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Ultra low noise EMCCD camera considered to test a novel superresolution microscopy technique: the 3-B technique

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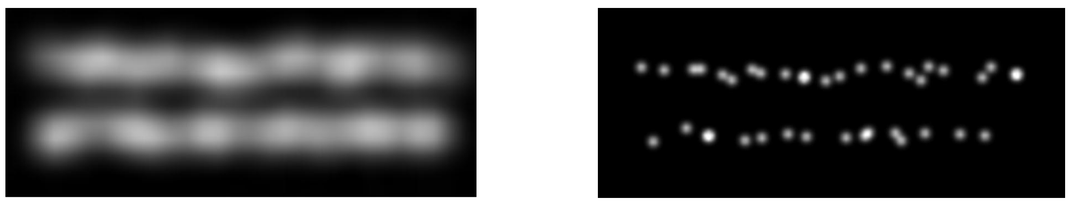
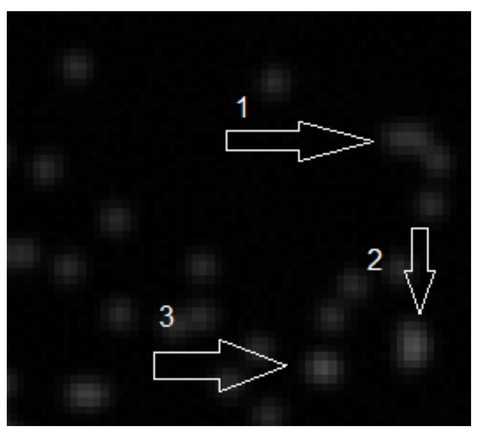
Abstract: Time series recording and analysis of fluorophore blinking result in image resolution beyond the diffraction limit. This superresolution microscopy technique would benefit from new ultra low noise EMCCD cameras.

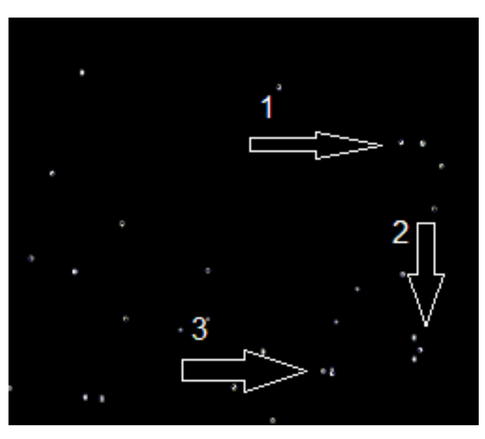
3-B superresolution microscopy technique (called for 'Bayesian analysis of Blinking and Bleaching') is based on time dependent properties of the fluorescent markers. This single molecule localization technique achieves high spatial resolution: appearance and disappearance of a single fluorescent spot against a low out-of-focus background allow to highlighting it by subtraction of time series successive frames. We establish the position of the molecules as a single spot using a method of maximum likelihood for estimation. It returns localized spots interpreted by a Gaussian regression as one fluorescent molecule. In contrast to STORM (PALM) method, this approach allows to imaging overlapping fluorophores with high resolution. This permits to speed up data collection. That's why this technique is very attractive for the dynamic optical imaging at intracellular level.



Digital simulation of blinking image spheres with diffraction-limited resolution and result of 3B-algorithm reconstruction.

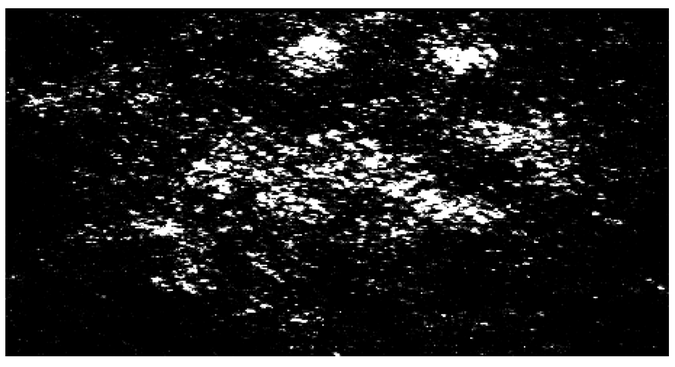


One of the frames of blinking test-objects (fluorescent microspheres) with diffraction limited resolution.



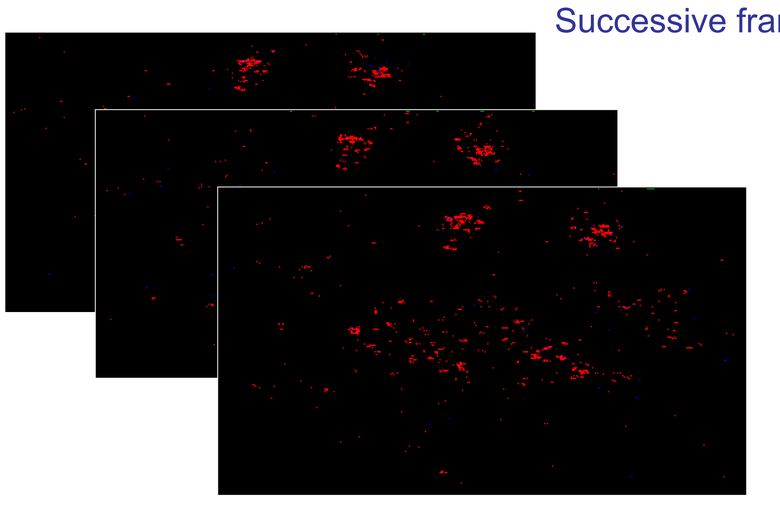
Reconstructed image.
In regions 1, 2 and 3, we can see separated markers.

