Partially coherent phase microscopy with arbitrary illumination source shape

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Abstract: We propose a method that recovers phase from a stack of through-focus intensity images taken with a partially coherent microscope employing illumination of any arbitrary source shape. Our algorithm uses a Kalman filtering approach, which is fast, accurate and robust to noise. We validate the method in simulation and with experimental data taken on a commercial microscope with varying condenser apertures in Köhler geometry.

OCIS codes: 100.5070, 100.3010.

Phase can be reconstructed from a series of through–focus intensity images taken by a microscope or *4f* system (see Fig. 1) [1–4]. In previous methods, the illumination is assumed to be spatially coherent, although it may actually be partially coherent. The coherence of a brightfield microscope, for example, can be controlled by changing the condenser aperture shape; smaller apertures improve coherence at the cost of light throughput and resolution. We demonstrate here a phase imaging method that can handle arbitrary source (aperture) shapes, giving accurate phase results with any illumination coherence. Further, we show that not only can coherence be incorporated, but there may be some advantages in using non-traditional source shapes for phase imaging. These ideas are inspired by the lithography industry, where it is well known that the source shape can be optimized - for example, dipole or multi-pole illumination is widely used for accentuating specific spatial frequencies.

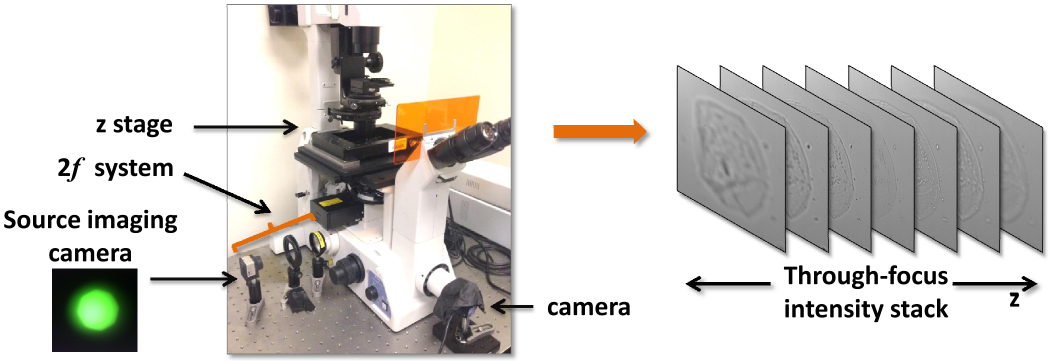

Fig. 1. A microscope is a widely used *4f* system setup. It can capture intensity images at various defocus distances by moving the camera to recover phase.

Our method is a simple extension of an existing phase-from-intensity algorithm that uses Kalman filtering. The method is conceptually related to the Transport of Intensity equation (TIE) [1,2], which approximates the first derivative of intensity with respect to the optical axis in order to recover phase. However, the approximation is sensitive to noise [3]. In [4], we show that by instead using a Kalman filtering approach, we can provide the information theoretic near-optimal phase solution, even in severe noise. The original Kalman filter in [4] was severly limited by computational complexity and storage requirements; however, by using a sparse Kalman filter [5], the computational complexity of [4] was reduced from $\mathscr{O}(N^3)$ to $\mathscr{O}(N\log N)$, where N is the number of pixels in the phase reconstruction. This model is therefore fast, accurate and robust to severe noise, but only works for coherent illumination. In this paper, we extend the sparse Kalman filtering model in [5] to the case of arbitrary partially coherent illumination. The new Kalman filtering method retains all the benefits of the sparse Kalman filter, but is also able to handle arbitrary source shapes for microscopes with Köhler illumination.

In order to incorporate partially coherent illumination into our Kalman filter model, we assume an incoherent extended source in the Köhler configuration that is typical of most microscopes, but the shape of the source may be any (known) arbitrary distribution. After a defocus distance of z, the intensity $I(x,y,z)$ can thus be written as a convolution between the intensity that would have occured given coherent illumination $|A(x,y,z)|^2$ and a scaled source intensity distribution $S(x,y)$:

$$I(x,y,z) = |A(x,y,z)|^2 \otimes S(-\frac{f}{z}x, -\frac{f}{z}y), \tag{1}$$

where f is the focal length of the condenser lens.

