Transmission Microscopy without Lenses: Principles, Benefits and Applications

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BIOGRAPHY

John Rodenburg has worked for about 25 years on the theory, instrument development and application of novel imaging techniques in transmission electron microscopy (and more recently, light and X-ray microscopy). He has been based in the Universities of Cambridge, Sheffield Hallam and (since 2003) Sheffield. In 2006 he co-founded Phase Focus Ltd, based at The Kroto Innovation Centre, University of Sheffield, Broad Lane, Sheffield, S3 7HQ, to exploit commercially lensless microscopy.

ABSTRACT

Using a recently-developed computational algorithm, it is now possible to generate wide field-of-view transmission images by recording a number of diffraction patterns from different parts of a specimen. By disposing of the lens, various experimental advantages arise. The waves scattered by the object can be calculated in modulus and phase, the latter containing important information on the thickness or refractive index of transparent objects; the working distance of the microscope can be large without seriously compromising resolution; and the data recorded can be processed after the experiment is complete, say to alter the plane of focus of the micrograph.

KEYWORDS

microscopy, ptychography, lensless, phase, diffraction, unstained, focus

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INTRODUCTION

The history of transmission microscopy has largely depended upon the improvement of the focussing properties of lenses and the invention of new optical configurations of lenses. An alternative approach (a variation on ptychography [1]), first demonstrated experimentally in 2006 [2], is to dispose of the lens and instead form a transmission image with a 'virtual' computational lens. The method may at first seem to complicate our intuitive understanding of how an image is formed, but it has many potential advantages in all fields of transmission microscopy. The main motivation for the development of the technique was in the field of electron and X-ray microscopy [3], where it is difficult and expensive to manufacture good quality lenses. However, it turns out that the technique also has important applications at visible light wavelengths, which is what we discuss in this article.

We will see that the virtual lens can have a very large working distance (of the order of centimetres) without significantly compromising resolution, leading to a range of applications where it is difficult to arrange for an objective lens to be close to the object; it can measure the absolute phase retardation of the object (an improvement over Zernike contrast); and it allows post-acquisition focussing of the image, so as to obtain a focal 'swim through' of different layers of the object after the data has been collected.

HOW LENSLESS MICROSCOPY WORKS

Let's first think about how a conventional lens works. With reference to Figure 1a, an object is illuminated by light waves. Each point in the object scatters a small proportion of the waves into a spherically-expanding pattern of waves. At a particular instant in time, we can describe the part of the scattered wave which arrives at one point in the entrance pupil of the lens by two numbers: its magnitude and phase. By analogy to sea waves approaching the shore, the magnitude is the height of the wave, and the phase measures the relative time of arrival of different parts of the wave along the shore. A lens retards the waves that travel near its centre and carefully engineers an alteration in the phase of the outer regions, thus turning the spherical wave scattered from a point in the object 'inside out', so that it now converges to form a point in the image plane (Figure 1a).

Now consider the very simplest lensless configuration, shown in Figure 1b. Coherent plane wave illumination is incident upon a simple aperture which is placed in front of the object. A conventional CCD detector is placed somewhere a long way downstream of the object, so that it satisfies the Fraunhofer diffraction condition. This requirement is not necessary for the virtual lens to work, but it will simplify our discussion here. It should be emphasised that the aperture can be many times larger (by a factor of hundreds or thousands) than the resolution of the final image we will obtain: this is not a 'pinhole' microscope. The detector only measures the time-averaged power delivered by the scattered wave to each of its pixels, i.e. the magnitude-squared of the wave. The phase is lost. An example of such a (diffraction) pattern is shown in Figure 2a. Since phase is such an important part of the imaging process, it would seem to be impossible to use only the intensity of a diffraction pattern to recover a picture of the object.

