# Super-resolution structured illumination imaging in the living brain with adaptive optics

**Raphaël Turcotte, Yajie Liang, Qinrong Zhang, Ziwei Li, Eric Betzig, Na Ji**
**Janelia Research Campus, Howard Hugues Medical Institute**
**19700 Helix Dr, Ashburn, VA 20147, United States**
**and**
**Department of Physics, Department of Molecular & Cell Biology, Helen Wills Neuroscience Institute, University of California, Berkeley**
**E-mail: raphael.turcotte@pharm.ox.ac.uk**

**KEY WORDS:** super-resolution, adaptive optics, in vivo imaging, neuroscience, structured illumination microscopy

Understanding neurobiological processes requires studying the living brain because neurons form a complex network and neuronal functions are better assessed in a behaviourally relevant context. Structure illumination microscopy (SIM) has been demonstrated to provide twice the lateral resolution of a widefield fluorescence microscope[1] and therefore offers an avenue for visualising the structural and functional details of neurons at a level not achievable with diffraction-limited techniques. Here, we implemented one-photon SIM for super-resolution imaging of the living brain. SIM was combined with adaptive optics[2] for correction of sample-induced optical aberrations, which were more deleterious for super-resolution than diffraction-limited imaging. We further optimised data acquisition and the reconstruction process of super-resolution SIM images by using phase up-sampling and image registration to alleviate the effect of brain motion, and estimated illumination parameters on a 2D fluorescence sample composed of sub-diffractive objects. Altogether, these strategies allowed us to apply SIM to *in vivo* imaging in the brain of mice at a depth of up to 50 $\mu$m and to demonstrate high-speed (9.3 frames per second), robust imaging of synapses with super-resolution (FWHM of 0.1-$\mu$m fluorescent beads at 525 nm: 190 ±11 nm).

1. M. G. L. Gustafsson, "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," *J. Microsc.* **198,** 82–87 (2000).
2. K. Wang, *et al,* "Direct wavefront sensing for high-resolution in vivo imaging in scattering tissue," *Nat. Commun.* **6,** 7276 (2015).