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Report on workstations and techniques for label-free imaging

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Deliverable Nature	
R = Report, P = Prototype, D = Demonstrator, O = Other	R
Dissemination Level	
PU = Public PP = Restricted to other programme participants (incl. the Commission Services) RE = Restricted to a group specified by the consortium (incl. the Commission Services) CO = Confidential, only for members of the consortium (incl. the Commission Services)	PU

A. Abstract / Executive Summary

We have successfully realised a label-free imaging platform based on Stimulated Raman Scattering (SRS) microscopy.

B. Deliverable Report

1 Introduction

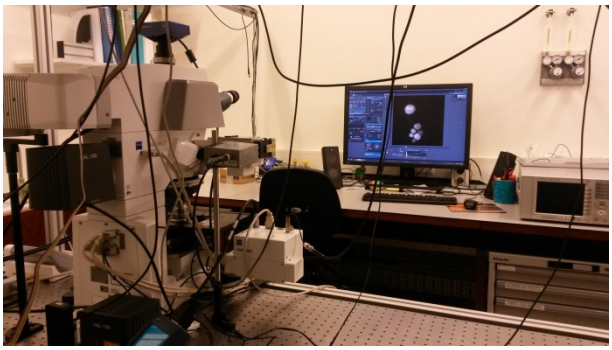
In SRS, two different coloured laser beams are incident on a sample. If the energy difference between them matches a molecular vibration of a molecule, energy can be transferred from one beam to the other. By applying amplitude modulation to one of the beams, the modulation transfer to the other beam can be measured. The efficiency of this process is a direct measure for the number of molecules of interest in the focal volume. Combined with laser scanning microscopy, this technique allows for fast and sensitive imaging with submicrometre resolution [1].

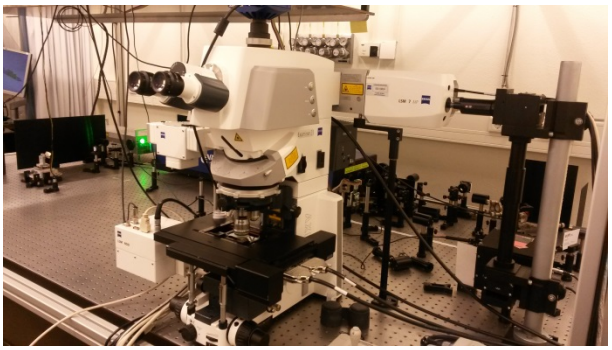
2 Objectives

Development of an SRS microscope for flexible label-free imaging

3 Work performed / results / description

We have built a flexible imaging set-up for Stimulated Raman Scattering microscopy [2]. An 80 MHz laser (Lumera Plecter Duo) with 8 ps pulses at 532 nm is used to pump an Optical Parametric Oscillator (OPO) (APE Levante Emerald) with wavelength output ranges of 775 - 990 nm and 1150 - 1700 nm. These outputs is overlapped in space and time with the 1064 nm fundamental laser line. A combination of a half–wave plate and a polarizing beamsplitter in each beam is used to control the polarization state and laser intensity. An acousto–optic modulator is used for sinusoidal intensity modulation of the 1064 nm beam. A Zeiss laser scanning upright microscope (type LSM 7MP) with 32x objective is used to image samples with non–descanned detection in forward- or backscattering mode. Optical filters reject the 1064 nm modulated beam and the pump beam light is collected on a photodiode. The signal is amplified with a homebuilt transimpedance amplifier before demodulation in a lock-in amplifier (Stanford Research Systems SR844). The ZEN microscopy software is used for image acquisition and processing. For imaging lipids, a shot noise limited detection window is achieved from 0.45 - 60 mW of total power on the sample. These laser powers are a useful range for imaging of biological samples.
This system allows for flexible and user-friendly imaging of a variety of samples. Further improvements are continuously being implemented to make the system more versatile and accessible for non-expert users. Very few SRS microscopes exist in Europe, and after presentations at national and international conferences and symposia, multiple researchers have shown interest in using the set-up for complementing their research. Collaborations have been initiated within the host university with the Institute for Environmental Sciences, within the Netherlands with an inorganic synthesis group at the University of Leiden and abroad with medicinal chemists at ETH-Zürich and pharmaceutical company Roche.





4 Conclusions

We have successfully realised a label-free imaging platform based on Stimulated Raman Scattering (SRS) microscopy.

5 References/Publications

1. C. W. Freudiger, W. Min, B. G. Saar, S. Lu, G. R. Holtom, C. W. He, J. C. Tsai, J. X. Kang, and X. S. Xie, "Label-Free Biomedical Imaging with High Sensitivity by Stimulated Raman Scattering Microscopy," Science **322**, 1857-1861 (2008).

2. M. J. B. Moester, F. Ariese, and J. F. de Boer, "Optimized signal-to-noise ratio with shot noise limited detection in Stimulated Raman Scattering microscopy," Journal of the European Optical Society: Rapid Publications **10** (2015).