Biomolecular orientational organization is a crucial factor in biological processes where functions (such as cell motility, vesicular trafficking, signalling, protein interactions, etc.) can be closely related to orientation and ordering. For instance, the investigations of structural behaviours of bio-molecular assemblies are sine qua none condition towards a better understanding of the fundamental mechanisms governing the cell membrane.

One-photon fluorescence microscopy provides a convenient and powerful tool toward this goal. Indeed, both absorption and emission of light are strongly dependent on the orientation of the fluorescence dipole with respect to the polarization of the excitation and emission fields. In this aim, we have developed a general polarimetric fluorescence microscopy technique that relies on the analysis on the fluorescence image that is recorded for several directions of exciting polarizations. With this technique, the angular distribution of an ensemble of dipoles present in the confocal volume can be monitored without a priori knowledge on the average orientation [1].

The principle of the technique will be detailed. The determining parameters that allow to improve the measurement duration and accuracy will be discussed, as well as the statistical data processing methodology. The power of the method will be illustrated on biological systems, including the MHC Class I proteins in the plasma and nuclear membranes, the lipid orientational order in the plasma membrane. Using pharmacological treatments, we will show how it can effectively monitor structural changes (Fig. 1).

Figure 1: (a) Illustration of the angular distribution width $\Psi$ for different levels of molecular order of the membrane. (b, c) Fluorescence images and resulting value of $\Psi$ recorded on a live cell with lipid labeling before (b) and after (c) disruption of the actin cytoskeleton.