# Pair correlation analysis of Fixed Photoactivatable Localization Microscopy (PALM) and Powerspectral Analysis of Live PALM applied on the Water Channel Aquaporin-3

**Arnspang, E. C.; Sengupta, P.; Jensen, H. H.; Hahn, U.; Andersen, I. T.; Jensen, Ellen Birgitte Agerbo; Mortensen, Kim; Lippincott-Schwartz, J.; Nejsum, L. N.**

*Published in:*
Molecular Biology of the Cell

*Publication date:*
2016

*Document Version*
Publisher's PDF, also known as Version of record

diffraction limit. While many of these approaches require specialized instrumentation and are difficult to implement with biological specimens, expansion microscopy (ExM) provides an alternative approach that is compatible with a conventional confocal microscope. Here, we report our progress in adapting ExM for use with Drosophila tissues.
ExM relies on physical expansion of samples to effectively increase resolution. At all stages in its life cycle Drosophila has a rigid cuticle. We therefore implemented an additional enzymatic digestion step to remove the cuticle, allowing for robust expansion of diverse Drosophila samples including embryos, larval body walls, and larval brains. To ascertain whether the sample expansion causes distortion, we performed correlative pre- and post-expansion imaging and found through quantitative image analysis that sample distortions are typically less than 1%. To assay the capacity of ExM to improve resolution, we performed comparative analysis of conventional microscopy and ExM in visualization of a variety of cellular structures and organelles, and in all cases we observed a significant improvement in resolution using ExM. A variety of superresolution techniques have been used to characterize presynaptic active zones in Drosophila, therefore we stained Drosophila larval neuromuscular junctions (NMJs) with antibodies to the active zone marker Bruchbilot (Brp) and assessed the ability of ExM to resolve structural features of active zones. In contrast to conventional confocal microscopy, ExM allows for resolution of structural features of active zones, and correlative imaging demonstrates that confocal-based analysis leads to undercounting of synapse number. Currently, we are using ExM to monitor age-related changes in active zone structure. Finally, we have developed techniques for multicolor labeling for ExM to facilitate analysis of cell-cell interactions, and have begun to use these techniques to study epithelial ensheathment of somatosensory dendrites. Similar to our findings with Brp staining, we find that confocal-based analysis underreports on epithelial ensheathment of somatosensory dendrites. Altogether, these results demonstrate the utility of ExM for superresolution imaging of Drosophila tissues.

## P1665

**Board Number: B208**

### Pair correlation analysis of Fixed Photoactivatable Localization Microscopy (PALM) and Powerspectral Analysis of Live PALM applied on the Water Channel Aquaporin-3.

E.C. Arnspang[1,2,3], P. Sengupta[1,4], H.H. Jensen[5], U. Hahn[6], I.T. Andersen[6], E.B. Jensen[6], K. Mortensen[7], J. Lippincott-Schwartz[1,4], L.N. Nejsum[3];
[1]The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, United States, [2]Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Odense, Denmark, [3]Department of Clinical Medicine and Interdisciplinary Nanoscience Center , Aarhus University, Aarhus, Denmark, [4]Janelia Research Campus, Ashburn, United States, [5]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark, [6]Department of Mathematics, Aarhus University , Aarhus, Denmark, [7]Department of Micro- and Nanotechnology, Technical University of Denmark, Copenhagen, Denmark

Water transport across the plasma membrane of epithelial cells is mediated by aquaporin (AQP) water channels. In the renal collecting duct, water is reabsorbed from the renal filtrate through apically localized AQP2 and exits the cells through AQP3 and AQP4, which are expressed in the basolateral plasma membrane, where AQP3 is the main exit pathway. The antidiuretic hormone Arginine Vasopressin (AVP) increases urine concentration via an increase in cAMP, leading to insertion of AQP2 containing vesicles into the apical plasma membrane. This instantly increases water reabsorption and urine concentration. We previously found that a short-term increase in cAMP leads to an increase in lateral diffusion of AQP3, revealing short-term regulation. To further study if AQP3 is regulated at the

nanoscale level, we first combined single molecule detection using Photoactivatable Localization Microscopy (PALM) imaging of fixed cells with pair correlation (PC). This showed that AQP3 molecules organize in nano-domains smaller than 60 nm and upon stimulation mimicking vasopressin, change organization to 60 – 200 nm sized nano-domains. Thus, PC-PALM revealed regulation at the nanometer resolution. Furthermore, we performed live-PALM of AQP3 upon cAMP stimulation and have initiated analysis by power spectral analysis. The analysis was done by first identifying isolated spots and fitting with a two-dimensional point-spread function. The localization errors were found theoretically and the diffusion coefficient for each trajectory was calculated using a covariance-based estimator. To demonstrate that the identified molecules were indeed freely diffusing with identical diffusion coefficients, we calculated the power-spectrum of each trajectory. The power-spectral values were rescaled with their expected values given theoretically as a function of the averaged diffusion coefficient and the localization errors. Thus, fixed PALM revealed that AQP3 changed nano-organization in the plasma membrane upon stimulation mimicking vasopressin; from an even distribution to an organization in nanoclusters. This indicates short-term hormone regulation of AQP3 at the nanoscale level, which may be important in urine concentration. PC-PALM may be used to reveal previously undetectable protein regulation at the nanoscale. We furthermore did live-PALM and are currently analyzing the data using power spectral analysis.

## P1666

**Board Number: B209**

### Engineering nanobodies for super-resolution imaging and native protein complex isolation.

T. Pleiner[1], M. Bates[2], S. Trakhanov[1], C.T. Lee[3], J.E. Schliep[4], H. Chug[1], M. Böhning[1], H. Stark[4], H. Urlaub[3], D. Görlich[1];
[1]Cellular Logistics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [2]NanoBiophotonics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [3]Bioanalytical Mass Spectrometry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [4]Structural Dynamics, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Nanobodies are single-domain antibodies derived from atypical antibodies of camelids. They are commonly produced in *E. coli* by periplasmic secretion - a process that is incompatible with many tagging and engineering strategies. Hence, nanobodies were previously immobilized covalently via lysines for affinity chromatography and this required denaturing elution conditions. Likewise, fluorescent labeling of nanobodies at lysines for imaging often directly interfered with antigen recognition.
We demonstrate functional cytoplasmic expression of nanobodies with protease-cleavable tags for native affinity purification and with engineered cysteines for site-specific fluorescent labeling. We chose the *Xenopus* nuclear pore complex (NPC) as a model target and developed high-affinity nanobodies against its constituent proteins, called nucleoporins (Nups). Using specific nanobodies, we purified their target protein complexes from *Xenopus* egg extract in a single step with native elution based on proteolytic matrix-release. This allowed a direct structural analysis of nanobody-purified endogenous Nup complexes by electron microscopy. We further implemented a simple strategy for site-specific fluorescent labeling of nanobodies for imaging. It involves a selective modification of engineered cysteines at the nanobody surface with maleimide fluorophores and keeps the internal framework cysteines fully intact. This strategy allowed super-resolution imaging of NPCs with a negligible label displacement and very low background. The introduced cysteines further proved useful for rapid epitope mapping via crosslinking mass spectrometry.
The presented strategies greatly facilitate the use of nanobodies in affinity isolation and imaging and are applicable to any nanobody and nanobody-target.