High-resolution and label-free neuroimaging of a living zebrafish by simultaneous angular scanning microscopy

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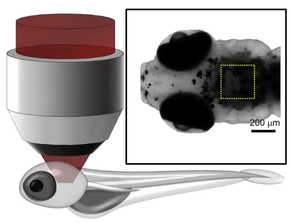
Abstract

We reported methods termed collective accumulation of single scattering (CASS) microscopy and closed-loop accumulation of single scattering (CLASS) microscopy. However, its application to in vivo imaging has remained unrealistic so far to the long acquisition time of the time-gated reflection matrix, a unique matrix containing wide-field and time-gated maps of elastically backscattered waves for various illumination angles. To speed up the measurements of wide-field and time-gated detection, a simultaneous angular scanning microscope (SASM) is developed to synchronously scan the angle of the sample and reference waves. Here, using this SASM, we obtained the volumetric images of the fine structures of myelinated axons located deep within the hindbrain of a living zebrafish without using any exogenous labeling agents.

Results

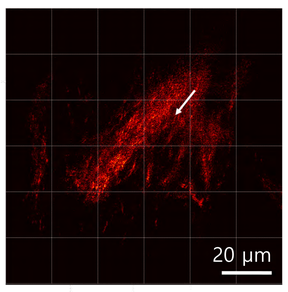
Correction of local position-dependent aberrations for in vivo neuroimaging of a larval zebrafish.

After hatching (96h)

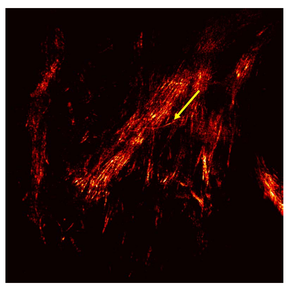


- Source : super continuum laser (tunable between 450~650 nm, center wavelength 515 nm)
- NA : 0.8, resolution ~ 0.4 μm (lateral, diffraction limited), coherence length : 10~30 μm
- An anesthetized zebrafish larva at 21 days post-pertilization (dpf)

