Institute of Molecular Biology

Ad Hoc Seminar

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Lightwedge and Lightsheet-Raman Microscopy

Friday 14th October 2016, 10:00 (*s.t.*)

Venue: 2nd Floor Seminar Room

Institute of Molecular Biology (IMB)

Johannes Gutenberg University Campus

All are welcome to attend.

Hosts: Prof. Christoph Cremer & Microscopy Core Facility, IMB
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Lightwedge and Lightsheet-Raman Microscopy

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Two recently developed modes of lightsheet imaging will be presented. **Lightwedge microscopy** aims at mesoscopic imaging of fixed and optically cleared samples at 1 µm isotropic resolution without the need for sample rotation. The key-idea is to focus a lightsheet at an unusually high NA (thus the name "lightwedge") and still obtain a large field of view due to refocusing of the lightwedge and stitching the multiple small regions of thin illumination back together. This has been simplified by electrical tunable lens technology, which has become available recently.

The second mode is **hyperspectral Raman imaging** in a lightsheet illumination configuration.

To recover the spectral information a full-field Fourier-spectroscopic approach has been chosen [3]. The difficulty here is that in a Michelson approach, it would be technically very hard to maintain the angular stability and common path approaches usually tolerate a relatively low product of étendue and maximal optical path difference. We thus developed an optically stable Mach-Zehnder like scheme based on the use of retro-reflecting corner cubes, which is inherently stable.

This enabled us to obtain full spectrally resolved Raman images consisting of over four million spectra in about 10 minutes. Advantages over the conventional Raman imaging are the reduced maximum power on the sample and out of focus heating, the lightsheet inherent good suppression of crosstalk from the illumination side and the avoidance of glass close to the sample mounting.

Light sheet illumination for Raman imaging at few specific wavelengths was previously reported [1, 2]. With a total laser power of 2 W at an illumination wavelength of 577 nm, we obtained images (2048 × 2048 pixels) of polystyrene beads (fig. 1b), zebrafish and a root cap of a snowdrop at a spectral resolution of 4.4 cm-1 with only few minutes of exposure. The olefinic and aliphatic C-H stretching modes, as well as the fingerprint region are clearly visible along with the broad water peak of the embedding medium (fig. 1a). Spectrally resolved spontaneous Raman microscopy therefore promises high-throughput imaging for biomedical research and on-the-fly clinical diagnostics.

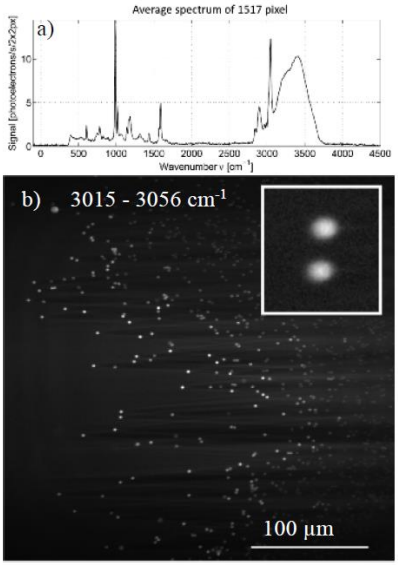

Figure 1: 2.2 µm Polystyrene beads

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REFERENCES

[1] Y. Oshima, H. Sato, H. Kajiura-Kobayashi, T. Kimura, K. Naruse and S. Nonaka, "Light sheet-excited spontaneous Raman imaging of a
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[2] Ishan Barman, Khay Ming Tan, Gajendra Pratap Singh, "Optical sectioning using single-plane-illumination Raman imaging", *J. Raman*
Spectrosc., **41**, 1099–1101 (2010)
[3] W. Müller, M. Kielhorn, M. Schmitt, J. Popp, R. Heintzmann, Light sheet Raman micro-spectroscopy, Optica 3, 452-457, 2016.