Optical window for immersion objectives to improve long-term and high throughput imaging

Abstract
Scientists from the Goethe University Frankfurt am Main developed a optical window, which is a attachment for water immersion and water dipping objectives. Water dipping and immersion objectives are frequently used for different types of light sheet microscopes (LSMs), such as inverted high-throughput (HT) LSMs. The optical window serves as a fluid liquid reservoir to avoid the evaporation of the immersion fluids during long-term and high throughput imaging of large biological samples in multi-well plates.

(A) Photograph of the optical window attached to a water dipping objective. (B) Close-up of the optical window attached to an illumination objective, highlighting the edges of the optical window (magenta).

Invention
Water dipping and immersion objectives are frequently used in combination with HT-LSFM, due to their high numerical aperture and consequently high resolution. Immersion fluids allow the constant contact of the immersion objective lens and the multi-well plate. However, during long-term and high-throughput measurements, the immersion fluids evaporate, which requires replenishing of the immersion fluid. To overcome this challenge, scientist from Goethe University developed the optical window. By applying the optical window for immersion and water dipping objectives, the problem of evaporating immersion fluids can be overcome. The optical window is composed of a transparent material that allows the passing of the illumination light in the ultraviolet, visible and near infrared range, along the illumination axis. Additionally, a reservoir is included, which can be replenished with the matching immersion fluid such as water, oil, or a gel. The material can be adapted to the refractive index of the immersion fluid to prevent light scattering.

The optical window can be applied during acquisition of biological samples using inverted HT-LSFM. The optical window can be attached to immersion or water dipping objective lenses. During long-term acquisitions of high amounts of biological samples, the optical window prevents evaporation or refilling of the immersion fluid. The reservoir of the optical window can be filled with different immersion fluids, which match the refractive index of the sample medium.