COMPUTATIONAL MICROSCOPY: ILLUMINATION CODING AND NONLINEAR OPTIMIZATION ENABLES GIGAPIXEL 3D PHASE IMAGING

Lei Tian

Electrical & Computer Engineering
Boston University
8 St. Mary's St., Boston, MA 02215, USA

Li-hao Yeh, Regina Eckert, Laura Waller

Electrical Engineering & Computer Science
University of California Berkeley
Cory Hall, Berkeley, CA 94720, USA

ABSTRACT

Microscope lenses can have either large field of view (FOV) or high resolution, not both. Computational microscopy based on illumination coding circumvents this limit by fusing images from different illumination angles using nonlinear optimization algorithms. The result is a Gigapixel-scale image having both wide FOV and high resolution. We demonstrate an experimentally robust reconstruction algorithm based on a 2nd order quasi-Newton's method, combined with a novel phase initialization scheme. To further extend the Gigapixel imaging capability to 3D, we develop a reconstruction method to process the 4D light field measurements from sequential illumination scanning. The algorithm is based on a 'multislice' forward model that incorporates both 3D phase and diffraction effects, as well as multiple forward scatterings. To solve the inverse problem, an iterative update procedure that combines both phase retrieval and 'error back-propagation' is developed. To avoid local minimum solutions, we further develop a novel physical model-based initialization technique that accounts for both the geometric-optic and 1st order phase effects. The result is robust reconstructions of Gigapixel 3D phase images having both wide FOV and super resolution in all three dimensions. Experimental results from an LED array microscope were demonstrated.

Index Terms— Computational microscopy, coded illumination, phase retrieval, Fourier ptychography, light field

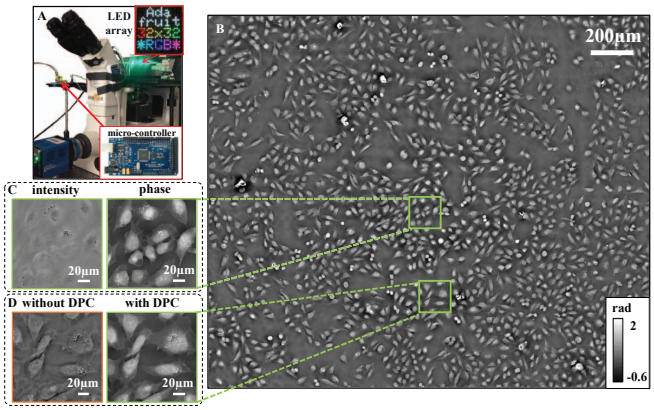

Fig. 1. Coded illumination based computational microscopy enables Gigapixel phase and intensity imaging [3]. (A) Experimental setup is a microscope with an LED array source and a wide FOV objective. Multiple images are captured with coded illumination to reconstruct higher resolution. (B) Phase reconstruction across the full FOV of a 4× objective with 0.7 NA resolution. (C) A zoom-in region shows the intensity and phase reconstructions. (D) FPM algorithm with DPC initialization provides a better reconstruction of low-frequency phase information. A zoom-in region shows comparisons between phase reconstructions with and without our DPC initialization scheme.

1. INTRODUCTION

Microscope lenses can have either large field of view (FOV) or high resolution, not both. Recent advances in computational microscopy circumvent this physical limit by computationally fusing information from multiple images [1, 2]. The results are Gigapixel-scale intensity and phase images, having both wide FOV and high resolution, i.e. large space-bandwidth product (SBP).

