SIMULTANEOUS SPATIOTEMPORAL SUPER-RESOLUTION FLUORESCENCE MICROSCOPY

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Super-resolution microscopy and single molecule fluorescence spectroscopy often require mutually exclusive experimental strategies optimizing either time or spatial resolution. While the measurement of biomolecular dynamics on the single molecule level requires fast measurements on the millisecond scale, super-resolved images require acquisition times on the second scale to achieve the required signal-to-noise ratio. These complementary requirements render the combination of spatiotemporal super-resolution microscopy challenging. Past solutions either restricted time resolution, limited the field of view and number of recorded points, or they required specialized instrumentation or fluorescent labels, restricting access to the techniques. To achieve high spatiotemporal resolution, we implemented a GPU-supported, camera-based measurement strategy that resolves high spatial structures (~60 nm), temporal dynamics (≤ 2 ms), and molecular brightness analysis from the exact same data within 5 minutes, including measurement time. If the data is analyzed during recording, data analysis can be performed online in real time.

In this work, we investigated the connection between the epidermal growth factor receptor (EGFR) dynamics, its oligomerization state and the cytoskeleton. For this purpose, we acquired images of mApple labeled EGFR and LifeAct, an actin binding protein labelled with EGFP, on whole cells with high sensitivity and high-speed using EMCCD or sCMOS cameras and GPU-based processing with spatial or temporal binning to optimize extraction of various parameters. The resulting single datasets are evaluated by a combination of spectroscopy and super-resolution techniques that include: imaging fluorescence correlation spectroscopy (imaging FCS) to measure dynamics; the FCS diffusion law to obtain information about the dynamic sub-resolution organization; Number and Brightness analysis (N&B) to determine oligomerization or aggregation states; and super resolved radial fluctuation microscopy (SRRF) to obtain super-resolution images. The simultaneous acquisition of these multiple fluorescence parameters allows a direct cross-correlation analysis, which would not be possible in sequential measurements, that allows us to determine how EGFR diffusion is dependent on its oligomerization state, and whether the cytoskeleton has any influence on the receptor diffusion mode and dynamics.

This approach is easily extendable to other fluorescence parameters, does not require specialized instrumentation, and thus is immediately applicable to a wide range of situations.