

## **Non-expert summary**

(Bernd Rieger, Ultra-resolution with visible light)

In the study of intricate machinery of life, microscopes are indispensable tools, preferably revealing molecular details in 3D on length scales down to 1 nm.

Optical nanoscopy is a technique to study biological structures and functionalities with 30-nm resolution using fluorescent labels. A further improvement by a factor of 10 would enable 3D imaging of the structure and protein composition of (macro)molecular assemblies such as the cell's vitally important nuclear pore complex. The composition of this complex is still much debated but essential to understand its function as gateway between cell nucleus and the rest of the cell, and its role in autoimmune diseases.

How to achieve this much-needed improvement in nanoscopy resolution? Fluorescence imaging at cryogenic temperatures ( $<-150^{\circ}\text{C}$ ) is highly promising as it enables a localization precision below 1 nm. Yet, the actual achievable 3D resolution is insufficient due to unavoidable incomplete fluorescent labelling and inherent poor axial resolution in microscopy.

I propose a combination of image-reconstruction schemes and a new cryogenic imaging technique to enable, for the first time, 3D localization of single molecules with 1-nm resolution. I will 1) combine information from many chemically identical complexes into one single image reconstruction in a manner that compensates for incomplete fluorescent labelling, and 2) realize an experimental setup and methodology for imaging at cryogenic temperatures with improved localization precision and axial resolution.

Importantly, the cryogenic fluorescence imaging can be directly followed by cryo-electron microscopy in one workflow, creating an imaging modality with unprecedented power for studying the building blocks of life.