be an ideal support for such experiments including coherent imaging of single proteins, organelles or ordered 2D structures such as membrane systems [51].

5. Conclusions

In summary, we demonstrated, for the first time, the imaging of dehydrated biological material in the water window with radiation from a free-electron laser. The obtained contrast of the dried material is very promising for future experiments on hydrated or vitrified specimen. The implementation of zone plates rather than pinholes allowed us to effectively use 3rd harmonic radiation from FLASH, which provides photons at the desired energy between the K-edges of oxygen and carbon. The insufficient temporal coherence is a challenge on the path to high-resolution holographic imaging in the zone plate geometry – especially given the flux limitations of using the higher harmonics of FELs. To make a successful reconstruction holography may be less demanding on the coherence properties, of the radiation than CXDI is, though at the expense of contrast and resolution. Since higher temporal coherence can be achieved by narrower filtering of the FEL radiation, the attempt of pushing the coherence-limited resolution further up is accompanied by a reduction of photon flux, leading to a drop in the SNR and subsequently in resolution. Thus, the increased photon flux and higher coherence emitted from the currently constructed more powerful light sources like the upgraded FLASH, LCLS or the European XFEL and the option to use photons in the first harmonic are very promising in this respect. A more efficient use of photons by using zone plates in tandem with next generation light sources and new soft X-ray detectors that could offer an enhanced dynamic range and a bigger SNR will be of major importance for high resolution holography. This becomes especially promising if X-ray results obtained on hydrated specimen are considered jointly with other imaging techniques (e.g. correlative microscopy) to solve specific biological questions. Our experiments show that high efficiency zone plates and robust ultrathin substrates can significantly contribute to realizing the vision of single pulse holography of hydrated biological material, where drift related problems and first and foremost limitations set by radiation damage are elegantly overcome with ultrashort flashes. Since FLASH is now capable of delivering photons in the water window at fundamental wavelengths, the vision of imaging hydrated material with high contrast starts to become reality.

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