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Separation of three-dimensional scattering effects in tilt-series Fourier ptychography

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ABSTRACT

We investigate a strategy for separating the influence of three-dimensional scattering effects in tilt-series reconstruction, a method for computationally increasing the resolution of a transmission microscope with an objective lens of small numerical aperture, as occurs in the transmission electron microscope (TEM). Recent work with visible light refers to the method as Fourier ptychography. To date, reconstruction methods presume that the object is thin enough so that the beam tilt induces only a shift of the diffraction pattern in the back focal plane. In fact, it is well known that the diffraction pattern changes as a function of beam tilt when the object is thick. In this paper, we use a simple visible light model to demonstrate a proof-of-principle of a new reconstruction algorithm that can cope with this difficulty and compare it with the aperture-scanning method. Although the experiment uses a model specimen with just two distinct layers separated along the optic axis, it should in principle be extendable to continuous objects.

1. Introduction

Tilt-series reconstruction is a method of improving the resolution of a transmission electron microscope (TEM), or any microscope that has an objective lens with small numerical aperture [1–3]. The illumination is tilted to a number of incident angles so that the diffraction pattern lying in the back focal plane of the objective is shifted laterally (see Fig. 1(b)). In this way, parts of the diffraction pattern that would normally lie outside the transfer function (or the objective aperture) when the illumination is parallel with the optic axis can be made to pass through it. An image is recorded for each illumination tilt, each one of which has the resolution predicated by the objective lens. However, by using a suitable reconstruction algorithm, the diffraction pattern in the back focal plane can be synthesized by tiling together the different regions of it that have passed through the limited transfer function, thus computationally creating a synthetic objective lens with a much larger numerical aperture than the actual physical lens.

There has recently been reported a significant amount of work on visible light tilt-series reconstruction, referred to in that literature as Fourier ptychography [4]. In light optics, a good quality lens can have near-unity numerical aperture, so at first there may seem to be little motivation for undertaking a tilt series reconstruction at visible light wavelengths. In fact, by deliberately stopping down the objective lens, a very wide field of view image can be recorded at low resolution on a detector with a conventional number of pixels (say $1k \times 1k$). The tilt series reconstruction greatly increases this, generating a gigapixel image, thus producing a wide field of view image at high resolution [4]. The other key advantage of the technique is that the phase of the image is recovered accurately, without loss of low frequencies.

Recent work in visible light Fourier ptychography has drawn upon algorithms that have been developed for what we call here real-space ptychography. In the latter, now widely adopted in the X-ray community [5], a patch of radiation is scanned to a number of positions over a specimen, which lies in real space. The pattern of scattered radiation intensity is recorded somewhere downstream of the specimen, usually in the far-field Fraunhofer plane, i.e. in reciprocal space. In early work [6], the main benefit of ptychography was perceived to be the fact that it could overcome the resolution limits in electron and X-ray microscopy imposed by the very small numerical aperture of the lenses available for those radiations. However, the complex-valued image it delivers (like tilt-series Fourier ptychography), corresponding to the absorption (modulus) and phase change induced into the transmitted wave as it traverses the specimen, is now also recognized as a great strength of the technique. In the visible light regime, the high contrast phase signal is ideal for imaging unstained and unlabeled live cells [7].

Now in tilt-series Fourier ptychography, the roles of real and

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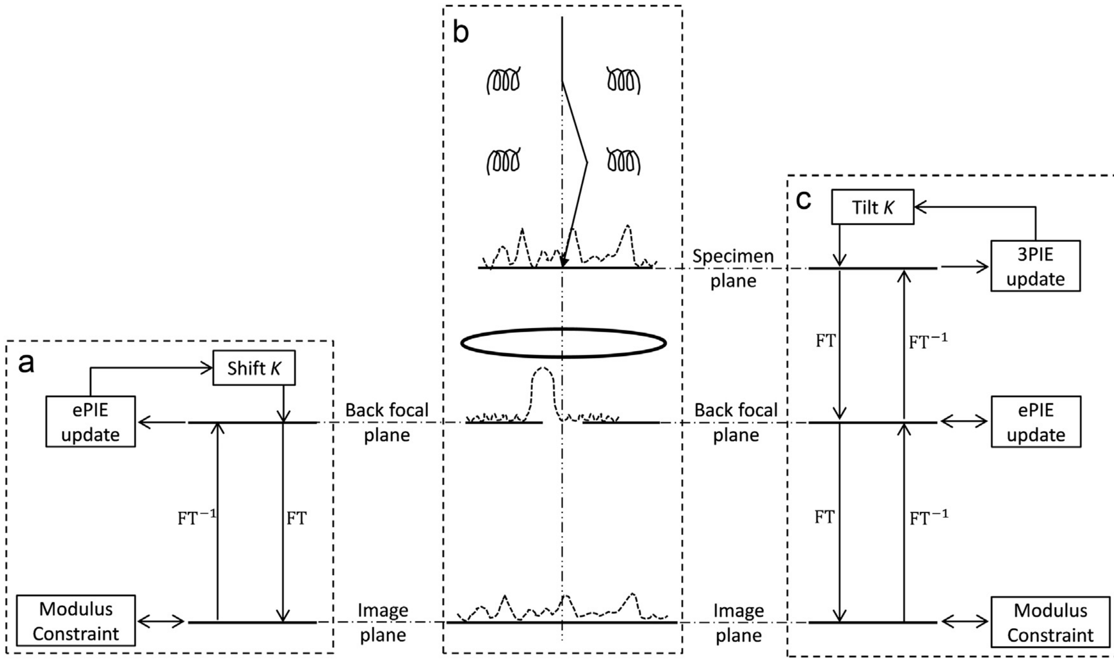

