phasefocus™

Technical Brief

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Optical transmission mode imaging with the Phase Focus Virtual Lens®

The Phase Focus Virtual Lens® technology can be applied to a range of optical transmission geometries. In this technical brief we describe in detail the principle behind optical transmission mode imaging with the Phase Focus Virtual Lens®, present a variety of transmission set-ups, and discuss what physical properties of the sample can be obtained from measured data. These include optical thickness, refractive index and optical transparency. As with other Virtual Lens® measurements it is also possible to refocus the images after acquisition.

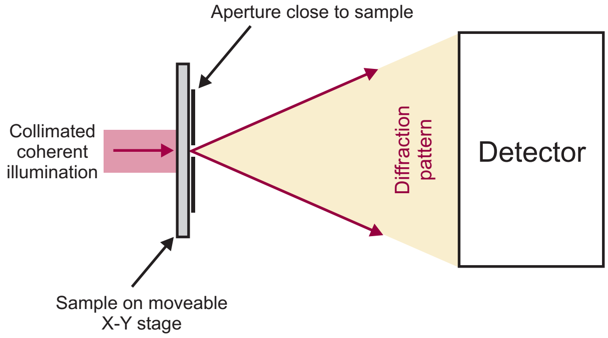

Figure 1: *Conceptually the simplest optical transmission geometry of the Virtual Lens. Collimated light is transmitted through, and diffracted by, a sample. A detector in the far-field regime collects the resulting diffraction pattern from a small region of the sample.*

Lensless geometry

A conventional microscope forms an image either from light transmitted through a transparent sample, or from light reflected from the surface of an opaque sample. In transmission mode, the wavefront entering a sample is modified through refraction, diffraction and absorption, resulting in an exit wave that propagates outwards. Conventionally, this wavefront is refocussed using a lens and interferes with itself to form an image of the sample. If the exit wave is incident on a flat surface instead, the wavefront interferes with itself to form a diffraction pattern.

The Phase Focus Virtual Lens® algorithm can take a series of such diffraction patterns and reconstruct the wavefront that exited the sample. Figure 1 shows the simplest implementation of the virtual lens. A detector is located sufficiently far from the sample that a Fraunhofer diffraction pattern is formed on it. An localised area of illumination formed by an aperture is either projected onto the sample, or is placed near to it to define a “probe” region on the sample. The sample is moved laterally to an array of positions and a diffraction pattern is collected at each location. The positions are chosen such that each “probe” region overlaps its nearest neighbours, as shown in Figure 2. Once all the diffraction patterns have been collected, the Phase Focus Virtual Lens® algorithm is used to reconstruct the complete wavefront exiting the sample. Since both the phase and amplitude of the wavefront are known, it can be propagated forwards and backwards, enabling images to be reconstructed in any plane. This

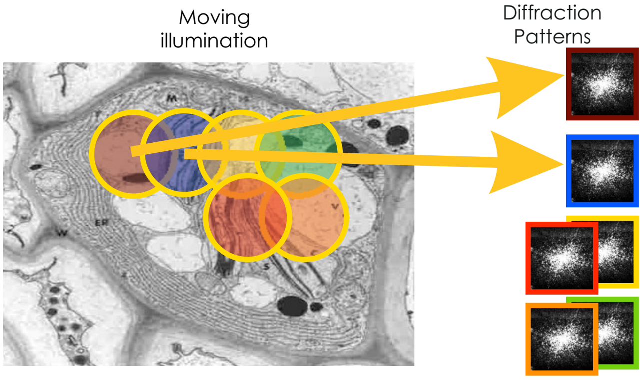

***Figure 2:** Image data collection; a series of overlapping regions are illuminated and the corresponding diffraction patterns recorded.*

results in the ability to perform post-acquisition re-focussing and allows measurements to be made at multiple depths from a single set of data. For a more detailed description of how the Phase Focus Virtual Lens® algorithm works, see Technical Brief TB01.

The area of diffraction pattern collected determines the resolution of the reconstructed image. In the simple system illustrated in figure 1, an inconveniently large detector would be required in the far field plane. To address this issue, the detector (typically a CCD camera with dimensions 7-15mm wide) can simply be moved closer to the sample, moving from the Fraunhofer regime to the Fresnel regime, where the wavefront can no longer be assumed to be flat. The Virtual Lens algorithm seamlessly compensates for the curvature introduced in the Fresnel regime by adding curvature to the probe wavefront; no changes to the algorithm are necessary.

