A pixel-by-pixel correcting autobalanced detector for SRS microscopy

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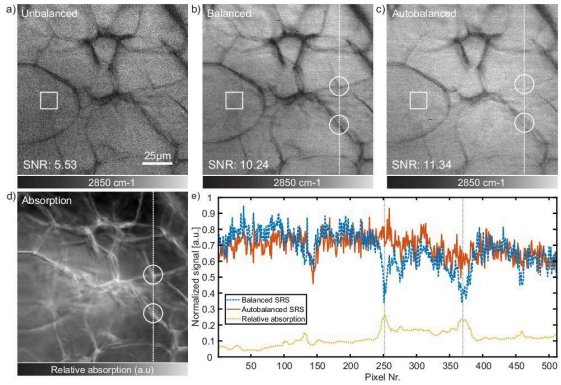
Abstract

Stimulated Raman scattering (SRS) microscopy is a powerful tool in biological and medical research due to the chemically-selective and label-free nature of the Raman process. However, excess noise of the used light source limits the achievable signal-to-noise ratio (SNR) below the SNR given by the shot-noise limit [1,2].

In this submission we present an easy to use, compact, and modular autobalanced detector capable of up to 55 dB correlated noise suppression, 110 ° possible phase compensation at 20 MHz, and 300 ns PID settling time allowing for true pixel-by-pixel sample absorption compensation for pixel scanning rates of up to 1 MHz. Our autobalanced detector is able to provide an SRS image which is absorption compensated as well as shot-noise limited for photocurrents above 1 mA. As a novel addition providing image context, a noise-suppressed absorption image of the sample is simultaneously provided, allowing for combined SRS and absorption imaging. Our autobalanced detector was applied to a home-build SRS microscope setup utilizing a fiber-based dual-color picosecond light source with a 40 MHz Stokes and 20 MHz pump repetition rate, detecting the stimulated Raman gain (SRG) as a 20 MHz modulation on the Stokes beam with a lock-in amplifier. A special high dynamic range photodetector was developed, suppressing the 40 MHz repetition rate signal of the Stokes beam by up to 80 dB before transimpedance conversion, allowing for sensitive and saturation-free detection even for photocurrents above 10 mA. The image improvement by using our autobalanced detector is demonstrated in fig. 1(a)-(c) for sliced chicken tissue. The SNR was increased by a factor of 2, which is limited only by shot-noise due to the available laser power of 10 mW per detector as well as the already low excess noise of the used light source. Additionally, notable in fig. 1 b), c), and e), the autobalancer suppresses parasitic absorptive features in the image. These image features can be introduced by spatially varying optical densities across the sample attenuating the pump and Stokes beam and therefore leading to reduced SRG modulation amplitude. To the lock-in amplifier, a reduction in the modulation amplitude caused by a lower concentration of Raman active molecules or caused by absorption in the sample is indistinguishable. For structured and absorbing samples, the intrinsic absorption compensation of an autobalanced detector therefore offers an additional improvement in addition to the noise reduction.



a)-c) SRS image of lipids in sliced chicken tissue probed at 2850 cm-1 with unbalanced, balanced, and autobalanced detection respectively with 512 by 512 pixels and a pixel dwell time of 10 µs. Calculation of the SNR was performed within the white square.

d) Simultaneous to c) acquired relative absorption image of the sample. Bright pixels correspond to higher absorption.

e) Normalized signal values for the measured balanced SRS, autobalanced SRS, and relative absorption for a vertical slice of the image (dashed white line in b), c), and d)). Two absorption features are marked with a dashed gray line which are suppressed by the autobalanced detection, corresponding image features are marked by white circles in b), c), and d).

References

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