Recently numerous high-resolution structures were obtained for macromolecular complexes by single particle cryo-EM techniques. The importance of the development of new generation pixel detectors and the possibility to correct for motion by the alignment of image frames has been particularly stressed and is considered to be one of the main important recent hardware developments. These detectors are also used within the context of modern high-end microscopes, and in parallel, considerably improved computational image processing tools were developed over the years. It is thus difficult to determine the exact contribution of the new detectors to the latest success in high-resolution cryo-EM. We wanted to understand the influence of the detector and other hardware components in more detail and systematically recorded large image datasets of a biochemically well-defined macromolecular complex (70S ribosome-EF-Tu-kirromycin complex) varying only one imaging parameter at the time. We studied data recorded on CCD and the Falcon pixel detector (DDD) using either a normal FEI Schottky field emitter (SFEG) or the high-brightness gun (XFEG) in a Cs corrected Titan Krios. Using the DDD/XFEG setup, we obtained the structure of the ribosome at 3.1 Å resolution which is identical to the resolution obtained by X-ray crystallography for the same complex from a different organism. Surprisingly, using images recorded on CCD camera still results in a 3.9 Å reconstruction which shows the possibility of high-resolution structure determination on CCD cameras. A more detailed analysis revealed however, that a ~3 times higher image statistics is required for CCD images to obtain the same resolution as for DDD images.

One conclusion from this analysis is that most structural studies are not necessarily limited by the detector but by the lack of biochemical control during purification and handling of the macromolecular complex. Macromolecules may easily become damaged during the purification procedure and further destabilized being in a non-optimum buffer environment. We have therefore developed a screening method to find maximum stabilization conditions by systematically screening the chemical space. Having analyzed >80 different macromolecular complexes, we found a very broad pH distribution in buffers that were most stabilizing. This is in severe conflict with an EMDB database analysis where the current entries reveal a narrow pH distribution around pH 7.5. The correct pH is one of the most important factors for stabilization of macromolecules and we speculate that screening optimum buffer conditions will provide a substantial boost in the functional understanding and structure determination of macromolecular complexes.