The advance of X-ray Free Electron Lasers (FELs) opens the door for single-shot studies of non-crystalline nanoparticles with high spatial and temporal resolutions. Ultrafast X-ray imaging provides high resolution information on individual fragile specimens such as aerosols, metastable particles, superfluid quantum systems and live biospecimen, which is inaccessible with conventional imaging techniques [1].

Coherent X-ray diffractive imaging, however, suffers from intrinsic loss of phase, and therefore structure recovery is often complicated and not always uniquely-defined. Here, we introduce the method of in-flight holography, where we use nanoclusters as reference X-ray scatterers in order to encode relative phase information into diffraction patterns of a virus [2]. The references (small spherical nanoparticles) and the biosamples are injected into random positions within the FEL focus. Using in-flight holography, we were able to reconstruct the unknown relative orientation of the reference and the sample. In a second step, we used Fourier X-ray holography to reconstruct the shape of the specimen.

The basic principle of in-flight holography is illustrated in figure 1. The X-rays scatter off two spheres and form a characteristic diffraction pattern (left panel). Changes in size and distance of the spheres are reflected in the patterns which can be directly translated from the diffraction image alone (center). If the spheres are shifted out of plane, the fine lines of the diffraction pattern become curved. These unique signatures can be used to reconstruct sized and position of the spheres. The small sphere can be viewed as a reference scatterer and the large sphere can be replaced by any unknown sample. In a proof-of-principle experiment, we recorded X-ray in-flight holograms with an unambiguous three-dimensional map of a virus and two nanoclusters with the highest lateral resolution so far achieved via single shot X-ray holography [2].

In my talk I will report on several studies exploring the in-flight holography principle. The experiments were carried out at LCLS and FLASH X-ray FELs within an international collaboration including TU Berlin, Uppsala University, Argonne National Lab, XFEL and others. Our approach unlocks the benefits of holography for ultrafast X-ray imaging of systems in gas-phase and paves the way to direct observation of complex electron dynamics down to the attosecond time scale.
