Our research focuses on cell signaling and kinetics as well as cell adhesion, migration and cancer progression. Cell adhesion and migration are investigated via growth and development as well as molecular signaling pathways, e.g. serine and tyrosine kinases. Development and cancer progression is studied with an emphasis on the role of growth factors, such as the Bone Morphogenetic Protein (BMP)/Smad pathway. We have also taken on new challenges such as the imaging of neurodegeneration.

FRET microscopy allows us to observe both the dynamics and cellular localization of protein conformational changes and protein-protein interactions with improved interpretation based on both anisotropy and fluorescence lifetime. We observe the diffusion dynamics of lipids and proteins by long-range techniques such as FRAP, to complement short-range methods such as FCS. We also measure single-molecules by wide-field imaging and total internal reflection fluorescence microscopy (TIRF), since we now have the latest technologies to monitor long and short diffusion ranges with tracking, FRET, and co-localization events.

Sheet illumination microscopy for improved, sensitive imaging in tissues and organisms

We developed our own versions of selective plane illumination microscopy (SPIM) and ultramicroscopy. SPIM and ultramicroscopy use light sheet illumination parallel to the focal image plane of a microscope objective, a concept introduced by Siedentopf in 1903. Our setup enables penetration depths beyond 1 mm in living embryos with resolution comparable to confocal microscopy. Growing evidence points to the importance of tracking low numbers of proteins in tissues and living organisms. We successfully applied our SPIM setup to detect single proteins and single nano-crystals. Ultramicroscopy applies sheet illumination to image large tissue sections cleared to achieve optical transparency by methods developed by Spateholz in 1914. To overcome the limitations of only 0.4 mm penetration in mice of up to 5 weeks old, we improved the clearing procedures, instrumentation, and analysis to perform imaging in adult mouse tissue (> 2 years) yielding cellular and anatomical details of disease models. As a first model, we used an AAV-DsRed expression system and showed that our improved setup can achieve over 4 millimeter penetration with subcellular resolution in various tissues of the central nervous system (CNS).

Fig. 1: Microscopy systems in the RVZ.
**Impaired axonal transport in motor neurons correlates with clinical prion disease**

Together with Michael Klein from the Institute of Virology in Würzburg, we investigated issues of neurodegeneration in prion disease. Prion diseases are fatal neurodegenerative disorders causing motor dysfunctions, dementia and neuropathological changes, such as spongiosis, astrogliosis and neuronal loss. The chain of events leading to the clinical disease and the role of distinct brain areas are still poorly understood. The role of axonal impairments in prion pathology remains controversial, since there is no evidence for either functional impairments or their connection with prion pathology. Our study demonstrates that functional axonal impairments in motor neurons correlate with the onset of clinical prion disease for several mouse lines despite profound differences in the incubation times. Axonal transport impairments were documented in 30 to 45% of red nucleus neurons bilaterally after intracerebral prion inoculation, and unilaterally after inoculation into the right sciatic nerve. Up to 94% of motor cortex neurons also demonstrated transport defects. The alterations in protein localization implicate a mechanism of transport disruption at the level of cargo attachment to the dynein-dynactin complex. Our work reveals a connection between axonal transport impairment and disease symptoms in vivo for different prion strains and inoculation routes. These findings establish further insights into prion pathology development and suggest novel targets for therapeutic and diagnostic approaches.

**Anisotropy and FRET microscopy: PH domain and PTH receptor**

We developed a method and instrument of polarized fluorescence resonance energy transfer (FRET) and anisotropy imaging microscopy carried out in parallel for improved interpretation of photophysical interactions. This set-up could better determine the protein-protein interactions of the pleckstrin homology domain and conformational changes in the Parathyroid Hormone Receptor, a G-protein coupled receptor, both fused to the cyan and yellow fluorescent proteins for either inter- or intra-molecular FRET. From the anisotropy measurements of donor and acceptor of these FRET interactions, we find that our instrumentation and method also characterize crucial effects due to homo-transfer, polarization-specific photobleaching and background molecules.

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**Extramural Funding**

DFG GK 1048
DFG GK 1342

**Selected Publications**


