Large Time Series Single-Molecule Tracking Including Defocus and Motion Blur Control

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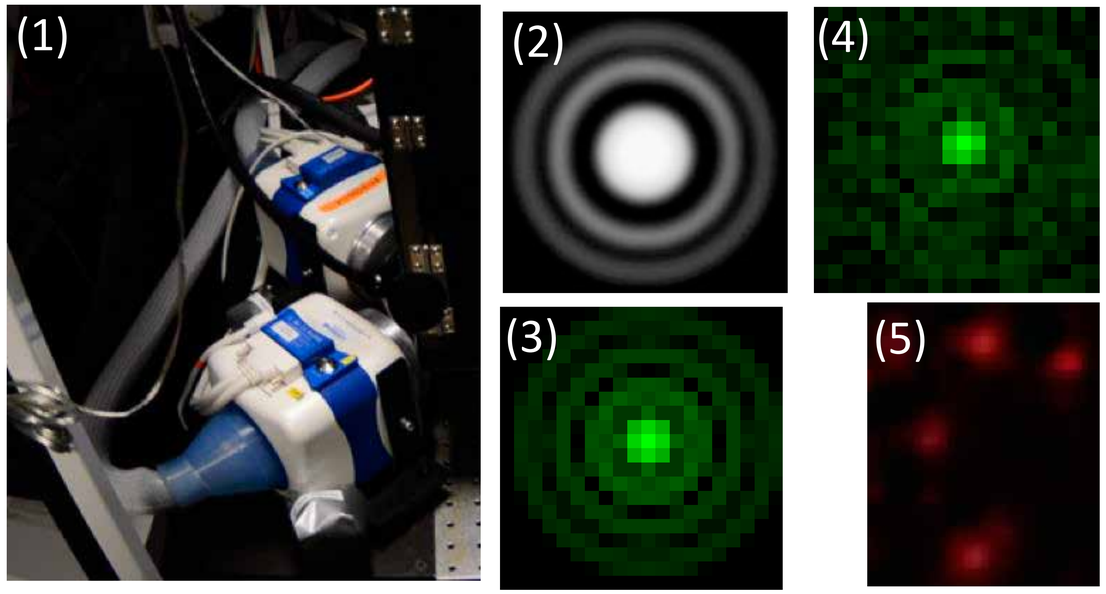
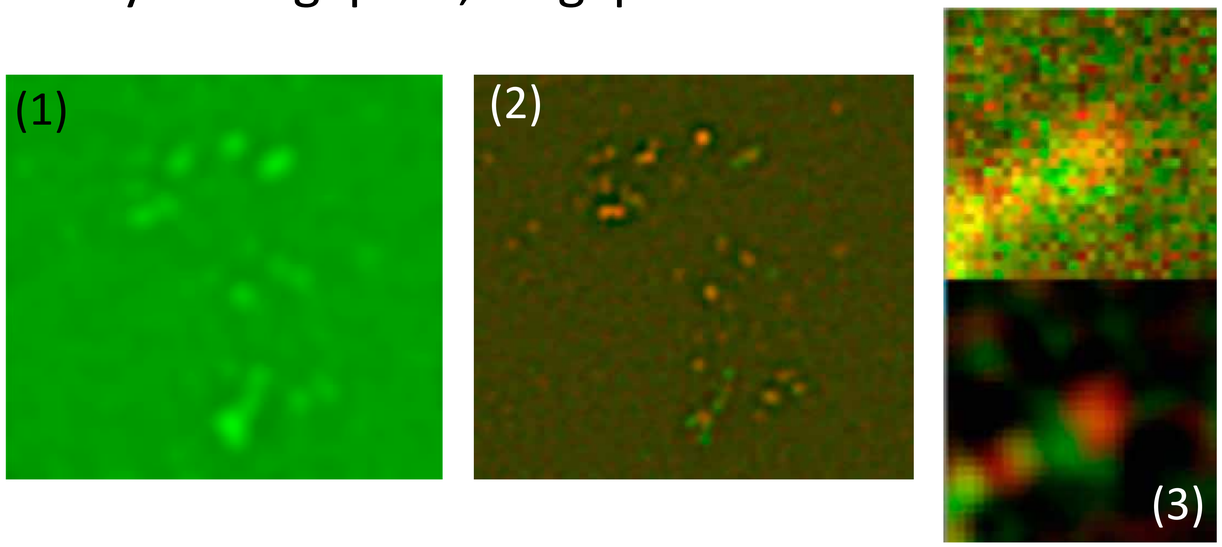
We present an operational tracking implementation for multi-channel microscopy time series from hundreds to tens of thousands of frames, depicting the dim traces of single fluorescent molecules moving over time. The characteristic shape of an optical point source is used to localize and trace thousands of molecules fast, accurately, and reliably over a timespan of several minutes.



(1) Dual cooled camera setup to record low photon counts,(2) ideal in-focus airy disk from a single emitter, (3) its pixelleted form, and the (4) photon starved form with statistics noise. (5) a recorded image at different foci.

The circular ringed disk-like image of a single emitter extends over multiple pixels and forms a small, not always centered image of that disk.

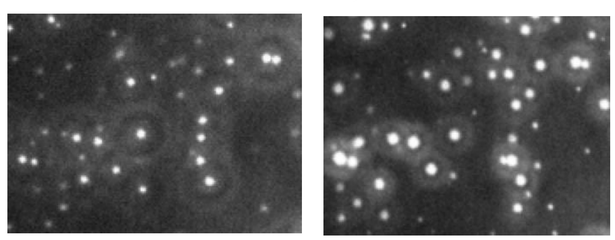
In order to find these images in a series of larger images, sub-pixel shifted models have to be compared across all recorded data. Parallelization is achieved by first filtering all frames for local maxima (implemented as texture mapping), then distributing the small environment around each maximum to individual GPU cores which then optimize a matching to the model signal at sub-pixel accuracy and deliver a floating-point coordinate or a fail result.



(1) Image processing for the background and (2) amplitude estimation This dramatically sharpens the images and (3) often isolates formerly overlapping channels in space.

As a series contains hundreds of thousands of local maxima the granularity is naturally fine and speed ups are significant. Also the maximum likelihood process for a ~100 pixel image can be compressed into single precision float operations and so can the obtained coordinate systems.

Adding motion blur and defocus adds lots of flexibility but hundred folds the computational cost.



Same dataset at slightly varying foci of about 50nm difference.

A complete analysis without defocus can be performed at about 30 to 100 frames per second, each optional matching decreases the performance about tenfold.

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