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Holography and coherent diffraction with low-energy electrons: A route towards structural biology at the single molecule level

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ABSTRACT

The current state of the art in structural biology is led by NMR, X-ray crystallography and TEM investigations. These powerful tools however all rely on averaging over a large ensemble of molecules. Here, we present an alternative concept aiming at structural analysis at the single molecule level. We show that by combining electron holography and coherent diffraction imaging estimations concerning the phase of the scattered wave become needless as the phase information is extracted from the data directly and unambiguously. Performed with low-energy electrons the resolution of this lens-less microscope is just limited by the De Broglie wavelength of the electron wave and the numerical aperture, given by detector geometry. In imaging freestanding graphene, a resolution of 2 Å has been achieved revealing the 660.000 unit cells of the graphene sheet from a single data set. Once applied to individual biomolecules the method shall ultimately allow for non-destructive imaging and imports the potential to distinguish between different conformations of proteins with atomic resolution.

1. Introduction

Q3 Today, structural information about biological molecules at atomic resolution is predominantly obtained by X-ray crystallography and NMR spectroscopy, where samples in the form of crystals or in liquids are studied. This, however, implies averaging over many molecules whereby diverse and important conformational details remain indistinct. Besides this drawback, these methods can only be applied to a small subset of biological molecules that either readily crystallize to be used in X-ray studies or are small enough for NMR investigations [1]. A third approach for imaging molecules is cryo-electron microscopy. In the case of biological molecules the resolution is limited by radiation damage caused by the high electron energy in transmission electron microscopes. With a critical dose of (5–25 $e^-/Å^2$) [2] an individual molecule is destroyed long before a decent quality image has been acquired. The radiation damage problem is countered by averaging over several thousand noisy images to end up with a satisfactory signal-to-noise ratio of the averaged molecule structure [3]. The aligning and averaging routines inherent to cryo-electron microscopy limit its application range to symmetric and particularly rigid objects, like specific classes of viruses for example. In X-ray experiments, radiation damage is even more severe. Out of one million photons interacting with a biological molecule, only one is scattered elastically and carries structural information to the detector while all the other photons cause damage, be it by ionisation or other inelastic processes.

Despite all limitations of the three conventional structural biology tools discussed above, one needs to express respect for the vast amount of data that has been generated over the past decades, reflected by the impressive volume of the current protein database [4].

Nevertheless, prospective structural biology definitely asks for methods and tools that do away with averaging over an ensemble of molecules but enable structural biology on a truly single molecule level. To obtain atomic resolution information about the structure of any individual biological molecule, entirely new concepts and technologies are needed. One approach of this kind is associated with the emerging X-ray Free Electron Laser (XFEL) projects. They appeared initially as the novel technology to gain information from just one single biomolecule at the atomic scale by recording its X-ray diffraction pattern within just 10 fs before the molecule is decomposed by radiation damage [5]. Meanwhile, it became clear that averaging over a large number of molecules (of the order of one million) will be inevitable to enable numerical

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reconstruction with atomic resolution [6]. However, individual larger biological entities of 700 nm in diameter have been imaged at a resolution of 32 nm [7]. While single protein imaging has been the initial trigger for the XFEL projects, a current focus for XFEL applications in biology is nano-crystallography [8], not single molecule imaging anymore.

In Transmission Electron Microscopy (TEM), the current trend points towards decreasing the kinetic electron energy from formerly 300 to 100 keV down to even 60 keV where radiation damage appears tolerable for imaging graphene without knocking out too many carbon atoms during investigation [9]. With modern aberration corrected electron lenses [10], atomic resolution is still achievable at an energy of 60 keV [11]. However, based on electron-optical constraints, it is foreseeable that future TEMs will still operate in the keV regime but never reach electron energies as low a few 100 eV where the problem of radiation damage is avoided [12]. Hence, despite this technical progress concerning aberration corrected TEMs, they will not allow imaging an individual biomolecule in a foreseeable future.

Furthermore, when aiming at three-dimensional imaging, the scattering mechanism inherent to high-energy electrons constitutes a severe problem. Unlike photons, all electrons feature anisotropic scattering described by a partial wave expansion [13]:

$$f(\vartheta) = \sum_{l=0}^{\infty} (2l+1)\frac{1}{k} e^{i\delta_l(k)} \sin\delta_l(k) P_l(\cos\vartheta), \tag{1}$$

where k is the wave number, $P_l(\cos\vartheta)$ are the Legendre polynomials, ϑ is the scattering angle, l is the angular momentum number for each partial wave ($l=0$ corresponds to isotropic s-waves), and $\delta_l(k)$ are the phase shifts. The amplitude of $f(\vartheta)$ exhibits a pronounced maximum in the direction of the incident wave as illustrated in Fig. 1.

However, in case of high-energy electrons, the intensity of the scattered wave is extremely straightened in forward direction within just a few degrees inhibiting three-dimensional imaging in high-energy electron holography. Low-energy electron holography, on the other hand, has the intrinsic potential for three-dimensional imaging: electrons in the range of 50–250 eV scatter within an angle up to 30° (see Fig. 1).