Lens-assisted geometries

Moving the detector closer to the sample can be impractical, resulting in extremely small working distances. Hybrid "lens-assisted" modes can represent a good compromise, as shown in Figure 3.

In the first example, a Fraunhofer diffraction pattern forms in the back focal plane of the objective lens. This is then imaged directly with the tube lens, with the distances S_o and S_i chosen such that a de-magnified image of the diffraction pattern is formed at the detector. The magnification is given by $-S_i/S_o$. Using this method, a long working-distance can be maintained yet high resolution reconstructions ~1um can be obtained with a standard-sized CCD detector.

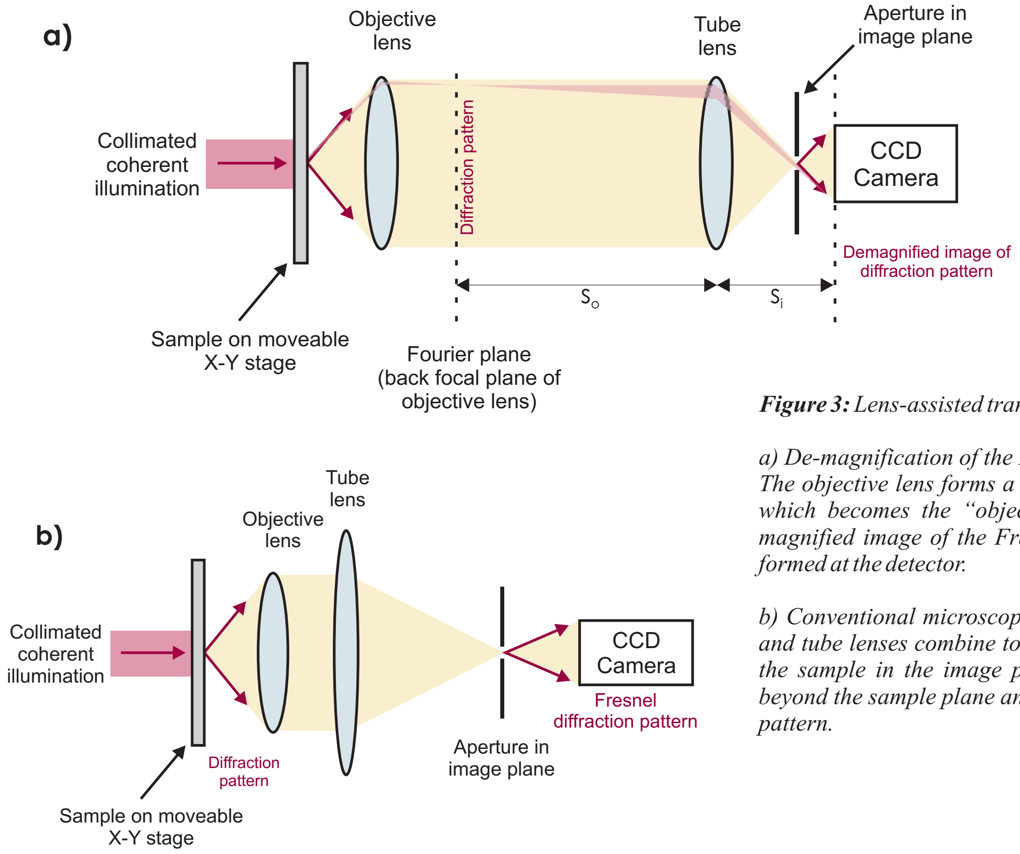

***Figure 3:** Lens-assisted transmission mode configurations.*

a) De-magnification of the Fraunhofer diffraction pattern. The objective lens forms a Fraunhofer diffraction pattern which becomes the "object" for the tube lens. A de-magnified image of the Fraunhofer diffraction pattern is formed at the detector.

b) Conventional microscope arrangement. The objective and tube lenses combine to produce a magnified image of the sample in the image plane. The detector is placed beyond the sample plane and collects a Fresnel diffraction pattern.

