COMPARISON OF FRET QUANTIFICATION IN TIRF AND WIDEFIELD RECORDINGS

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For research in various biological fields quantitative methods in Microscopy is getting more and more important. However to receive reliable and quantitative results the experimental effort especially for applying Foerster Resonance Energy Transfer (FRET) is daunting for most scientists [1, 2]. On the other side the utilization of quantitative FRET in fluorescence microscopy allows evaluating and quantifying sub-cellular signalling and physiological protein interactions.

Moreover if the interaction is physiologically restricted to specific compartments (e.g. the cell membrane) it might be beneficial to narrow the observation down to this area of interest. Therefore we used the Total Internal Reflection Fluorescence (TIRF) technique, which allows to restrict the observed area to a very thin layer (below 200 nm) [3], in combination with FRET to improve signal quality.

This makes TIRF a very interesting method to examine for processes in cellular membranes and its adjacent cell-plasma areas. The restriction to this thin layer also reduces background noise and fluorescent crosstalk from the complete cell volume.

To evaluate this potential benefit we compared FRET measurements of membrane-bound proteins using widefield and TIRF. We investigated the protein interactions between the membrane bound serotonin receptor 5-HT7 and one of its downstream effectors Gα12 [4].