Elucidating the nanoscale architecture of the plasma membrane with super-resolution spectroscopy

Erdinc Sezgin, Christian Eggeling

University of Oxford, MRC Weatherall Institute of Molecular Medicine, OX39DS, Oxford, UK

Email: erdinc.sezgin@rdm.ox.ac.uk

Diffusion and interaction dynamics of molecules at the plasma membrane play an important role in cellular signalling. Nanoscale mobility of lipids and proteins in the plasma membrane is highly heterogeneous. This heterogeneity gives invaluable information on the bioactivity of these molecules. Thus, it is crucial to accurately measure the diffusion dynamics of the membrane molecules. Here, we will explain how we utilise super-resolution STED microscopy combined with fluorescence correlation spectroscopy (STED-FCS) to access the nanoscale diffusion characteristics of fluorescently labelled lipid analogues and proteins in the live cell plasma membrane. Moreover, we establish an advanced STED-FCS measurement method; line interleaved excitation scanning STED-FCS (LIESS-FCS) which discloses the molecular diffusion modes at different spatial positions with a single measurement. Our data demonstrate a powerful experimental approach to decipher specific influences on molecular plasma membrane dynamics. By using this experimental setup, we show the role of cortical actin cytoskeleton on nanoscale membrane architecture by measuring the diffusion dynamics in cytoskeleton-free cell derived giant plasma membrane vesicles (GPMVs).

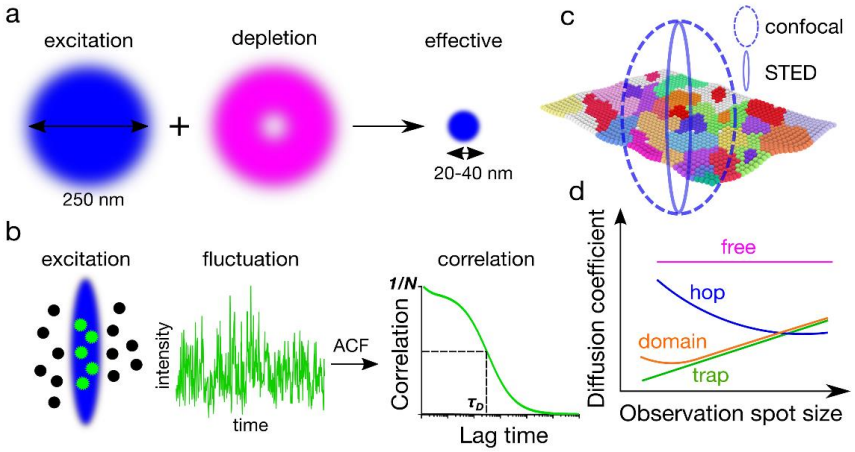

Figure 1. Principles of (a) STED and (b) FCS. (c) STED-FCS can yield nanoscale diffusion behaviour. (d) Diffusion law in the plasma membrane.

References

Schneider, F., Waithe, D., Clausen, M.P., Galiani, S., Koller, T., Ozhan, G., Eggeling, C., and Sezgin, E. (2017). Diffusion of lipids and GPI-anchored proteins in actin-free plasma membrane vesicles measured by STED-FCS. **Molecular Biology of the Cell** 28, 1507-1518.

Schneider, F., Waithe, D., Galiani, S., Bernardino de la Serna, J., Sezgin, E., and Eggeling, C. (2018). Nanoscale Spatiotemporal Diffusion Modes Measured by Simultaneous Confocal and Stimulated Emission Depletion Nanoscopy Imaging. **Nano Letters** 18, 4233-4240.

Sezgin, E., Schneider, F., Galiani, S., Urbancic, I., Waithe, D., Lagerholm, B.C., and Eggeling, C. (2019). Measuring nanoscale diffusion dynamics in cellular membranes with super-resolution STED-FCS. **Nature Protocols**.