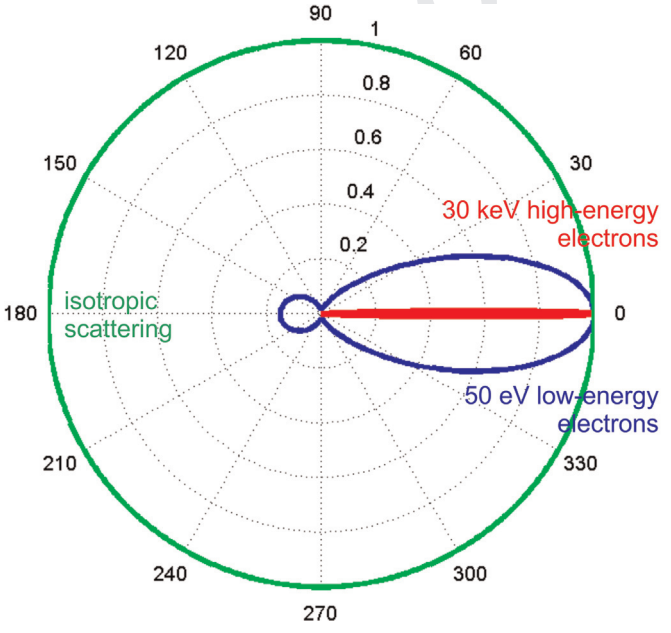

Fig. 1. Angular dependence of electron scattering in different energy regimes. Displayed here are the normalized squared amplitudes $|f(\vartheta)|^2$ of scattered low- and high-energy electron waves off a single carbon atom. The green circle indicates isotropic s-wave scattering as for photons. The phase shifts $\delta_l(k)$ for simulating $f(\vartheta)$ were provided by the NIST library (NIST electron elastic-scattering cross-section database, 2000).

2. Solutions to the phase problem – holography and coherent diffraction imaging

In 1947, Dennis Gabor proposed a novel microscopy principle, later named holography that allows capturing the phase distribution of the scattered object wave by superimposing it with a well-defined reference wave [14,15]. In the original experimental arrangement envisioned by Gabor, also called inline holography, part of the coherent incident wave is scattered by the object and the un-scattered part of the wave constitutes the reference wave, as Illustrated in Fig. 2. In the resulting interference pattern, named hologram, the phase distribution of the scattered object wave is thus captured in a two-dimensional record. Holography unambiguously solves the phase problem in one step thanks to the presence of a reference wave. Numerical hologram reconstruction is achieved by back propagating the complex-valued wave from the hologram plane to the object position (based on Huygens' principle and Fresnel's formalism):

$$U(\vec{\rho}) = \frac{i}{\lambda}\int\int H(\vec{r})\frac{\exp(ikr)}{r}\frac{\exp(-ik|\vec{r}-\vec{\rho}|)}{|\vec{r}-\vec{\rho}|}\mathrm{d}\sigma_S, \tag{2}$$

where $H(\vec{r})$ is the hologram transmission function distribution, $\vec{r}$ and $\vec{\rho}$ point towards coordinates at the detector plane towards positions of scattering centres making up the object. Integration is performed over the hologram plane and the result of this integral transform is a complex-valued distribution of the object wavefront at any coordinate $\vec{\rho}$ and hence a *three-dimensional* object reconstruction. For the Low Energy Electron Point Source (LEEPS) microscope [16], a first theory and a numerical reconstruction scheme has been developed in 1992 [17]. In 2007, the long standing twin image problem inherent to Gabor type holography has finally been solved [18] and in 2009 it became possible to reconstruct phase and amplitude separately from a single holographic record [19].

In contrast to holography where a reference wave is employed to determine the phase, Coherent Diffraction Imaging (CDI) is a technique that offers a solution to the phase problem for experimental conditions where only the intensity of the scattered wavefront is detected:

$$I(\vec{r}) = \left|\int\int o(\vec{\rho})e^{-ik\vec{r}\vec{\rho}}\mathrm{d}\vec{\rho}\right|^2, \tag{3}$$

as illustrated in Fig. 2. In 1952, Sayre suggested that it should be

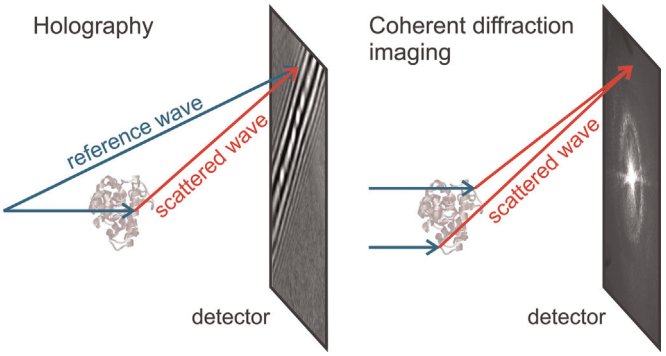

Fig. 2. Illustration of the principle of holography and coherent diffraction imaging.

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