In figure 3b), a slightly different arrangement is shown. Here, the objective and tube lenses combine to produce a magnified image of the sample in the image plane (at the back focal plane of the tube lens), with a magnification of $-f_{tube}/f_{objective}$. This is the arrangement used in a standard optical microscope. If the detector is placed further back from the tube lens than the image plane, it will record a diffraction pattern. This is directly equivalent to the lensless arrangement shown in figure 1, however the diffraction pattern is formed by a magnified image of the sample as opposed to the sample itself. Consequently, the detector can be placed in the Fresnel regime without changing the true working distance of the system. In both lens-assisted geometries, the ultimate resolution of the system is limited by the numerical aperture of the objective lens.

Lens aberrations

It is informative to consider the effect of aberrations due to the lenses in a lens-assisted system. When an image is formed by a lens, a converging wavefront exits the lens and only if the entire wavefront converges to the same point will a good quality image form. The highest spatial frequency components of the image depend in rays from opposing edges of the lens (usually the least perfect regions) re-interfering correctly. Conversely, a diffraction pattern is formed by a diverging (or planar) wavefront. Features in the diffraction pattern are formed by rays leaving the lens at very similar locations. As a result, the diffraction patterns are much less sensitive to the quality of the lenses used. In addition, aberrations that do affect the diffraction pattern will cause simple geometric distortions as opposed to the more complex distortions present in the image plane.

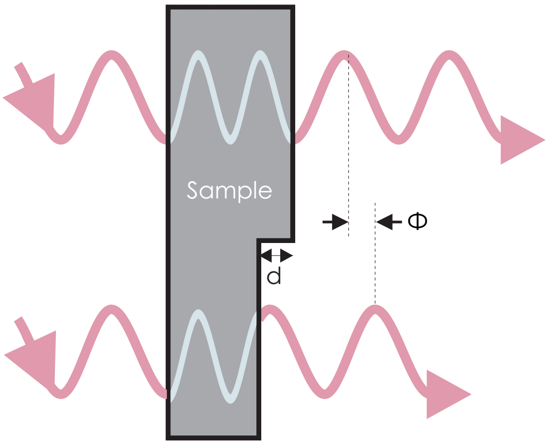

Figure 4: *Phase shift due to a step of height d in the thickness of a sample with uniform refractive index.*

Measurement of physical properties

Reconstructed images created by the Virtual Lens consist of both amplitude and phase information. The amplitude data is a direct quantitative measure of the optical transparency of the sample, and is equivalent to brightfield images produced by a standard optical microscope. The phase data represents how much one part of the exit wavefront lags behind another. Such a lag is caused by the incident wave travelling through regions with differing refractive index or of varying thickness, according to Eqn 1 below:

$$\Phi = \frac{2\pi n d}{\lambda} \qquad \text{Eqn (1)}$$

Where Φ is the phase shift, n is the refractive index, d is the thickness (or change in thickness) and λ is the wavelength of the illuminating light. Using Eqn 1, the phase data can be used to calculate the thickness or refractive index of a sample.

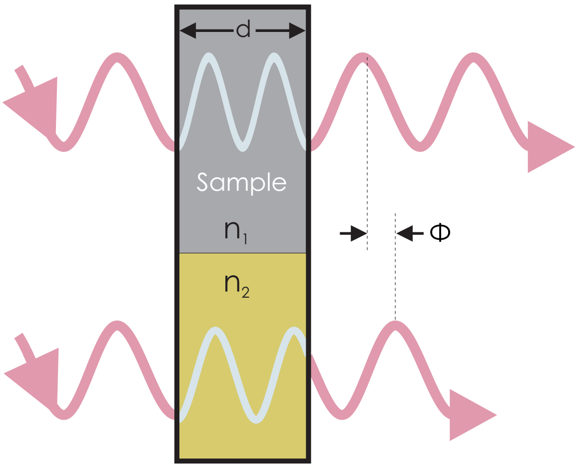

Figure 5: *Phase shift due to a variation in refractive index in a sample of uniform thickness.*

If refractive index is known to be constant, as shown in figure 4, the phase map can be used to measure the thickness of the sample. If, however, the thickness of the sample is constant a map of refractive index can be obtained, as shown in figure 5.

Conclusion

Using the Phase Focus Virtual Lens® in optical transmission mode it is possible to directly measure optical thickness and refractive index of transparent samples. A range of geometries can be implemented from fully lensless to lens-assisted. The lens-assisted geometries are less susceptible to aberrations than standard microscopy schemes.

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