Our work is based on Fourier ptychographic microscopy (FPM) [1], which is a coded illumination-based computational imaging technique. The hardware involves a simple replacement of the light source of a commercial microscope with an LED array (Fig. 1A). FPM combines ideas from synthetic aperture [4, 5], translational-diversity phase retrieval [6, 7] and ptychography [8]. Intensity images are first captured from different illumination angles. When the illumination angles are within the objective's numerical aperture ($\mathrm{NA}_{\mathrm{obj}}$), one captures brightfield images. Conversely, when the illumination angle is larger than the objective NA, one captures darkfield images. Although darkfield images alone do not have higher resolution than the objective allows, they do contain information about sub-diffraction-limit sized features, which occupy a shifted region of the sample's Fourier space. By collecting images that cover a wide region of Fourier space and stitching them together coherently using a

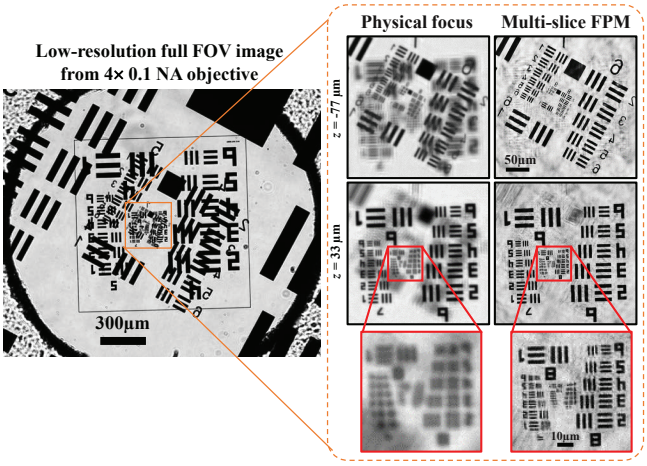

Fig. 2. Experimental results using multi-slice Fourier Ptychography to achieve enhanced resolution at two depths simultaneously [11]. Using a $4\times$ objective, we achieve resolution of 0.69μm ($5\times$ better than $\mathrm{NA}_{\mathrm{obj}}$). (Left) Low-resolution raw image. (Right) Zoom-in at two depths comparing our multi-slice recovery to physical refocusing.

phase retrieval algorithm [1, 9, 10], one can achieve resolution beyond the objective's diffraction limit, corresponding to the sum of illumination and objective NAs. FPM's scan-free high SBP imaging capability has great potential for revolutionizing microscopy, with applications including digital pathology [1] and *in vitro* cell culture imaging [3] (Fig. 1B,C).

The original FPM only applies to 2D thin objects. We have recently developed a new model and reconstruction algorithm that also enables wide FOV and high resolution imaging of thick samples [11]. This is possible because angular diversity provides *both* phase contrast *and* 3D information. The phase information is encoded through angled illumination from asymmetries introduced in the pupil plane [12]. The same space-angle dataset also encodes 3D information, similar to light field capture [13]. This enables us to employ the standard light field digital refocusing algorithm, which fully incorporates 3D geometric effects. However, diffraction and phase effects cause the light to deviate from the geometrical model, and degrade the resolution with defocus. Using the light field refocused result as an initial guess, we have developed a multi-slice Fourier ptychographic reconstruction algorithm that iteratively estimates phase and diffraction effects [11]. The multi-slice approach models the sample as a series of thin slices. The wave propagates through the sample from slice to slice. Each slice modulates the field and the objective NA limits the resolution of the captured image. To solve the inverse problem, we iteratively update the 3D complex object function for each illumination angle, effectively implementing a nonlinear 3D deconvolution which removes out-of-plane blur. Further, we use darkfield images to build up a larger effective NA, limited by the sum of illumination and objective NAs. Thus, we can recover high-resolution Gigavoxel-sized 3D intensity and phase images (Fig. 2).

2. EXPERIMENTAL ROBUST FOURIER PTYCHOGRAHPIC RECONSTRUCTION

2.1. Influence of cost functions and forward model errors

A critical metric of the Fourier ptychograhpic algorithm is the performance under forward model errors due to experimental imperfections. This is of particular importance for this type of computational imaging technique, which is often not robust enough to provide consistent high-quality results.

