FAST PALM-TIRF COMPARATIVE IMAGING

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Over recent years, a number of super-resolution techniques appeared following the increase of possibilities offered by fluorescent probes. One of these techniques, PALM microscopy (Photoactivation localization microscopy), uses sequential and stochastic switching ON and OFF of specific fluorophores. This slow and repetitive process requires thousands of iterations until the majority of molecules have been accurately detected and mapped. These long acquisition times (up to 30 minutes for 15.000 planes) combined with very high resolution (<50 nm) reveal stability defects in XY and Z both with a z autofocus or a manual stage.
Therefore we improved the acquisition parameters to reduce the acquisition time as much as possible.
In order to do so, we use a dual channel TIRF prototype equipped with a 561 nm 1W solid state laser among other light sources. Compared to a conventional laser (around 100mW per line), this power enables us to reduce significantly the exposure time and thus the bleaching phase of the mEOS2 probe. We have also optimized the acquisition step by replacing the numerous 405nm laser pulse by a continuous very low UV activation. On this setup equipped with two of the latest generation of EMCCD camera, we reach a streaming acquisition at a 100 fps frequency on PA-FP molecules where other setup are limited to 10 fps.
Consequently, a 15.000 planes acquisition drops down to less than 2 minutes, reducing the possible drift in XY and Z. This improvement is critical to reach the very high resolution expected. PALM images of cells expressing PA-FP mEos2 and mCherry coupled proteins will be presented and compared with TIRF images of the same cells acquired in two channels.