Design and validation of a new confocal FRET polarization anisotropy microscope

Frank Tobias, Rayees Khan, Marco Durante, Burkhard Jakob, Gisela Taucher-Scholz
GSI Helmholtzzentrum für Schwerionenforschung GmbH
Department of Biophysics
Planckstraße 1, 64291 Darmstadt, Germany
E-mail: f.tobias@gsi.de

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INTRODUCTION
Protein interactions constitute a fundamental process in almost all regulatory pathways in living cells but are highly challenging with regards to experimental accessibility. With the use of GFP and the production of numerous kinds of fluorescent derivates it became possible to measure Förster Resonance Energy Transfer (FRET) between fluorescently labelled proteins in living cells. FRET can be detected via different techniques. Most popular are sensitized emission, acceptor photobleaching and fluorescence lifetime imaging. Here we present a setup to measure FRET by detecting fluorescence polarization anisotropy. The system is based on a Yokogawa spinning disk confocal microscope. The setup is shown and validated with live cell measurements of GFP and mCherry constructs.

RESULTS
The experimental setup is based on a Nikon TiE inverted microscope equipped with 488 nm and 561 nm LASERs, a Yokogawa CSU-X1 confocal spinning disk unit and an image splitter with two Andor EMCCD cameras. A polarizer is placed in the excitation light path right before the first spinning disk within the CSU-X1. Hence the fluorescence is excited with linear polarized light. A polarizing beamsplitter is used in the emission light pass after the CSU-X1 and a filter wheel. The two different polarization components of the emission light are separated and directed to two different cameras. The images of both cameras are used to calculate fluorescence polarization anisotropies. FRET is detectable by a reduction in the fluorescence polarization anisotropy [1]. This technique prevents false positives, as it is less sensitive to excitation and emission crosstalk and has all the advantages of a confocal spinning disk microscope for live cell applications.

To validate the system for live cell applications cells were either transiently transfected with GFP or mCherry only, with GFP linked to mCherry by 13 amino acids (GFP-mCherry) or cotransfected with GFP and mCherry. Only in the case of GFP-mCherry the chromophores get close enough for FRET to occur. As expected cells transfected with GFP-mCherry showed a clear reduction in the acceptor fluorescence polarization anisotropy compared to GFP, mCherry, and cotransfection of GFP and mCherry. Hence this confocal spinning disk setup is well suited to measure FRET by fluorescence polarization anisotropy.

CONCLUSION
A microscopic setup was established which enables the measurements of FRET with all the advantages of a spinning disk confocal microscopy for live cell applications.

REFERENCES

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