METROLOGY IN NONLINEAR MICROSCOPY USING HARMONIC GENERATION NANOPROBES

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Keywords: nonlinear microscopy, harmonic generation nanoprobes, chromatic aberrations, multicolor imaging.

Technological developments in nonlinear or multiphoton microscopy are steadily improving, and their field of application constantly broadening. For example, current trends consist of extending the range of excitation wavelengths to perform multi-parametric imaging [1,2]. However, these new approaches remain challenging to implement. Indeed, some of them take advantage of the extended range of excitation wavelengths available with new laser sources, where optics are not necessarily corrected, while others require perfect foci co-alignment over large field of views and wavelength ranges, making them critically sensitive to chromatic aberrations. In these cases, the precise calibrations of nonlinear systems over a broad range of wavelengths is therefore critical. However, appropriate protocols are very challenging to implement.

In this study, we present a new and straigthforward method to calibrate nonlinear microscopes over a broad range of excitation wavelengths [3]. We show that harmonic generation nanoprobes are a unique tool to map the spatial resolution, field curvature and chromatic aberrations across the whole field of view. We analyze and compare measurements obtained with several microscope objectives designed for multiphoton microscopy. Finally, we discuss strategies to minimize the impact of chromatic aberrations for multicolor two-photon acquisitions.

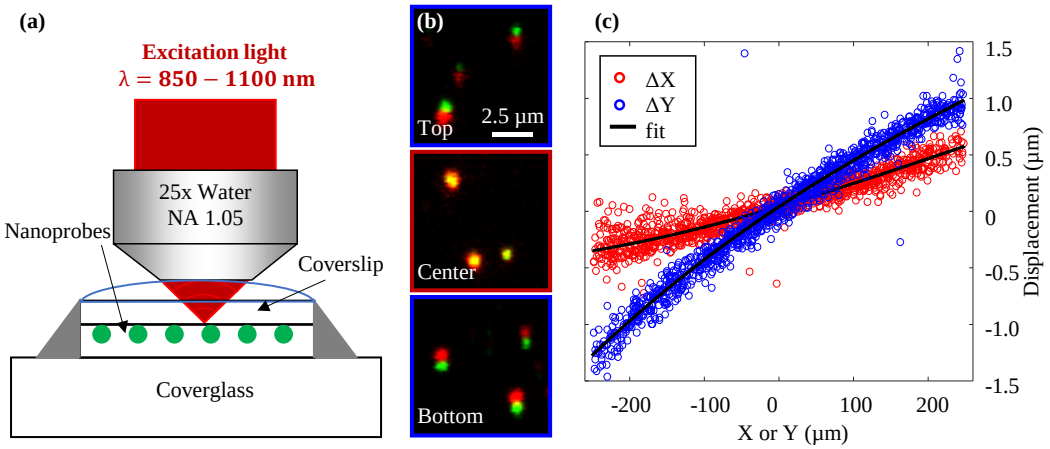

Figure: Quantification of lateral chromatic aberrations. (a,b) Nanoprobes are imaged at different excitation wavelengths (for e.g. λ = 850 & 1100 nm, green and red signal in (b), respectively). (c) Lateral chromatic aberrations are estimated across the field of view by comparing the positions of each nanoprobe imaged at different excitation wavelengths (for e.g. λ = 850 & 1100 nm).

[1] Mahou *et al. Nat. Methods* **9,** 815-18 (2012).

[2] Alexander *et al.* Curr. Opin. Cell Biol. **25** , 659-71 (2013)

[3] Mahou et al., in preparation.