In our previous work, we have shown that the cost function is of crucial importance in order for the algorithm to be experimentally robust [10]. One source of error in the FPM experimental data is measurement noise, including Gaussian noise and/or Poisson shot noise. Another main source of error is model mismatch, caused by experimental miscalibrations such as aberrations and LED misalignment. A particular problem of FPM datasets is that they contain both brightfield and darkfield images, which have drastically different intensity levels. Brightfield images can have several orders of magnitude higher intensity than darkfield images; thus, the amount of photon noise will also be significantly higher. If this difference in the noise levels is not properly accounted for, the brightfield noise may drown out the darkfield signal. In addition, aberrations and LED miscalibration also result in *intensity-dependent* errors. Thus, by carefully designing the cost function, we can develop algorithms that are significantly more robust to *both* noise *and* model mismatch.

Two classes of cost functions are of interest: *amplitude-based* methods refer to algorithms that minimize amplitude differences, and *intensity-based* methods minimize intensity differences. To better understand the differences of the two cost functions, we further developed a maximum likelihood framework to consider the Fourier ptychograhpic reconstruction with various noise models. The key insights are that *amplitude-based* algorithms implicitly incorporate a Poisson noise model, while *intensity-based* algorithms use a Gaussian noise model. As a result, *amplitude-based* algorithms are more experimentally robust than *intensity-based* algorithms.

Figure 3 summarizes our previous study. For the same cost functions, multiple algorithms are tested. It should be highlighted that algorithms with the same cost function give similar reconstruction artifacts. For example, the *intensity-based* algorithms suffer from high-frequency artifacts; *amplitude-based* and *Poisson-likelihood-based* algorithms give similar results. The exception is the original FPM (Gerchberg-Saxton) algorithm [1], which suffers from low-frequency artifacts. The reason is that the FPM phase retrieval problem is non-convex and the algorithm does not always converge to the correct solution.

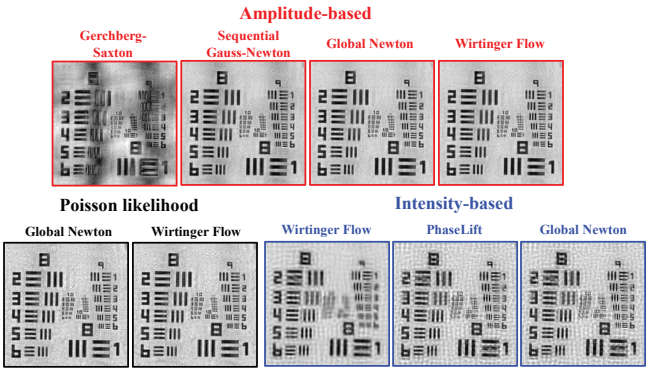

Fig. 3. Fourier ptychographic reconstruction with different algorithms, all using the same experimental dataset [10]. Algorithms derived from the same cost function (amplitude-based, intensity-based, and Poisson-likelihood) give similar performance, and first-order methods suffer from artifacts.

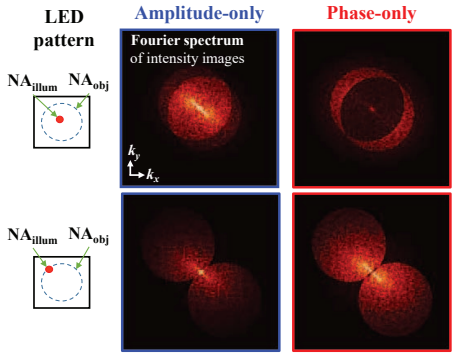

Fig. 4. Comparison between the Fourier spectrum of intensity images taken from amplitude-only (Left) and phase-only (Right) objects. Transfer function analysis shows that only large illumination angles (close to the objective NA) include low-frequency phase information (Bottom), while small illuminations angles capture illumination high-frequency information (Top), which is contrary to the intensity case.

2.2. Physical model-based initialization scheme

The phase retrieval problem like FPM is *non-convex* and nonlinear. Even with careful design of cost functions and updating algorithms, the reconstruction can still get stuck in local minima [14]. It is well known that the best way to avoid this is to provide a good initial guess [15, 16].