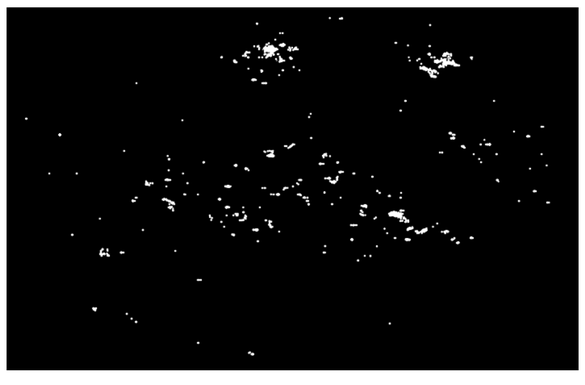
The accuracy of the localization of the blinking and bleaching molecules mainly depends on the camera signal-to-noise ratio and the photon flux emitted by markers. EMCCD cameras get rid of noises like the dark current and the read-out noise by well-established methods, but the innovations in the clock-induced charges diminution interest us in order to test the limits of performance of our 3-B super-resolution microscopy technique thanks to a state of the art low light imaging camera.



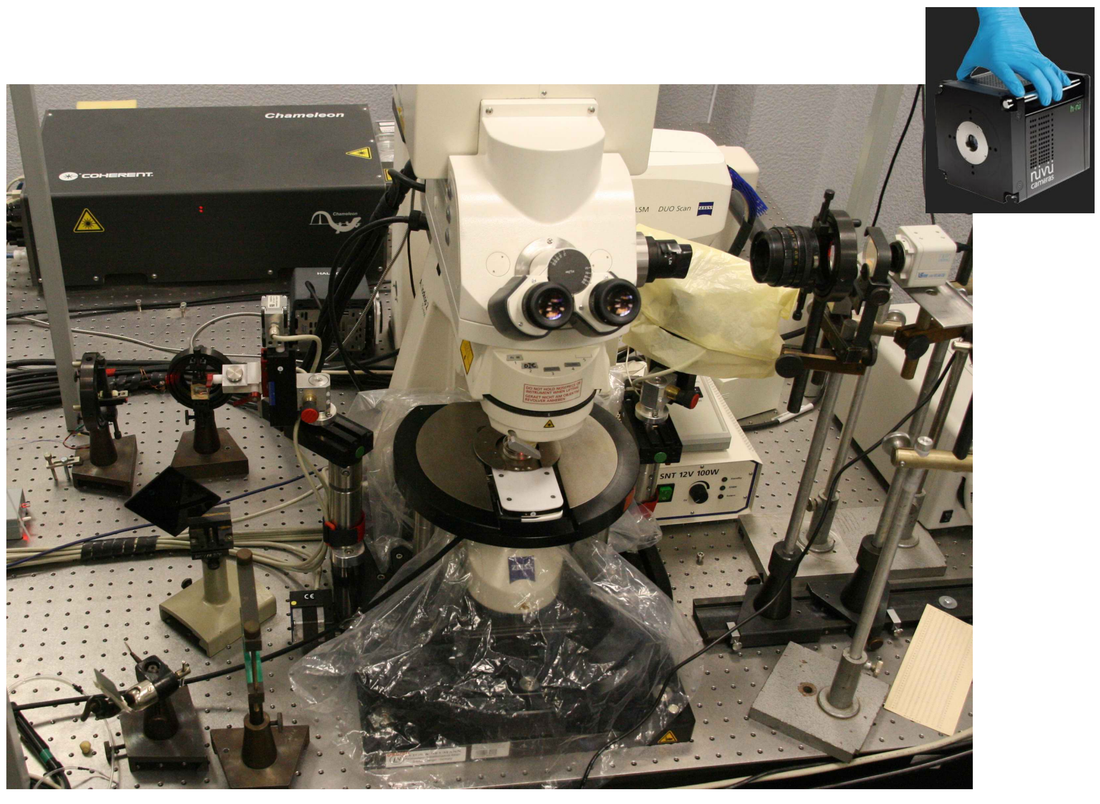
Fluorescent image of antibodies to neuron dendrite microtubules MAP2 labeled by Alexa Fluor 647.

Successive frames of time series.





Reconstructed superresolution image



Setup on the base of fluorescent microscope: Zeiss Axioscope 2 FS MOT with 'EVS' USB2 camera 'VEC-545' (photosensitive sensor - 'OmniVision' CMOS 'OV5620', matrix - 1/2.5", 2592x1944 pixels, 2.2 μm pixel size).

Hopefully one HNü camera will join our setup in a near future.