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Coherent diffractive imaging of biological samples at synchrotron and free electron laser facilities

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ABSTRACT

Coherent X-ray diffractive imaging (CXDI) is a new imaging technique that offers the potential to image non-crystalline materials to sub-nanometer resolutions. Here we review the progress in CXDI of biological samples at both synchrotron and free electron laser (FEL) sources. We outline the experimental design of a CXDI experiment and summarize the iterative phase retrieval techniques that are used to produce images from the measured diffraction patterns. We describe a selection of key experiments performed in bio-imaging with CXDI from synchrotron sources, and we discuss the proof-of-principle experiments performed at FLASH at DESY in Hamburg. Finally, we show through simulation that for realistic parameters of hard X-ray FELs a resolution of a few nanometers may be achieved for individual biological objects imaged with single pulses of FEL radiation. Furthermore, we revise how this resolution may be improved to the sub-nanometer range if we image multiple copies of samples with a reproducible structure.

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1. Introduction

Coherent X-ray diffractive imaging (CXDI) is a relatively novel imaging method that can produce an image of a sample without using optics between the sample and detector (see Fig. 1). This differs from conventional microscopy schemes which use objective lenses to produce an image of an object. Taking into account the difficulties of producing lenses at hard X-ray energies that are both highly resolving and efficient, we see clearly the advantages of so-called 'lensless' microscopy techniques. Many demonstrations of CXDI at synchrotron sources on non-biological samples have been made (Miao et al., 1999; Williams et al., 2003; Pfeifer et al., 2006; Chapman et al., 2006a). Due to the lack of resolution limiting optics and the penetrative ability of X-rays, this imaging technique ultimately offers new insights into the structure of intact, three-dimensional (3D) biological specimens.

While conventional X-ray imaging of biological specimens at synchrotron sources (Parkinson et al., 2008) suffers from radiation damage (Howells et al., 2009), which limits the resolution of the resultant images, breaking this resolution limit may be possible by the use of ultrabright, ultrashort pulses from free electron lasers (FELs). Indeed, one of the key goals of CXDI is to image beyond the conventional damage limit using these pulses of X-ray FEL radiation. If these pulses are short enough they may scatter from the specimen before it is destroyed by this pulse (Neutze et al., 2000). Structural information of the undamaged sample will be measured and reconstructed to produce an image of the object at a resolution higher than that typically obtained from conventional X-ray sources.

In this overview we describe some of the key experimental milestones towards CXDI at both synchrotron and FEL facilities. We examine, furthermore, a variety of CXDI techniques and consider the advantages and disadvantages of each. We conclude with statements regarding the expected resolutions achievable at hard X-ray FELs which have recently commenced operation (Emma et al., 2009) or are in the construction phase (Tanaka and Shintake, 2005; Altarelli et al., 2006).

Before we address in more detail the new microscopy that is CXDI, we first consider existing microscopies, in particular electron microscopy and optical confocal microscopy, to define the capabilities that CXDI is uniquely able to provide. After appropriate sample preparation (Reynolds, 1963; Dubuchet, 1995) transmission electron microscopy (TEM) can provide images of non-crystalline biological objects to some nanometers resolution (Beck et al., 2007) and a few ångstroms for crystalline specimens (Henderson, 2004). It is a standard tool for the biological community due to its ability to directly produce high resolution, real space images. The key limitation of electron microscopy for imaging biological specimens, however, is the strong interaction between the imaging electrons and matter. This results in a limited penetration depth of the electrons in a sample. In order to see through a biological specimen with an electron microscope the sample must be thinned, typically by slicing to a sub-micron thickness (Frank, 2006). This slicing has the potential to introduce unwanted artifacts in the imaging process.

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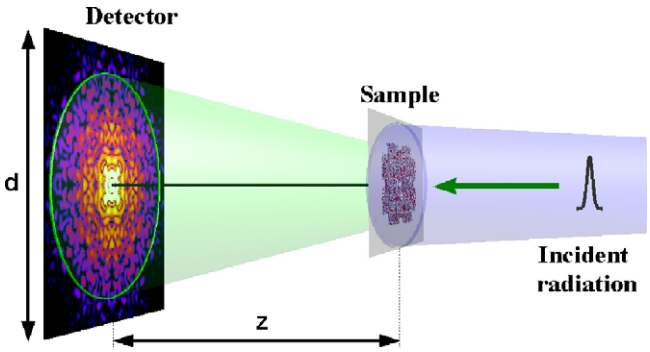

Fig. 1. Schematic of a coherent X-ray diffractive imaging experiment. Incident radiation, in this case a single FEL pulse, illuminates the sample, here a macromolecule, from the right. The scattered radiation propagates a distance, z, to an area detector of size d where the diffracted intensities are measured.

The use of X-rays allows us to bypass slicing due to their weaker interaction with matter.

Confocal microscopy (Pudley, 1995) is a point scanning microscopy that has the power to make 3D images of the locations of particular fluorescent dyes in a biological specimen. The dyes attach to certain features of the sample and when illuminated by laser light fluoresce with a given wavelength based on the dye used. This technique can provide up to 200 nm resolution images of these dyes in living cells and go beyond that with advanced techniques to a resolution of 20 nm (Hell, 2007). The limitation here is that the sample must be labeled with these fluorescent dyes (e.g. green fluorescent protein (Patterson and Lippincott-Schwartz, 2002)) and only the dyed components (typically proteins) are visible. These dyes also have a finite lifetime, limited by the finite number of photons they can fluoresce – a process known as bleaching (Pudley, 1995). Furthermore, the environment surrounding the labeled proteins remains invisible to the technique. Nevertheless, considerable structural studies of biologically relevant specimens have been performed using confocal microscopy (Pudley, 1995).