Amazingly, there is a way of recovering this

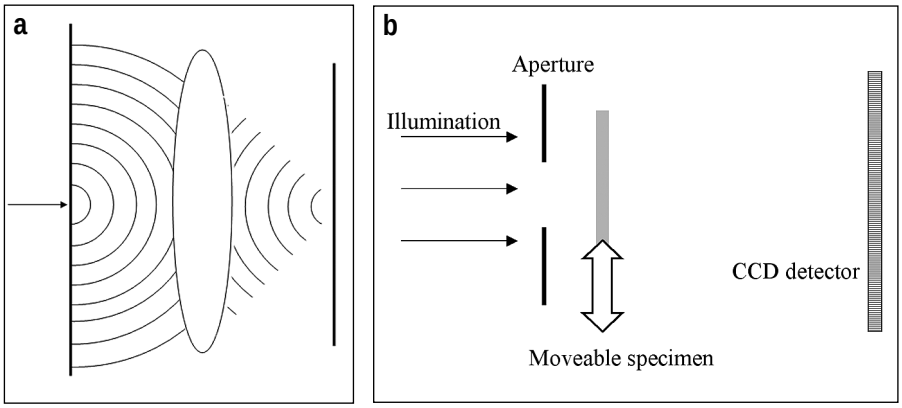

Figure 1:
(a) Conventional lens microscopy: the phase of waves scattered by the object (left) are altered by the lens (centre) to form an image in the image plane (right). (b) The simplest lensless configuration. From left: incident illumination, an aperture, a moveable specimen and a detector in the far-field. All phase information is lost at the detector, but can be recovered computationally by the virtual lens.

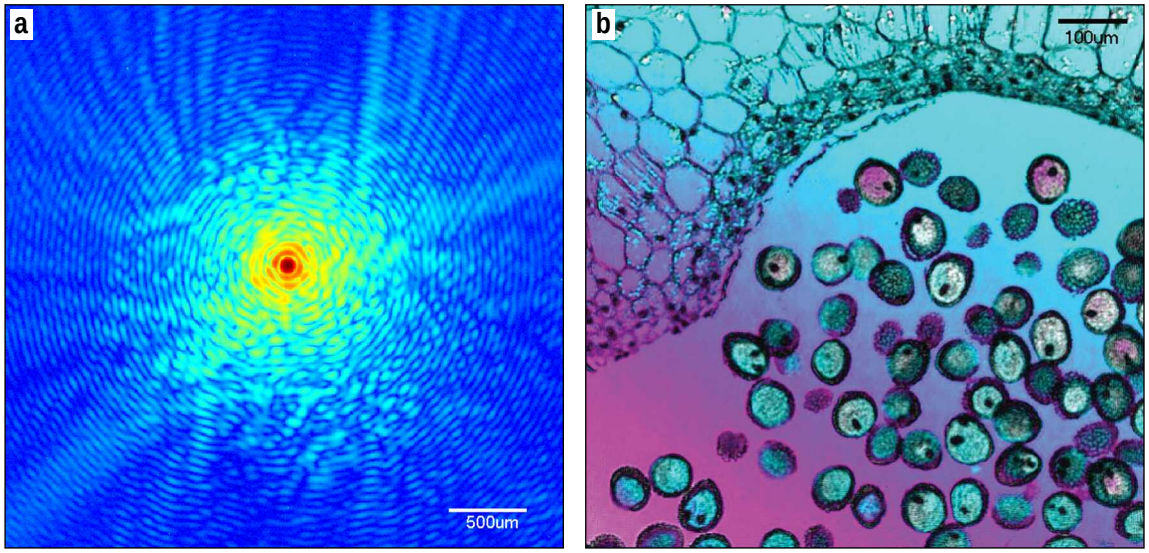

Figure 2:
(a) A diffraction pattern recorded at the detector plane in Figure 1b. A number of these are used to calculate the image (b), in this case of eggs within a lily anther. The image encodes both the modulus (rendered here as brightness) and phase (rendered as a colour map).

lost phase. What we do is illuminate one area of our object with a relatively large patch of radiation (say 0.5 mm in diameter) and collect a diffraction pattern. We then move the illumination and object relative to one another, by about half the diameter of the illumination patch, and record another pattern. The operation is repeated to span a wide field of view. Mathematically, there is only one combination of object and illumination field that is consistent with all these diffraction patterns. To find that unique combination we employ a fast iterative algorithm [2]. At first, we might suppose that we need to know illumination wave field very accurately, but it turns out that this can be solved at the same time as the image of the object, provided we know the relative positions of the illumination and object accurately [4, 5]. In Figure 2b we show an image, represented in modulus and phase, which has been calculated from a number of diffraction patterns similar to that shown in Figure 2a.

Once we have computationally solved the magnitude and phase of waves scattered by the object, then we actually have more information than we could obtain from a conventional microscope. Consider a thin transparent object, like a biological cell. In a conventional microscope the waves scattered by the cell are re-interfered in the image plane, but the image can itself only be recorded in intensity. Since the cell is transparent, we see virtually nothing. However, the cell has a refractive index, and so it changes the phase of the wave that has travelled through it. There are techniques for getting the phase information expressed in the image plane. For example, Zernike contrast alters the phase of the scattered waves (lying outside the unscattered beam in the back focal plane of the lens) relative to the unscattered wave by λ/2. For weak phase objects, the resulting image intensity becomes sensitive to phase. Defocusing the imaging lens also produces crude phase contrast. There are also methods of processing through-focal series of images in order to recover both the magnitude and phase of wave in the image plane. However, these methods lose the low resolution components of the image, and so cannot image large-scale phase changes. In contrast, the virtual lens can recover the absolute phase of the image over the entire field of view (see below) and, if so desired, can calculate any form of conventional image: because it has a full description of the wave entering its virtual pupil, it can process its numerical representation of the scattered wave field in any of the ways that a conventional lens can.