Fig. 1. Configuration of tilt-series Fourier ptychography with two associated iterative reconstruction algorithms. (a) The conventional algorithm for Fourier ptychography analogous to that for the real-space ptychography. (b) TEM configuration of tilt-series Fourier ptychography. (c) The new algorithm which can account for and separate the three-dimensional scattering effects from the specimen. However, it should be noted that (a) and (c) are equivalent when the specimen is infinitesimally thin.

reciprocal space in real-space ptychography are reversed [8]. The effect of tilting the illumination, at least in the case of the specimen being infinitesimally thin, is that the (complex-valued) diffraction pattern lying in the back focal plane of the lens is moved laterally (see Fig. 1(b)). If there is an aperture, filter or transfer function of some type lying in the back focal plane, then we now have a reciprocal version of a real-space ptychographic experiment: the diffraction pattern is like the specimen in real-space ptychography, the aperture is like the illumination function (which may be complex-valued if the lens has aberrations), and the image (also related to the back focal plane by a Fourier propagator) is like the diffraction pattern, also recorded in intensity alone. If the tilt angle interval of the illumination is chosen so that the spectrum of the object shifts by less than the aperture width in

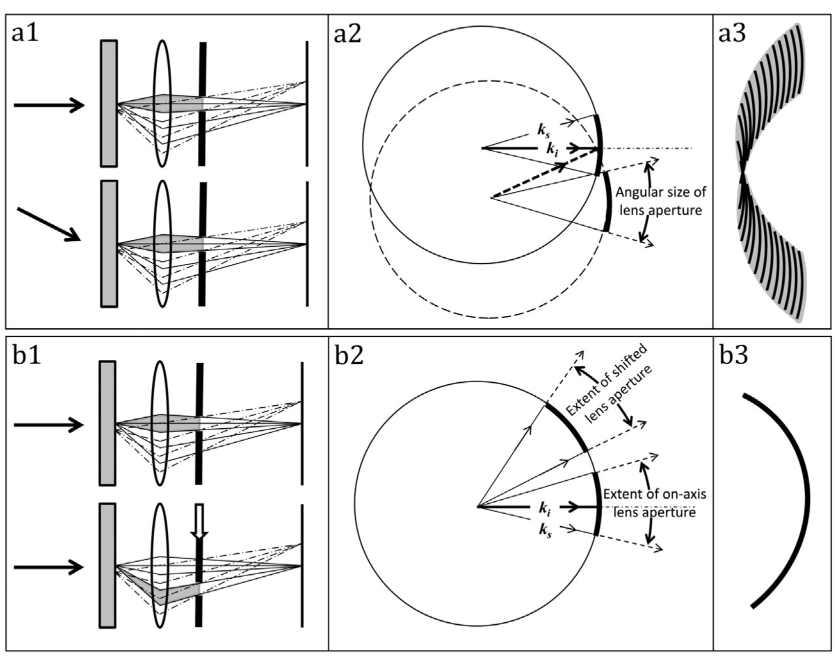

Fig. 2. Ewald sphere construction for tilt-series configuration and aperture-scanning configuration. (a1) Illustration of the experimental setup for the tilt-series configuration. (a2) The data representation on the Ewald sphere of tilt-series configuration. k_i is the k-vector of an incident plane wave along the optic axis. Allowed scattering vectors, k_s, must lie on the Ewald sphere (seen in cross-section, solid circle) associated with k_i ($|k_i| = |k_s| = 2\pi/\lambda$). For a tilted incident k-vector, the dotted Ewald sphere applies. The cone of scattered wave vectors admitted by the object lens remains constant, picking out a series of spherical caps in 3D reciprocal space. (a3) Total volume of reciprocal space (seen in cross-section) spanned by the tilt-series configuration. (b1) Illustration of the experimental setup for aperture-scanning configuration. (b2) The data representation on the Ewald sphere of aperture-scanning configuration. The method solves for the information over the surface of the Ewald sphere: the same information that is expressed in a conventional transmission microscope. (b3) Total volume of reciprocal space (seen in cross-section) spanned by the aperture-scanning configuration.