Giant unilamellar vesicles (GUVs) present an accessible model system to study diffusion, transport and self-organization phenomena in membranes under well-defined conditions. We used a light sheet fluorescence microscope to observe these processes at the single molecule level. Tracking of single fluorescent particles and molecules can reveal details of the underlying dynamics, which would remain uncovered in the ensemble average. The optical sectioning capability of light sheet microscopy [1] and fast parallel image acquisition by sensitive cameras enabled us to track lipids carrying a single fluorophore in the GUV membrane at a temporal resolution of 10 ms and below [2].

To overcome the limitation of most tracking techniques to 2D samples we placed a cylindrical lens in the detection path of our setup. This created an astigmatic point spread function (aPSF) and encoded information on the third spatial dimension in the exact shape of the aPSF [3]. We combined this approach and a real-time tracking algorithm, what allowed us to keep an emitter in the focal plane of the instrument by continuously adjusting the z-position of the sample stage, increasing the observation time for individual particles by more than one order of magnitude [4].

The setup was used to follow three-dimensional trajectories of fluorescent nanobeads with a diameter of about 40 nm attached to the surface of GUVs. GUVs of different lipid compositions (DOPC, Cholesterol, DPPC, DHPE) and 40 – 80 µm diameter were prepared by electroformation [5]. The GUV membrane contained small amounts of fluorescently labeled lipids to visualize the GUV membrane. Biotin anchors bound neutravidin-conjugated fluorescent beads. Even tracking of single fluorescent lipids, integrated into the GUV membrane could be achieved by using a qualified lipid composition. Although single fluorophores only give low signals, we were able to track single POPE-Atto647 molecules at concentrations of about 10⁻⁸ mol% within a DPPC/Cholesterol (1:1) membrane. Trajectories spanning several hundred frames in case of fluorescent nanobeads and up to 130 frames in case of single molecule tracking allowed us not only to analyse diffusive behaviour of single beads, but also of single lipid molecules within a curved GUV membrane.