Dual-color 3D PALM/dSTORM imaging of centrosomal proteins using MicAO 3DSR

Grégory Clouvel\textsuperscript{1}, James Sillibourne\textsuperscript{2}, Audrius Jasaitis\textsuperscript{1}, Ignacio Izeddin\textsuperscript{3}, Xavier Levecq\textsuperscript{1}, Maxime Dahan\textsuperscript{4}, Michel Bornens\textsuperscript{2}, Xavier Darzacq\textsuperscript{3}

\textsuperscript{1}Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France
\textsuperscript{2}Institute Curie, CNRS UMR144, 26 rue d’Ulm, 75248 Paris Cedex 05, France
\textsuperscript{3}Institute de Biologie de l’Ecole Normale Superiore, CNRS UMR8197, 46 rue d’Ulm 75230 Paris Cedex 05, France
\textsuperscript{4}Institute Curie, CNRS UMR 168, 26 rue d’Ulm, 75248 Paris Cedex 05, France

The determination of 3-dimentional arrangement of cellular structures has become a necessary requirement in cellular biology. Unfortunately, the size of such structures usually lies beyond the diffraction limit and therefore they cannot be visualized in studies using today’s widely popular fluorescence microscopy techniques. Photoactivation localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) enables us to locate fluorescent molecules with nanometric resolution. We used these microscopy methods together with MicAO 3D-SR – the first adaptive optics device which introduces the three dimensional imaging capability for PALM/STORM. It also corrects various types of aberrations, induced by optical elements inside the microscope and by the biological sample. This correction almost doubles the number of detected photons and increases the localization precision by 40%. At 1000 detected photons the localization precision of our setup is 8 nm in lateral and 16 nm in axial directions. The separate optimization performed for two different colors delivers the superb imaging quality. We demonstrate this by imaging two centrosomal proteins in HeLa cells.