High-fidelity functional and structural whole-brain imaging with Bessel-beam light-sheet microscopy

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1. ABSTRACT

Light-sheet microscopy (LSM)[1] has proven a useful and versatile tool in neuroscience and is particularly well suited to image the entire brain with high frame rates at single cell resolution. On the one hand, LSM is often employed in combination with tissue clearing methods like CLARITY[2] which allows for the reconstruction of the neuronal or vascular anatomy over cm-sized samples like the entire mouse brain[3]. On the other hand, LSM has been paired with intrinsically transparent samples for the real-time recording of neuronal activity with single cell resolution across the entire brain[4].

Despite its intrinsic advantages in terms of high imaging speed and reduced photobleaching, LSM is very sensitive to residual opaque objects present in the sample, which cause dark horizontal stripes in the collected images. In the best case, these artefacts obscure the features of interest in structural imaging; in the worst case, dynamic shadowing introduced by red blood cells significantly alters the fluorescence signal variations related to neuronal activity.

We show how the use of Bessel beams[5] in LSM can dramatically reduce such artefacts even in conventional one-sided illumination schemes, thanks to their non-diffractive and "self-healing" properties. On the functional side, Bessel-beam LSM allows recording neuronal activity traces without any disturbing flickering caused by the movement of red blood cells. Furthermore, using this approach it is possible to extend high-resolution calcium imaging in less transparent samples or regions, like older or slightly pigmented Zebrafish larvae. On the structural side, our proposed method is capable of obtaining anatomical information across the entire volume of whole mouse brains allowing tracing blood vessels and neuronal projections also in poorly cleared specimens.

References

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