Super-resolution microscopy has brought a significant improvement in nano-scale imaging of molecular assemblies in biological media. However, apart from recent work on proteins aggregates [1], its extension to imaging molecular orientation using fluorescence anisotropy has not yet been fully explored. Providing orientational order information at the nano-scale would be a considerable progress for the understanding of biological functions since the structure of biomolecular assemblies is strongly interrelated with fundamental processes such as in clustering in cell membranes. In addition, anisotropy imaging combined with super-resolution would open new possibilities to measure the temperature of single molecules in nano-structured local environments, following previous approaches already developed in standard microscopy [3].

In this work, we report a super-resolution polarization-resolved microscopy technique able to image molecular orientation behaviors in static and dynamic environments. Using direct Stochastic Optical Reconstruction Microscopy (dSTORM) [4] in combination with polarized detection, fluorescence anisotropy images can be reconstructed (Fig. 1) at a spatial resolution of 20 nm. Analysis of the data using a numerical model based on orientational diffusion allows retrieving molecular angular constraint information (molecular order $\psi$ in Fig. 1) as well as local environment properties such as local viscosity or temperature. We illustrate this technique on molecular order imaging in COS-7 Actin fibers (Fig. 1) and local temperature retrieval in molecules freely diffusing over heating metal structures.

References