

# Spatially and time-resolved fluorescence anisotropy imaging of membrane dynamics in living cells

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Fluorescence imaging – based on its intensity, lifetime, wavelength or polarization – is an important tool in biological science, and provides structural as well as functional informations. Since fluorescence lifetime and anisotropy depend on the lipid phase of cell membranes, their measurement permits the analysis of membrane dynamics in cells. However, in contrast to free rotation in liquid media the motion of fluorophores in organized structures such as membranes is limited in angular range, because the surrounding architecture usually imposes certain restrictions on the orientation of the probe.

In conventional wide field microscopy, the fluorescence image is composed by the signals originating from the focal plane as well as from out-of-focus planes of the object. However, by structured illumination an axial resolution in the micrometer range can be achieved [1], thus providing informations from different planes of the sample. Therefore, fluorescence anisotropy of different membranes (located at different depths of the sample) can be obtained. Further resolution on cell surfaces (e.g. plasma membranes) is achieved by variable-angle Total Internal Reflection Fluorescence Microscopy (TIRFM) [2].

In the present paper membrane dynamics of cultivated U373-MG human glioblastoma cells is characterized by fluorescence anisotropy measurements of the membrane marker 6-dodecanoyl-2-dimethylamino naphthalene (laurdan). In particular, steady-state and time-resolved fluorescence anisotropies as well as rotational relaxation times are evaluated. So far, membrane dynamics depended on temperature, growth phase as well as the on the intracellular amount of cholesterol. While highest membrane fluidities were found in the nuclear membrane, lowest fluidities were observed in the plasma membrane.

## References:

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