Depending on whether the sample is strongly absorptive or phase-only, different initialization methods for the FPM reconstruction are needed. The reason is that FPM only directly captures intensity and not phase at each angle. The absorption and phase contrast from the asymmetric illumination result in uneven sensitivities at different spatial frequencies. A linear transfer function analysis [3] based on the weak-object approximation shows that low-frequency phase information is poorly captured, since it results only from illumination angles that are close to the objective NA (Fig. 4). Thus, low frequency phase information is more difficult to reconstruct than high frequency phase information, contrary to the situation for intensity reconstructions.

Previous work in FPM uses a low-resolution intensity image as the initial guess [1, 9]. Samples with strong absorptions can reconstruct successfully with this method since the intensity-only initialization is close to the actual solution. However, for phase samples like unstained cells, the intensity-only initial guess does not provide a good starting point. To improve the phase reconstruction, we have proposed using a linearly approximated phase solution based on differential phase contrast (DPC) deconvolution [3] as a close initial guess for spatial frequencies within the 2 NA bandwidth. As compared in Fig. 1D, though high frequency phase features (e.g. edges) are reconstructed with or without using our DPC initialization method, low spatial frequency phase is much better recovered with the DPC initialization.

2.3. Physical model-based illumination coding design

The physical model used in our DPC initialization method also provides new insights to design better illumination coding strategies. The original FPM employs a 'sequential' illumination scheme. An image is collected while scanning through each of the LEDs in the array, leading to hundreds of images in each dataset. This is compounded by the fact that each LED has limited intensity, requiring long exposure times. We have previously proposed a random coding strategy across both brightfield and darkfield regions [9]. A set of multiple randomly selected LEDs are turned on simultaneously for each measurement, allowing larger coverage of Fourier space with each image.

Our new illumination coding strategy incorporates the DPC illumination patterns [3], since it provides a means for recovering quantitative phase and intensity images out to 2× the objective NA with only 4 images. This new method, termed source-coded FPM, uses a hybrid illumination scheme: it first captures 4 DPC images (top, bottom, left, right half-circles) to cover the brightfield LEDs, then uses random multiplexing with 8 LEDs to fill in the darkfield Fourier space region (outside 2 NA) (Fig. 5).

A major benefit of the illumination multiplexing is to significantly reduce both the acquisition time and data requirement. Faster capture times not only improve imaging speed, but also allow studies of live samples. With live samples imaged *in vitro*, dynamics create motion blur artifacts that can destroy the resolution improvements gained by large SBP methods. Thus, the final effective resolution is always coupled with acquisition speed and sample-dependent motion. Figure 5 compares the results of our source-coded FPM and sequential FPM. In both cases, the final result has a nomi-

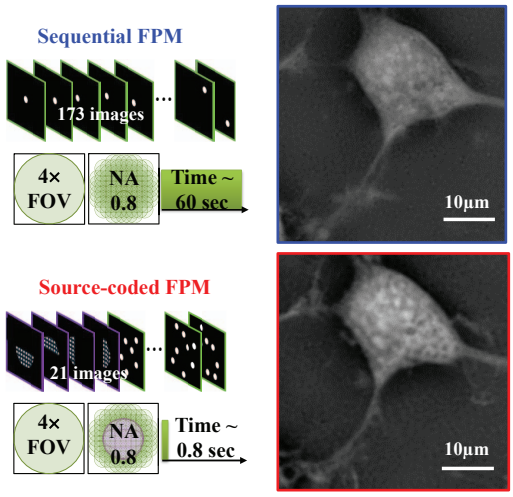

Fig. 5. Reconstructed phase of live cells using different illumination schemes with the same nominal resolution (0.8 NA), but different acquisition times [3]. Sequential FPM blurs out most subcellular features because of cell motion; source-coded FPM is able to capture details without motion artifacts.

nal NA of 0.8 and so each of these results *should* have the same resolution. However, sequential FPM results in significant motion blur, particularly along thin, extended processes and within intracellular vesicles and organelles. In contrast, source-coded FPM is able to accurately capture the full details of the sample without motion blur artifacts.

