Cryo-Fluorescence Microscopy of Single Molecules

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The most popular super-resolution fluorescence microscopy methods which can resolve structures far below the classical diffraction barrier of light microscopy are based on the localization of single fluorescent molecules. Typical examples of these methods are Stochastic Optical Reconstruction Microscopy (STORM), Photoactivation Localization Microscopy (PALM), or Ground State Depletion Imaging (GSDIM). All these techniques routinely achieve a lateral image resolution of ~ 30 nm. This resolution is directly related to the number of photons emitted from a single fluorescent molecule and roughly scales with the invers square root of the detected photon number. Therefore, photo-bleaching is the fundamental bottleneck that limits the achievable resolution.

One method to suppress photo-bleaching is to cool a sample down to cryogenic temperatures. For that purpose, we designed and built a dedicated cryostat suitable for single molecule fluorescence microscopy. The system is not only capable of cooling the sample to cryogenic temperatures, but gives also optical access to the sample for high-quality imaging with a conventional microscope employing an objective with high numerical aperture. Another important property of our system is its excellent mechanical stability, enabling long-time observations of samples over several hours with negligible drift. Using our system, we successfully performed photo-bleaching studies on single molecules showing a more than two order of magnitude enhancement in photo-stability, which results in an exceptional molecular localization accuracy in angstrom scale.