

Biological imaging using super resolution stimulated Raman scattering microscopy

Julien Guilbert¹, Awoke Negash¹, Simon Labouesse², Sylvain Gigan¹, Anne Sentenac³, Hilton B. de Aguiar¹

1. Laboratoire Kastler Brossel, ENS-Université PSL, CNRS, Sorbonne Université, Collège de France, 24 rue Lhomond, 75005 Paris, France

2. Department of Electrical, Computer, and Energy Engineering, University of Colorado, Boulder, Colorado, 80309, USA

3 Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France

Far-field super resolution imaging has allowed for a better understanding of the complex machinery of biological systems. Recently, a growing interest has emerged to use label-free Raman-based techniques exploiting the intrinsic nonlinear vibrational response of molecules [1]. Nevertheless, proposed super-resolution techniques in Raman processes either require high laser powers that may be phototoxic for biological specimens, or reduce the excitation volume resulting in lower signal levels [2]. Moreover, as light goes deeper inside tissues, wavefront distortion arises inside a scattering medium generating a speckle pattern, hence precluding most super resolution techniques to be used at depth.

Here, we present a single-pixel blind structured illumination microscopy technique (Blind S^3) that is able to go beyond the diffraction limit of the conventional stimulated Raman scattering (SRS) microscope. The technique uses speckles as structured illumination patterns and a second beam to generate a stimulated Raman scattering process. After various images taken, we reconstruct a super resolved image without knowing the exact speckle illumination used to illuminate the sample [3]. The aforementioned super resolution technique has been demonstrated on polystyrene beads and is compatible with biological specimens imaging (see **Fig. 1**).

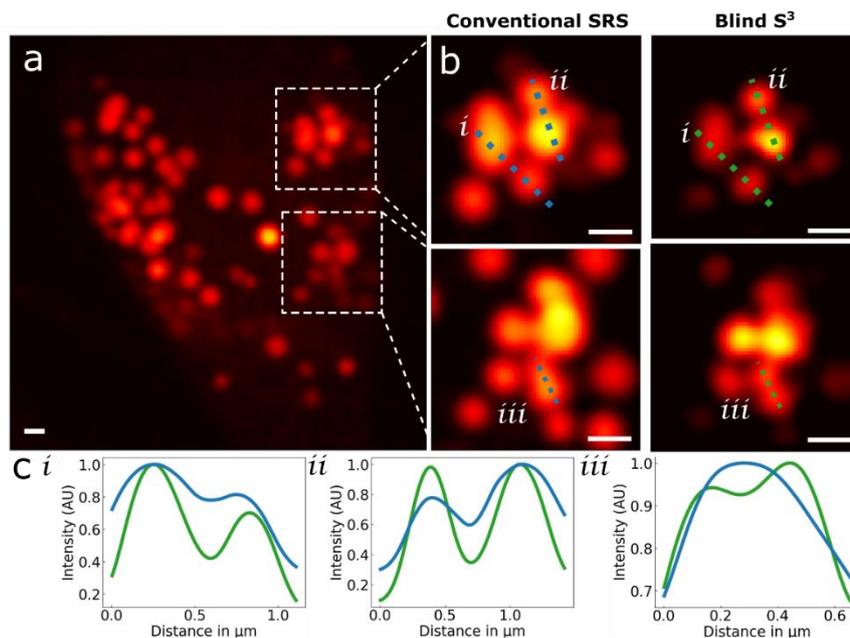


Fig. 1 HeLa cell lipid droplet imaging. (a) Large field of view. (b) Two zoomed-in region of interests imaged with conventional SRS (left) and blind S^3 techniques. (c) Line profiles corresponding to the dashed lines of (b).

References

- [1] J.-X. Cheng and X. S. Xie, Vibrational spectroscopic imaging of living systems: An emerging platform for biology and medicine, *Science* 350, aaa8870 (2015)
- [2] R. C. Prince, R. R. Frontiera, and E. O. Potma, Stimulated raman scattering: From bulk to nano, *Chemical Reviews* 117, 5070 (2018).
- [3] L.-H. Yeh, L. Tian, and L. Waller, Structured illumination microscopy with unknown patterns and a statistical prior, *Biomed. Opt. Express* 8, 695 (2017).