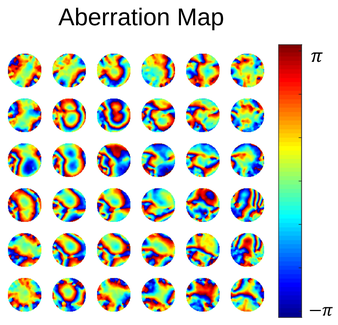
Before correction



After correction

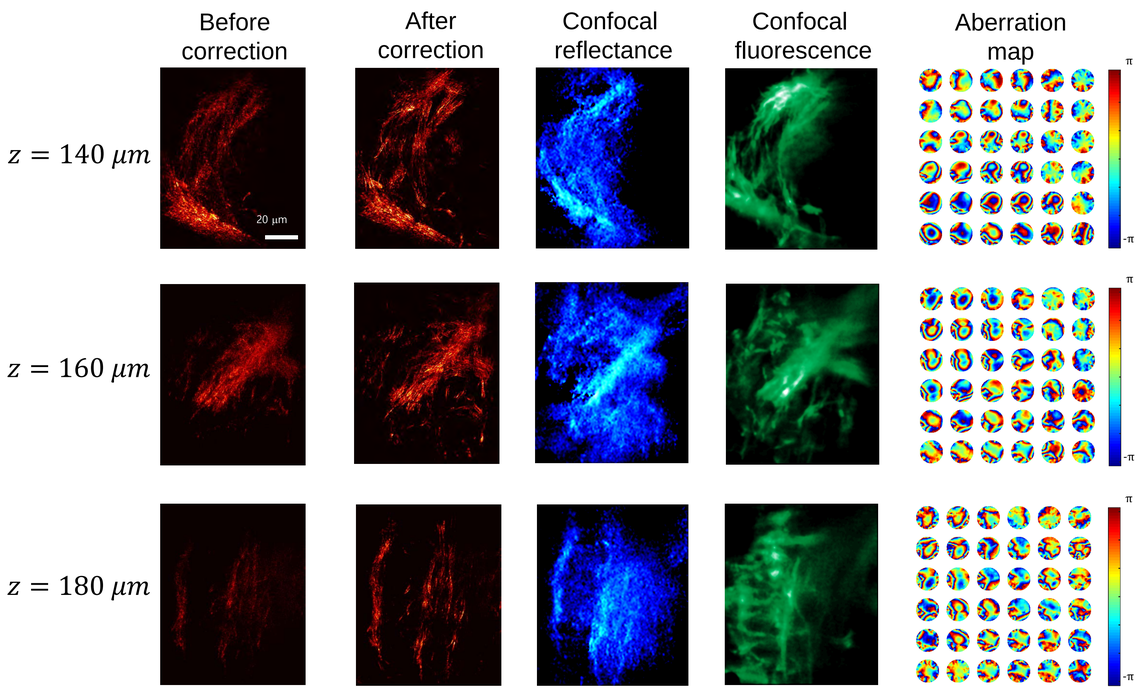


Aberration Map



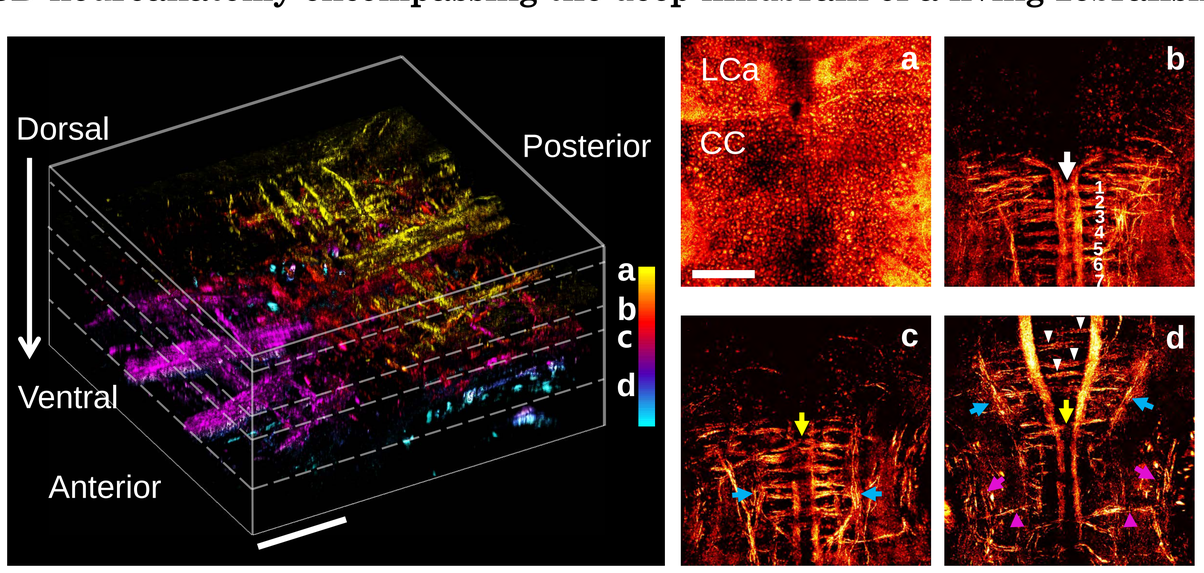
- ✓ After aberration correction, the fine filament structures of individual myelinated axons are clearly resolved over the entire field of view.
- ✓ In some areas, such as the one indicated by a yellow arrow, even the fine neuronal processes that used to be invisible before aberration correction appear.

Additional depth-dependent images for the zebrafish



- ✓ After correction, the structures of myelinated axons were clearly distinct at each depth.
- ✓ The aberration maps were also highly depth-dependent.

3D neuroanatomy encompassing the deep hindbrain of a living zebrafish



- 3D rendered image and maximum amplitude projection (MAP) images of aberration corrected images at the hindbrain of a 10 dpf transgenic zebrafish (**Tg(claudinK:gal4;uas:mgfp)**) embryo.
- Wavelength of light source : 633 nm
- Recording depth : approximately 220 μm with a depth scanning step of 5 μm
- **LCa** : lobus caudalis cerebella, **CC** : crista cerebellaris
- White arrow : the crossed axons of Mauthner cells, labels from 1 to 7 : a series of seven ladder-like commissural tracts
- Yellow arrows : two prominent commeasures in rhombomere 3
- Blue arrows : the ipsi- and contralateral lateral projections
- White arrowheads : commissural tracts connected with the anterior medial projections
- Magenta arrows and arrowheads: torus semi-circularis and interneuron projections, respectively
- Green arrowheads : commissural tracts connecting between medial and lateral projections
- The scale bar : 50 μm

- ✓ AO-SASM noninvasively showed the various structures of fine myelinated axon projections inside the hindbrain of a larval zebrafish.
- ✓ Confocal fluorescence images showed similar structures to AO-SASM images. This means the myelin sheath is the main source of reflectance signals. However, many of the fine myelin processes in fluorescent image were not clearly resolved due to the aberration.
- ✓ Confocal reflectance images were rather similar with those AO-SASM images before aberration correction with more background noise due to the absence of temporal gating.
- ✓ AO-SASM showed the detailed neuroanatomy to a greater depth and for a more matured developmental stage than the conventional imaging modalities.

Conclusion

- ✓ We presented a high-speed and label-free adaptive optical coherence imaging method that is fast enough to perform in vivo imaging, and visualized the internal structures of a living zebrafish with a diffraction-limit spatial resolution (370–480 nm) up to the depth where conventional imaging modalities fail.
- ✓ By dealing with both position-dependent sample-induced aberrations and multiple-scattering noise with no need for guiding stars and hardware feedback, AO-SASM noninvasively offered the atlas of the nervous system and the tomography of fine myelinated axon projections located deep within the hindbrain of a living zebrafish.
- ✓ This will extend the proposed method to in vivo imaging of animal and human subjects for various applications, including myelin-associated physiology in neuroscience, retinal pathology in ophthalmology.

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