We formulate a state–space Kalman filter from the mathematical model of the partial coherence. After we have the state–space Kalman filter, the standard Kalman filter can be used to estimate the phase. However, the computational complexity for the standard Kalman filter is $\mathscr{O}(N^3)$. So we follow [5] to develop a new sparse model to reduce the computational complexity to $\mathscr{O}(N \log N)$.

Simulation and Experiment Result

We first demonstrate the proposed method by simulating a phase and amplitude object illuminated under 4 different conditions for the source shape (see Fig. 1). As shown in the top of Fig. 2, the source shapes are a circle with radius of $100\mu m$, a circle with radius of $500\mu m$, a square with side of $1000\mu m$, and a ring with smaller radius of $500\mu m$ and larger radius of $700\mu m$, respectively. The focal length of the condenser is $10mm$. The level of partial coherence σ is defined by the ratio of numerical aperture (NA) between condenser and objective lenses. For each illumination shape, an intensity stack consisting of 101 images was generated by defocusing the object symmetrically about the focus with $10\mu m$ step size. The data was further corrupted by white Gaussian noise with variance 0.001. To demonstrate the effect of partial coherence, we first process the data using the fully coherent model in [5], shown in the middle row of Fig. 2. Significant blur is present in the phase result because the coherent Kalman filter fails to model the partial coherence. On the other hand, our new partially coherent Kalman filter successfully eliminates the blurring artifact, as shown in the bottom of Fig. 2.

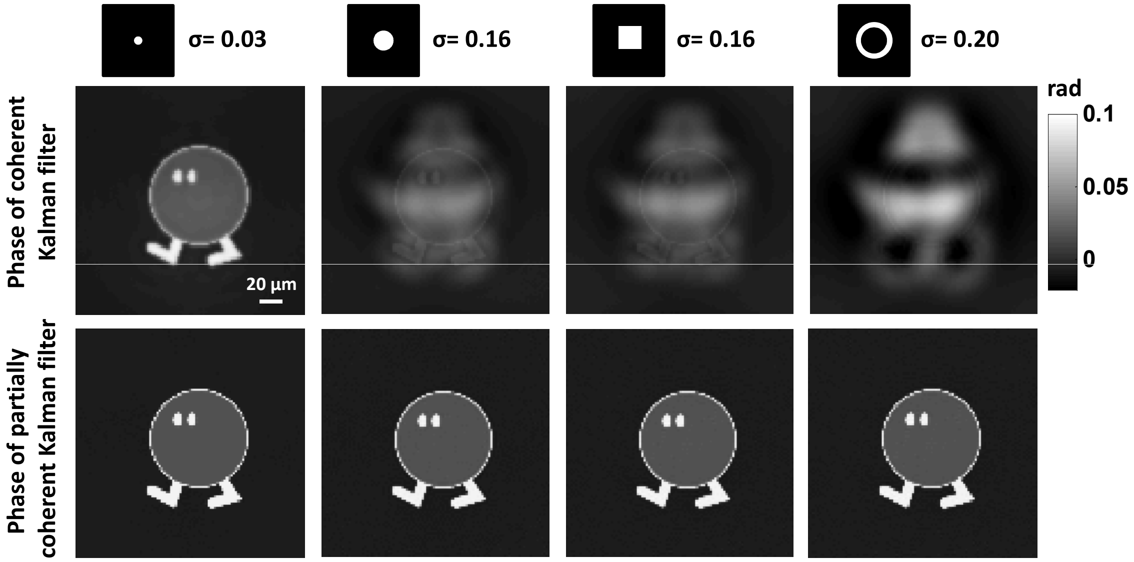

Fig. 2. Simulated data. (Top) Source shapes. (Middle) Recovered phase by coherent Kalman filter [5], which ignores partial coherence. (Bottom) Recovered phase by the proposed Kalman filter, which includes partial coherence.

