Two-photon line-scanning structured illumination microscopy (LIL-SIM) for super-resolution imaging in deep tissue

Patrick Byers[1,2], M.Sc. Thomas Kellerer[1], Prof. Dr. Thomas Huser[2], Prof. Dr. Thomas Hellerer[1]
[1]Multiphoton Imaging Lab, Munich University of Applied Sciences, Munich, Germany,
[2]Biomolecular Photonics, Department of Physics, University of Bielefeld, Bielefeld, Germany

Background incl. aims
In the field of cellular imaging, the demand for sub-cellular resolution and three-dimensional (3D) visualization of biological specimens has been a driving force behind the development of various microscopy techniques. This study explores a novel approach of combining structured illumination microscopy (SIM)1,2 and two-photon line scanning (2P-LS) microscopy to provide super-resolution imaging in thick biological tissue. We apply our method, named LIL-SIM, to cardiovascular artery tissue and tree trunk to demonstrate the penetration capability of our imaging system.

Methods
Structured Illumination Microscopy (SIM) is a well-established super-resolution method, breaking the diffraction limit by introducing a spatially modulated light pattern to the sample. However, SIM's performance traditionally decreases in highly scattering media typical of biological tissues. Conversely, Two-photon (2P) microscopy provides exceptional depth penetration due to its employment of near-infrared light, reducing scattering, and targeting excitation to the focal volume, thus limiting photodamage. Despite the advantages gained by combining 2P microscopy and SIM, the necessity for camera-based detection lowers the modulation contrast of the pattern in thick biological specimens and reduces the signal to background ratio (SBR) of the acquired SIM patterns. This leads to artefacts in the reconstructed images due to insufficient modulation contrast of the generated patterns. Interestingly, the SBR can be increased by confocalization of the emitted fluorescence emission, restricting out of focus contributions of scattered photons. This leads to increased SBR and high modulation depth of the generated SIM patterns in thick biological tissue, enabling volumetric super-resolution imaging.

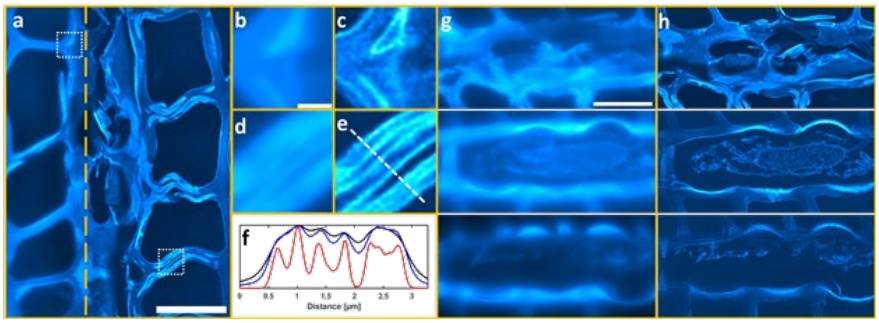
Results
We validate the super-resolution capability of our microscope by imaging tissue samples composed of pine wood and tree trunk, with z-depths of around 40 µm. For all experiments, we generated SIM patterns with five phase shifts to acquire homogeneous illumination of the sample and three rotation angles for an isotropic resolution enhancement. This amounts to a total of 15 acquired images for one SIM frame. Averaging of the 15 acquired frames leads to a homogeneously illuminated intensity distribution, which we refer to as the 2P widefield image in the following. As seen in Fig. 1a), LIL-SIM increases the resolution compared to diffraction limited 2P widefield imaging while simultaneously obtaining better optical sectioning by line confocalization. The 2P widefield image is blurred by the contribution of excited fluorophores close to the focal plane due to scattering. By the application of LIL-SIM and following computation of the acquired images with fairSIM3, the resolution is effectively doubled. LIL-SIM insets 1c) and e) show clear resolution improvement of nanostructures over the acquired 2P widefield structures 1b) and d). The line plot comparison in Fig. 1f) shows a FWHM distance of 384 nm in the 2P widefield image and 150 nm for 2P-LS-SIM.

Conclusion
Our results demonstrate that the fusion of SIM and 2P-LS microscopy leads to an imaging modality capable of resolving structures down to 150 nm in thick biological specimens. We provide data analyzing imaging parameters, e.g. penetration depth, modulation depth of the

illumination grating and phase stability of the illumination patterns and present reconstructed super-resolution images of thick tissue samples, e.g. cardiovascular artery and pine trunk.

Fig. 1 a) 2P widefield and LIL-SIM comparison. b-e) insets and f) line graph demonstrate resolution improvement. g-h) 2P widefield and LIL-SIM with increasing z-depth (10, 20 and 30 µm). Scale bars a) 10 µm, b) 2 µm, g) 5 µm.

Graphic:



Keywords:

Structured Illumination microscopy, Two-photon microscopy

Reference:

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