ENHANCING IMAGING SPEED IN THE PRESENCE OF NOISE AVOIDING SAMPLE DAMAGE

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In order to understand the structure and behaviour of a sample, it is necessary to achieve high imaging quality in a short time without influencing or damaging the sample. If imaging speed is not limited for technical reasons, the maximum imaging rate is constrained by the required signal-to-noise ratio (SNR) as well as the sample damage threshold.

There are different strategies for sample illumination leading to comparable imaging results but intrinsically different light load on the sample. For example, light sheet microscopy [1] is known to be gentle to sensitive fluorescent samples in contrast to confocal microscopy.

However, in order to assess the influence of an illumination geometry on the sample, the properties of the mechanism which is responsible for sample damage are crucial. In case of conventional microscopy, Raman microscopy [2] or fluorescence microscopy with robust fluorophores, the main issue is thermal load due to linear absorption in the sample.

Here we compare the achievable SNR of several microscope and micro-spectrometer approaches using different illumination geometries while being constrained by thermal sample damage.

As a result, we are able to explain the unintuitive behaviour of the damage threshold and the achievable SNR gain for different scanning and non-scanning microscopes. We explain the thermal advantage of light sheet illumination and why it can easily speed up a measurement by multiple orders of magnitude in comparison to confocal scanning. On the other hand, we also prove that light sheet illumination is not unconditionally a better choice than confocal illumination for fast hyperspectral imaging [3]. In addition, we examine new illumination approaches for microscopy and micro-spectroscopy.

These investigations enabled us to improve the speed of Raman micro-spectroscopy and to understand its limits. We believe that further examining the SNR and the sample damage threshold for other microscopy methods is the key for their further speed enhancement.

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