We test out our method experimentally in a Nikon TE300 microscope (see Fig. 1). The temporal coherence is not considered here since the white light is filtered by a color filter of 550 nm. We took intensity focal stacks with different source shapes to vary the partial coherence of the illumination (see top row of Fig. 3). For the first three shapes, the sizes are changed by opening the condenser aperture. The ring shape in the fourth case is obtained by choosing the annular diagram of the Ph1 condenser. Note that the objective lens is not a Ph1 type, so the images are still brightfield mode. The images of the source shapes were taken by adding a $2f$ system at the side port of the microscope. For each shape, the same cheek cell sample was defocused symmetrically about the focus at 131 z-planes ranging from $-32.5um$ to $32.5um$. Each image contains 800×800 pixels. Figure. 3 compares the recovered phase images using the coherent Kalman filter and the proposed partially coherent Kalman filter. As the coherence decreases (aperture opens up), the phase images from the coherent Kalman filter become more blurred. However, the recovered phase images from the partially coherent Kalman filter still have high contrast and contain fine details. Interestingly, the recovered phase from the Ph1 mode has smaller low frequency artifacts. This is likely due to the fact that the intensity images resulting from the Ph1 source have higher contrast and therefore larger SNR in the low frequency information.

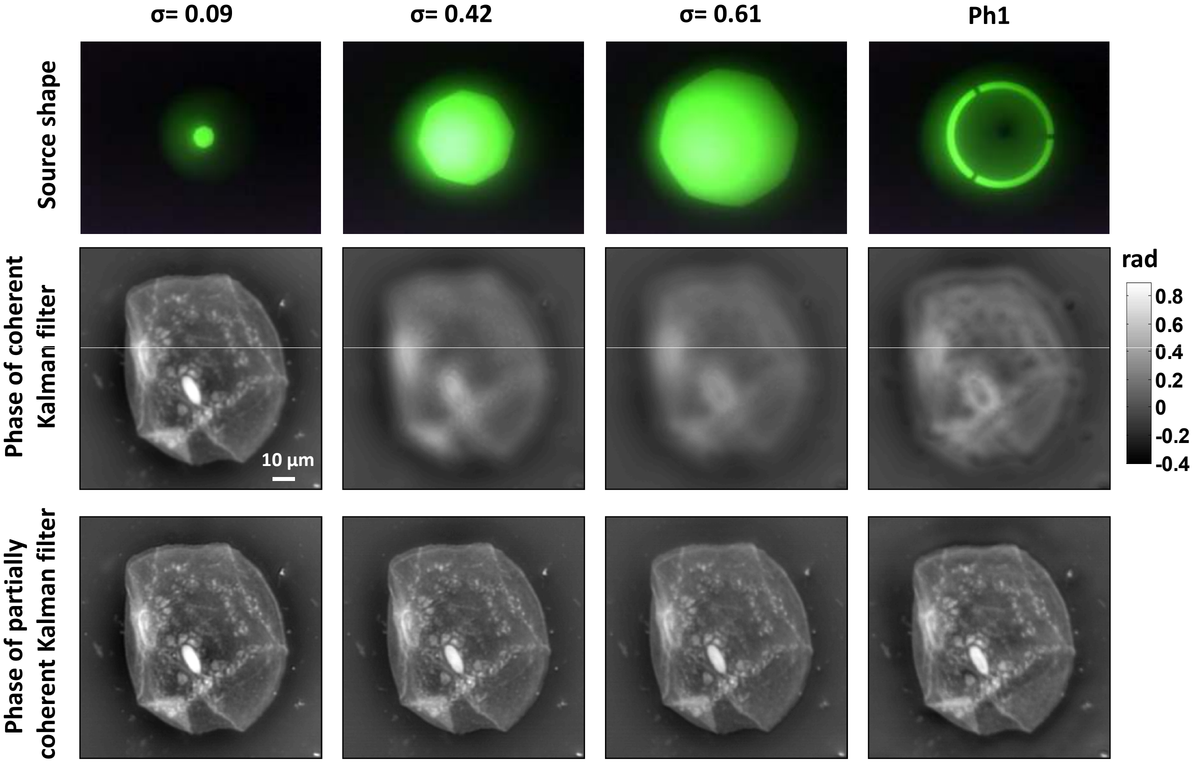

Fig. 3. Comparison of experimental results by coherent Kalman filter and partially coherent Kalman filter. (Top) Source shapes taken by a $2f$ system at the microscope's side port. (Middle) Recovered phase by the coherent Kalman filter. (Bottom) Recovered phase by the proposed Kalman filter method.

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