Widefield two-photon microscopy with temporal focusing and line-scanning for molecular motors tracking in C Elegans worms.

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In order to study dynamical processes at a single molecule level in living multicellular organism, fluorescence microscopy is a method of choice: it permits to observe dynamical behaviour of single molecules almost non-invasively. Nonetheless, in relatively thick samples with a background signal caused by auto-fluorescence, unspecific staining or light scattering, the brightness of a few fluorescent proteins of interest might not give a satisfactory signal over noise ratio. The "axial sectioning" power of confocal microscopy or conventional (point-scanning) two-photon microscopy can be used to remove most of the out of focus signal. However, the need to scan the whole field of view point by point limits the measurements time resolution, which is by definition critical for dynamical processes.

In order to get both a good axial sectioning power and a quick wide-field illumination, the combination of temporal focusing with line-scanning microscopy has been proposed in 2005 [1], and used for imaging purposes in combination with PALM several years later [2].

We propose to use this technique for *in vivo* particle tracking. More particularly, we are interested in intraflagellar transport (IFT) process in C Elegans chemosensory cilia [3], involving molecular motors (kinesins and dyneins).

Compared to previous standard WF fluorescence measurements (*e.g.* Fig. 1), significant quality improvements of the data collected are expected. Our versatile setup, allowing multiple 2-photon fluorescence imaging modes (widefield temporal focusing, temporal focusing + line-scanning, point-scanning), will be presented along with some early experimental results.

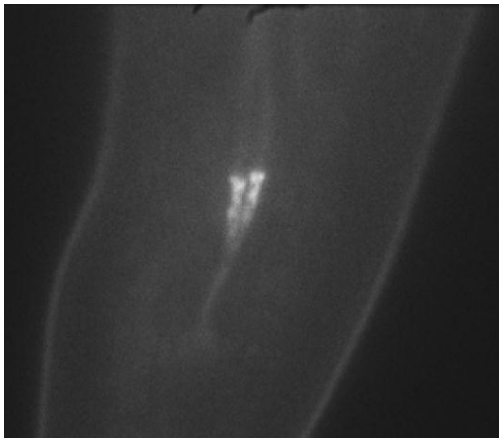

Figure 1. Single photon fluorescence image of kinesins in a *C Elegans* chemosensory cilia. *A strong fluorescent background is clearly visible and could be removed with line-scanning temporal focusing.*

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