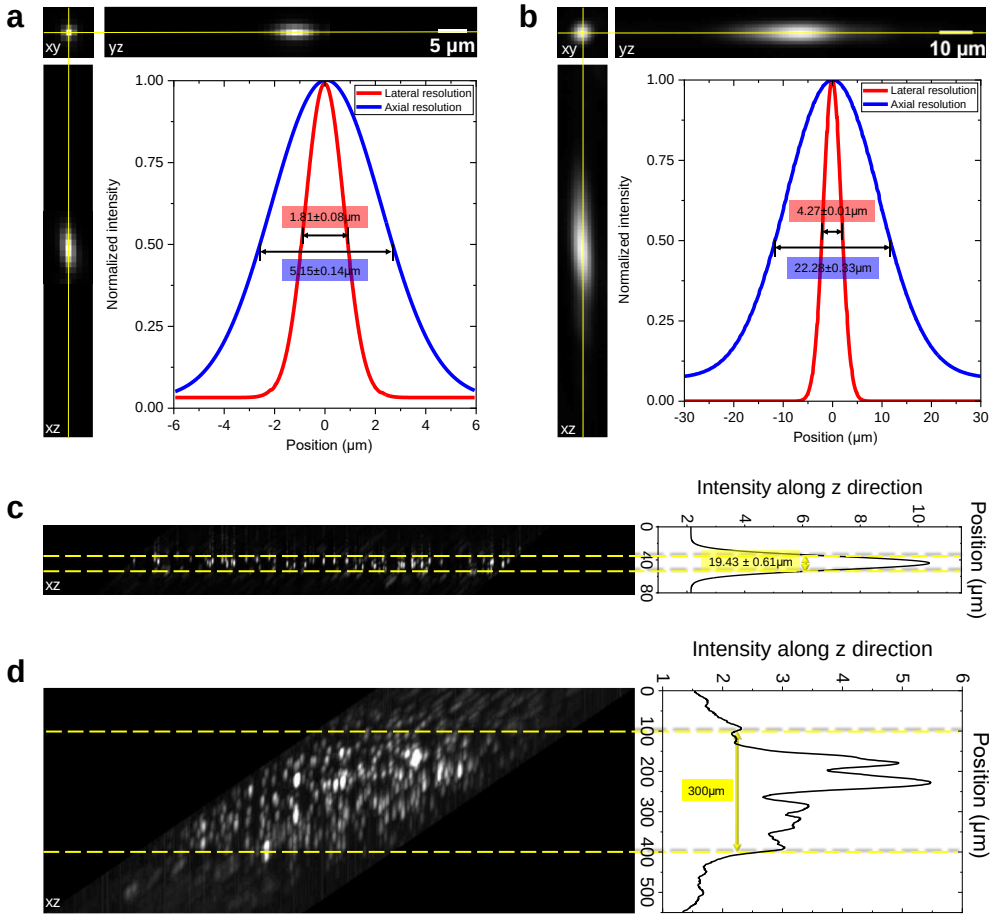

Fig. S1| Light sheet microscopy system characterization. (a) Resolution under high NA. **(b)** Resolution under low NA. **(c)** DOF under high NA. **(d)** DOF under low NA.

NOTE S2. Zebrafish larva head vasculature imaging: raw speckle images and speckle contrast maps

Raw speckle images were acquired from a zebrafish larva head at 15 light sheet (slice) positions, resulting 15 image stacks of 6,000 frames each. Some representative raw images were included in Fig. S2. Panels **a1** , **a2**, and **a3** were randomly chosen from the image stack for slice 3, while panels **b1** , **b2**, and **b3** were taken from the image stack for another layer (slice 5). Fig. S2(a) was created by computing the temporal speckle contrast in small time windows (100 frames) and then taking the time average over the entire time period of 2 seconds. As the exposure time was very short (20 μs), the time-averaged speckle contrast maps did not directly provide the flow information. Instead, they helped outline the vasculature structure and were used to generate masks to quantify blood flow velocities[40] in the vessels. Similarly, Fig. S2(b) was the speckle contrast map at slice 5.

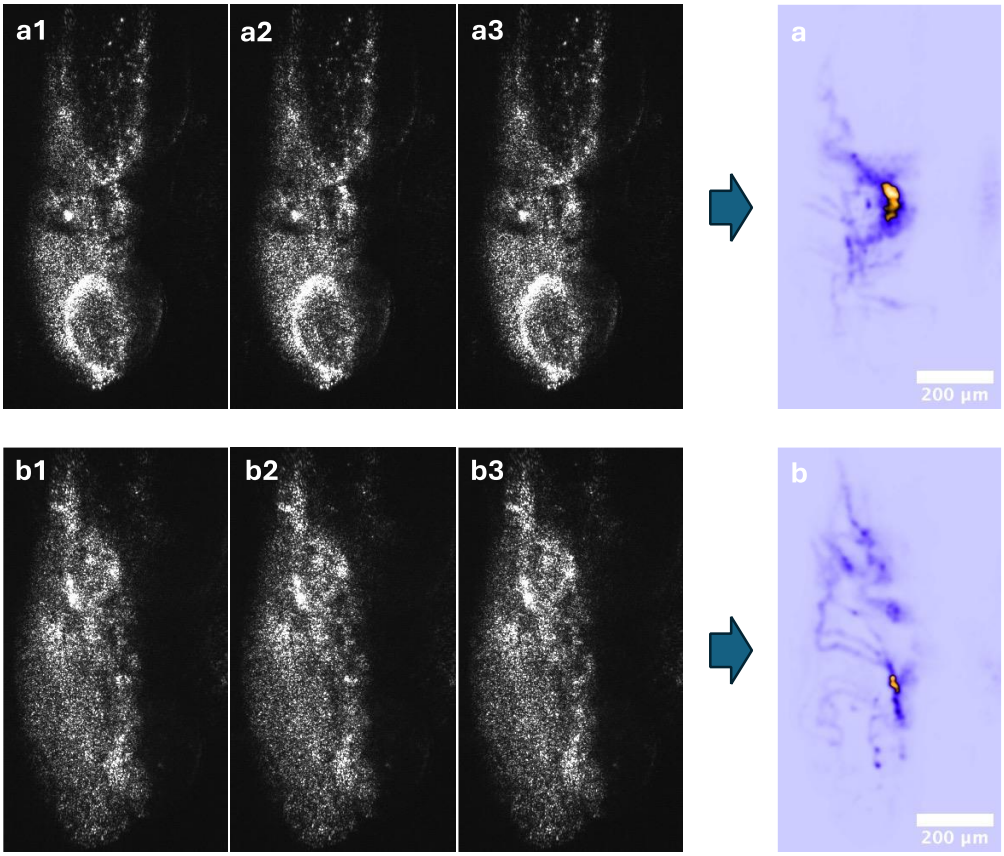

Fig. S2| Zebrafish larva head vasculature imaging: raw speckle images and speckle contrast maps. (**a**) The angiograph generated from the spckle image stack obtained at slice 3. Three frames at randomly chosen time points are displayed in (**a1**) to (**a3**). (**b**) The angiograph generated from the spckle image stack obtained at slice 5. Three frames at randomly chosen time points are displayed in (**b1**) to (**b3**).