Multiphoton Super-Resolution Microscopy

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Imaging biological samples with sub-cellular precision is important for our understanding of fundamental processes governing biological processes. Standard optical techniques allows to resolve structures as small as $0.51\lambda/\mathrm{NA}$, where λ is the wavelength of the light used, and NA is the numeric aperture of the imaging objective lens. A better resolving power can be achieved with advanced techniques referred to as super-resolution microscopy. When coupled with single photon excitation, super-resolution techniques are limited in imaging depth by absorption and photo-darkening. Photo-darkening is especially detrimental when acquiring image stacks, *i.e.* to obtain a 3-dimensional image. As the optical sectioning inherent to multiphoton imaging [1] relies on the nonlinear confinement of the excitation to the focus, out-of-focus photo-darkening is dramatically reduced compared to single photon excitation microscopy. As the illumination is done through the imaging objective, ultra-high NA optics can be naturally used with multiphoton excitation. Compared to single photon microscopy, the resolution that can be achieved with multiphoton microscopy is less good, because the effect of taking the excitation point spread function to the n-th power (for n-photon excitation) is more than offset by the n times longer wavelength needed. Nonetheless, the loss in achievable resolution can be re-gained by combining multiphoton excitation with super-resolution techniques.

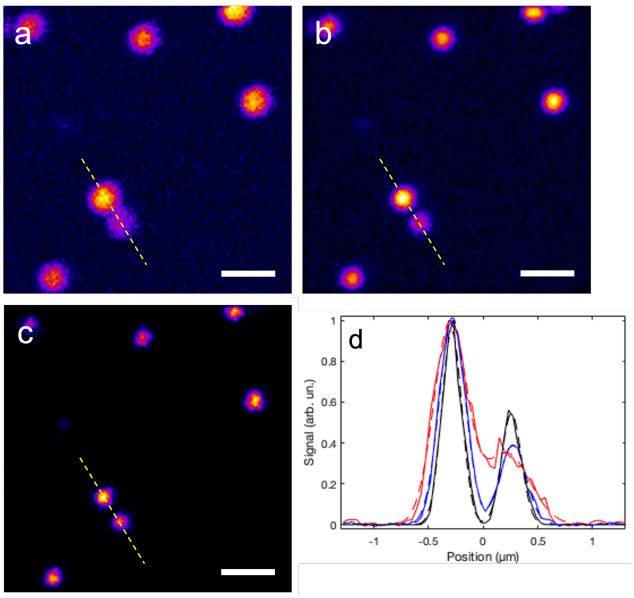

Figure 1: Resolution enhancement of multi-photon microscopy by using different super-resolution techniques. Image taken with (a) standard 2-photon microscopy, (b) ISM enhanced 2-photon microscopy, and (c) SOFI+ISM (SOFISM) enhanced 2-photon microscopy. (d) Profile along yellow line in the images, showing the consecutive enhance-ment of ISM and SOFISM

I will present recent results we have obtained demonstrating the combination of 3-photon excitation with image scanning microscopy (ISM) [2], which allowed us to obtain sub-diffraction limited images with a resolution of $\lambda/8$. Combining different super-resolution techniques allows to further improve the imaging resolution. We demonstrate 2-photon excitation with super-resolution optical fluctuation imaging (SOFI) [3] and ISM [4], which allows us to achieve an imaging resolution better than 100 nm, *i.e.* better than $\lambda/10$. We apply 3P ISM to image samples to a depth greater than 100 µm, where confocal imaging is limited to just a few µm due to photodarkening.

References

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