LARGE WORKING DISTANCE

The resolution of a microscope is determined by the wavelength of radiation used and the effective numerical aperture size of the condenser and objective lenses. This is because high-resolution (sharp) features in the object scatter waves to large angles. One problem with very high-resolution imaging is that the objective lens must be mounted close to the object in order to capture these high-resolution data. In the case of the virtual lens, resolution is determined by the angle subtended by the detector at the object plane. A large detector can be placed quite some distance downstream of the object, yet still capture a high range of scattering angles, and hence retain good resolution without requiring a short working distance. In practice, it is advantageous to have a large 'poor' lens (4-6 cm in diameter) mounted downstream of the object. This is used to tailor the demagnification of the diffraction pattern to match it to the CCD size. Unlike an imaging lens, this lens is not required to interfere accurately beams that pass through opposite edges of it; it is only required to focus narrow pencil rays. In this configuration, aberrations in the lens can be large because they only cause distortion of the diffraction pattern, which can be easily removed computationally.

Figure 3 shows an application where it is important to have a very large working distance while still maintaining reasonable resolution: in this case, living cells can be imaged within a standard T25 flask. There are important applications for this capability, for example in imaging human cells that cannot be removed from a sealed container because of the risk of infection to the microscopist; similarly, it can also be used to image live cell cultures that could be infected or polluted by exposure to air.

ABSOLUTE PHASE IMAGING

Because the virtual lens computes the entire scattered wave field, it can use this information to map the modulus and phase of the waves that exit the object. Changes in modulus represent absorbed light, whereas the phase corresponds to the optical thickness of the object (the combined effect of changes of refractive index and thickness of the object).

Figure 3:
The virtual lens can operate as large working distances, in this case imaging through a T25 flask. Although the detector can be placed directly over the specimen, a large diameter poor-quality lens is being used here (see text).

Figure 4a shows a phase image of unstained human metastatic melanoma cells. Unlike Zernike phase imaging, there are no distortions caused by low resolution data being lost. No staining is required and so the development of live cell cultures can be tracked as a function of time. Because the phase image is so smooth and sensitive to the refractive index of cells, images can be reliably segmented (Figure 4b) making it easy to count and characterise cells automatically. Combined with the long working distance described above, the method has a potentially very wide range of practical applications: for example, high throughput measurements of living cell cultures. A further benefit arises for the opposite scenario – very absorbent objects. Features which may be hard to discern in the conventional microscope because they are too dark can still impress a measurable phase change on the waves travelling through them, and thus be imaged with strong contrast.

A much more pathological phase object is shown in Figure 5. This is an image of a contact lens which is submerged and hydrated within a fluid medium. As would be expected, the reconstructed conventional (intensity) image (Figure 5a) is uniformly bright. However, the phase image accurately picks out the curved surface of the lens, even though the phase wraps around many times (Figure 5b). A conventional transmission microscope cannot obtain any contrast from such features; currently soft, transparent samples like these have to be freeze-dried (which can change their physical structure) and then imaged by atomic force microscopy (AFM).

POST ACQUISITION FOCUSING

Once we have reconstructed computationally the exit wave from the object, we can manipulate it in the same way as a real lens. Perhaps the single most useful operation is to alter the effective defocus of the image after the data has been collected. The resulting defocus 'swim through' mimics changing the focus setting of a conventional microscope. If the object we are looking at consists of discrete scattering centres in an otherwise relatively empty medium, then it is possible to map the three-dimensional distribution of such features. This is not technically the same as 3D imaging because if the features of interest are not sharply defined then it is impossible to differentiate structure above or below the plane of focus. However, there are many situations where the ability to refocus an image after collecting the raw data is advantageous – for example, when it is known that the objects of interest are small and dispersed and/or if we know they are confined to one plane – say cells growing upon the lower surface of a Petri dish. For high-throughput screening of cell populations, this obviates the need for autofocusing during the collection of data. Figure 6 shows an example of how the virtual lens can focus on different planes within a thick object.