The remainder of the paper is organized into five sections followed by the conclusions. Section 2 details the principles of coherent X-ray diffraction imaging and the iterative phase retrieval techniques used therein. Following that are two sections outlining examples of biological CXDI experiments performed using synchrotron sources (Section 3) and FEL sources (Section 5). A short discussion of radiation damage is presented between these examples in Section 4. In Section 6 we present an outlook extrapolating from these experiments and outlining some simulations concerning what can be expected from CXDI in the near future. The conclusions are presented in Section 7.

2. Principles of coherent X-ray diffraction imaging

The conventional CXDI experiment is performed with an isolated sample illuminated by a coherent, plane wave (Fig. 1). The incident wave may be described by a complex valued field of uniform magnitude and phase. The radiation interacts with the sample, which affects both the amplitude and phase of this field. This scattered radiation, or exit-surface wave (ESW), from the sample propagates to a two-dimensional detector in the far-field from the sample and the diffracted intensities are measured (Fig. 1). These diffracted intensities alone are insufficient to determine the exit-surface wave, as during the measurement process the phase information is lost (the measured quantity is intensity and not the complex amplitude). However, with some additional knowledge of constraints on the sample in object space the ESW from the sample can be reconstructed using phase retrieval algorithms based on an iterative approach (Gerchberg and Saxton, 1972; Fienup, 1982; Elser, 2003).

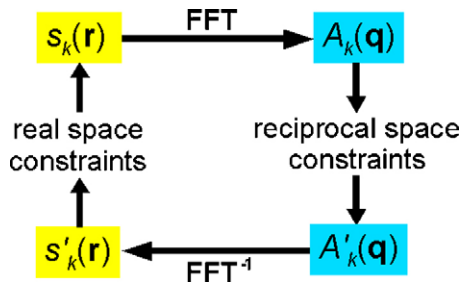

Fig. 2. Block diagram of the iterative reconstruction method.

More formally, we measure the modulus squared of the scattered amplitude,

$$F(\boldsymbol{q}) = \int \rho(\boldsymbol{r}) e^{i\mathbf{q}\cdot\mathbf{r}} d\boldsymbol{r}$$

and $\rho(\mathbf{r})$ is the electron density of the sample, $\mathbf{r}$ is the real space coordinate and $\mathbf{q}$ the momentum transfer. Note that the amplitude $F(\mathbf{q})$ is fully complex, while we can only measure its modulus squared. In general the inverse Fourier transform of $F(\mathbf{q})$ is not the electron density, but rather the ESW of the sample, however in the weak scattering case this reduces to the projection of electron density.

The image reconstruction process (Fig. 2) begins with assigning random phases to the known magnitudes in reciprocal space. This far-field wavefield, $A'(\mathbf{q}) = |F(\mathbf{q})|e^{i\phi(\mathbf{q})}$, is then inverse Fourier transformed to real space giving the first guess of the ESW, $s'(\mathbf{r})$. This first guess will look wholly unlike the correct ESW and constraints in the object space, most importantly the finite extent of the object, are applied to this guess. This typically involves setting the values of $s'(\mathbf{r})$ outside some bound to zero, known as the Error Reduction (ER) method, or forcing them towards zero, most commonly the Hybrid Input–Output (HIO) method (Fienup, 1982). After the constraints have been applied, the function, $s(\mathbf{r})$, is then Fourier transformed to the far-field. This new far-field guess then has its magnitude, $|A(\mathbf{q})|$, replaced by the measured amplitudes $|F(\mathbf{q})|$ while now the phases, $\phi(\mathbf{q})$, are kept. This process is then iterated over for typically thousands of iterations until it converges. The resulting function $s(\mathbf{r})$ is the exit-surface wave of the sample, and may be interpreted in terms of the sample's physical properties.

A necessary condition for the successful reconstruction of the ESW from a diffraction pattern is the appropriate sampling of that pattern (Sayre, 1952; Bates, 1982). A useful experimental rule of thumb is that at least two measurement points per fringe in the diffraction pattern are required for adequate sampling. This means that the autocorrelation function of the data is correctly sampled according to Shannon's sampling theorem (Shannon, 1949), which is twice that required to sample the fully complex wavefield. This essential sampling consideration leads this method to be sometimes referred to as the 'oversampling' method.

While CXDI requires no optics between the sample and the detector, and this is a great experimental simplification, there are also some limitations that are intrinsic to the method. Typically, a CXDI iterative procedure requires some thousands of iterations for an image to be reconstructed, meaning that the imaging is certainly not performed instantaneously. Moreover, CXDI is a photon hungry method requiring increasingly higher flux to achieve higher resolutions. The scattered intensity as a function of momentum transfer can be described by $I(q) \propto q^{-k}$, where k is between 3 and 4 depending upon the sample (Shen et al., 2004; Bergh et al., 2008; Huang et al., 2009). This means that for already weakly scattering samples a factor of three or four orders of magnitude in flux is required to improve the resolution by an single order, hence the need for very bright photon beams. Furthermore, a beam stop, or a hole in

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