To study the fine details of biological structures with microscopy, both the preservation of these structures during the preparation process and the achievable resolution of the imaging system are of equal importance. In the field of fluorescence microscopy, the latter has been addressed by various super-resolution methods that have been developed in recent years to overcome the diffraction-limited resolution of light microscopy. Super-resolution imaging in living cells remains very challenging. Typically, chemical fixation of the samples is required to achieve the best technical results, but unfortunately, this is associated with structural changes in the sample [1], especially at a size range that is relevant for light microscopic techniques achieving resolutions below the diffraction limit [2]. A preferable alternative is vitrification (i.e., cryo-immobilizing the structure in glasslike amorphous ice using rapid freezing techniques) that preserves the structures in a near-native state and is frequently used in the fields of electron and X-ray cryo-microscopy [3,4]. The advantages of vitrified specimens have not been fully exploited to date in fluorescence microscopy of subcellular structures. This is because one of the biggest challenges for fluorescence cryo-microscopy is currently its limited resolution of 400-500 nm due to the inherent technical challenges of the setup and in particular the lack of high NA cryo-immersion objectives [5].

We introduce a super-resolution technique for fluorescence cryo-microscopy based on photo-switching of standard fluorescent proteins in intact mammalian cells at low temperature (81 K) [6]. We demonstrate that the single molecule characteristics of reversible photobleaching of mEGFP and mVenus at liquid nitrogen temperature are suitable for the basic concept of single molecule localization microscopy. We show that single molecule localization microscopy is possible at cryo-conditions and achieve super-resolution imaging of vitrified biological samples with a structural resolution of ~125 nm (average single molecule localization accuracy ~40 nm), corresponding to a 3–5 fold resolution improvement. We expect that super-resolution cryo-microscopy will become a valuable imaging method for cryo-immobilized biological samples that is highly complementary to electron and X-ray cryo-microscopy for the study of cellular and subcellular complexity.

References:
Fig. 1: Single molecule super-resolution cryo-microscopy. Photoswitching of single fluorescent molecules at low temperature (81 K) enables single molecule localization microscopy of vitrified biological samples. Compared to basic wide-field fluorescence cryo-microscopy the resolution is improved by a factor of 3-5, achieving values in the 100 nm range [6].