CONCLUSIONS

We have seen that by disposing of the lens in transmission microscopy, and replacing it simply by a detector, a moveable illumination beam, and a computer, we can emulate all the properties of a conventional lens and at the same time exploit new imaging opportunities. What we have not discussed here is how the application of the technique could impact on transmission electron microscopy. It is possible that this is where the greatest gains in performance will occur, allowing for sub-atomic imaging without the need for expensive, aberration-corrected lenses.

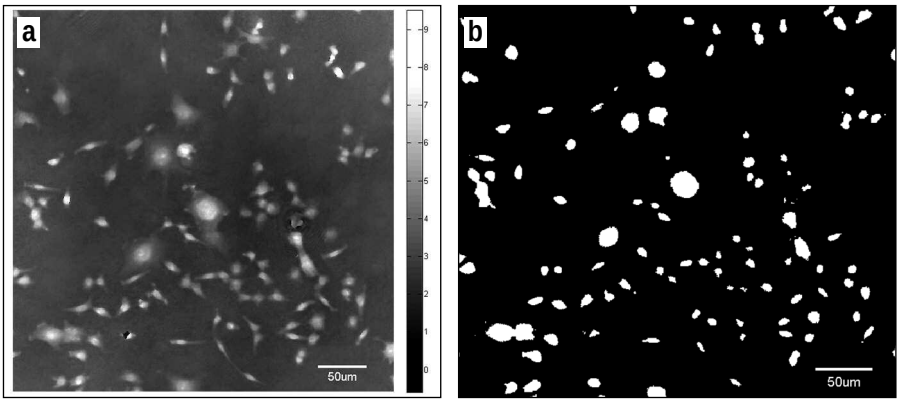

Figure 4:
(a) An example of the absolute phase image formed by the virtual lens: the sample is human metastatic melanoma cells. The intensity bar maps pahse in radians. (b) By simple thresholding, such images can be accurately segmented, say for the purposes of cell counting or shape analysis.

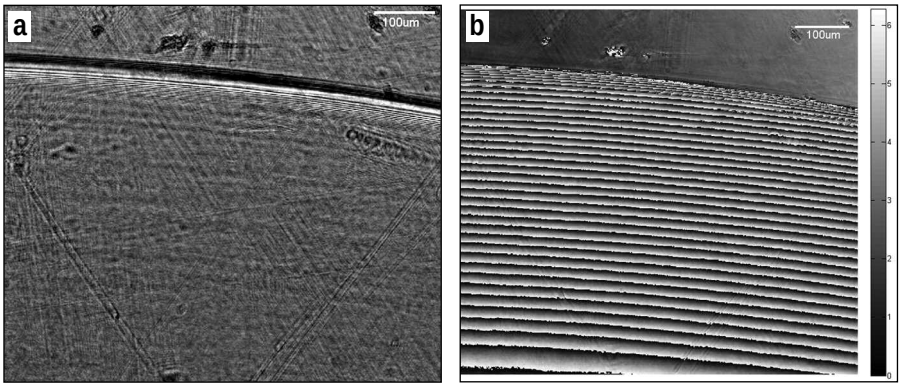

Figure 5:
(a) The amplitude image of a contact lens imaged through a hydration solution. (b) The phase profile of the contact lens clearly showing its refractive properties (see text for details). The intensity bar maps phase in radians

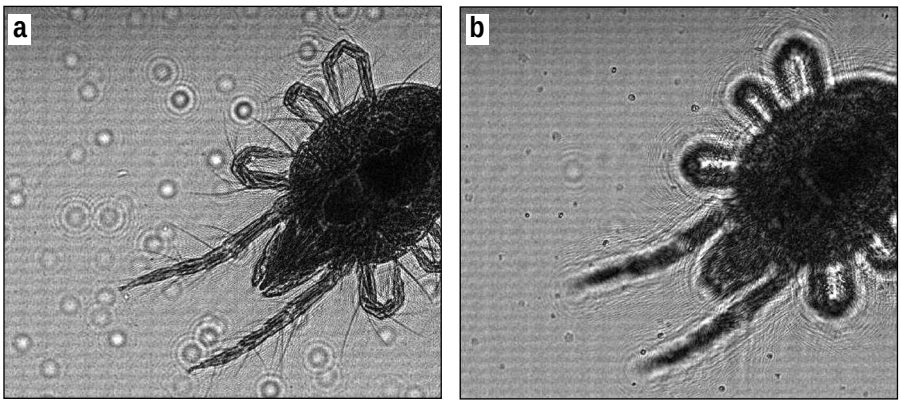

Figure 6:
(a, b) Two images of a red cotton spider calculated from a single virtual lens data set. It is possible to focus on any plane of the object. Here we see particles of dust outside the plane of the specimen come in and out of focus.

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