2.4. Physical model-based illumination angle calibration

An important model error for FPM is miscalibration of illumination angles. Though the amplitude-based algorithm is moderately robust to angle miscalibrations even without correction, the reconstruction still deteriorates for high resolution features and/or thick objects. Since high resolution information is only captured from large illumination angles, a small angular error may produce a significant unknown shift at a defocused plane. Therefore angle miscalibration has a more extreme effect on 3D reconstruction, where most of the sample will be out of focus in the raw data.

While the illumination angles could be exhaustively pre-calibrated, this can be extremely time-consuming and would not account for any change of angles induced by the sample (e.g. refraction from air-water interface in cell cultures). We have previously demonstrated a brute-force search-based algorithms based on simulated annealing [10]. However, it requires significantly longer computational times, and only accommodates small angular errors in practice.

Recently, we have proposed a new physical model-based method that exploits the asymmetric information encoded in the Fourier transform of the raw intensity data to estimate the

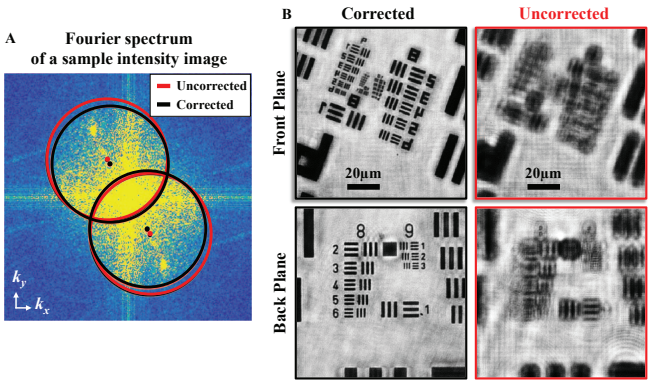

Fig. 6. Physical model-based illumination angle calibration method on a laser-illuminated setup [17]. (A) The actual illumination angle is estimated from the locations of the two circular regions in the Fourier space. (B) Multi-slice FPM reconstructions with (Left) and without (Right) angle self-calibration.

angles of illumination without pre-calibration [17]. The intensity Fourier spectrum corresponds to an autocorrelation of the optical field's Fourier spectrum, which is band-limited by the circular pupil. According to the transfer function analysis [3], the intensity Fourier spectrum contains two overlapped circular regions, each corresponding to a shifted pupil centered around the carrier frequency (Fig. 6A). Thus, applying image processing techniques to the Fourier spectrum allows us to accurately estimate the angle of illumination (i.e. carrier frequency). This method is simple, computationally efficient, and applies to both 2D and 3D FPM datasets. Figure 6B shows the multi-slice FPM reconstructions of a two-layer resolution target object. Without angle correction, the reconstruction suffers from severe artifacts due to large angular errors. Using the same dataset, we first employ the physical model-based calibration to correct the angles and then employ the same multi-slice FPM reconstruction algorithm. High-quality reconstruction across both planes demonstrates the effectiveness of our method.

3. SUMMARY AND OUTLOOK

This paper summarizes our recent efforts in developing experimentally robust FPM for imaging both thin and thick samples across a wide FOV with high resolution. We have shown that the *amplitude-based* algorithms are robust to the photon noise and model mismatch errors. Of particular interest are the physical model-based methods, which allow us to develop a better initialization scheme for robust low-frequency phase recovery and an efficient illumination angle calibration method. Further, our new source coding scheme enables fast, motion-free imaging of unstained live samples, opening up *in vitro* applications across multiple spatial and temporal scales.

4. REFERENCES

[1] Guoan Zheng, Roarke Horstmeyer, and Changhuei Yang, "Wide-field, high-resolution Fourier Ptychographic microscopy," *Nat. Photonics*, vol. 7, no. 9, pp. 739–745, 2013.

[2] Alon Greenbaum, Wei Luo, Ting-Wei Su, Zoltán Göröcs, Liang Xue, Serhan O Isikman, Ahmet F Coskun, Onur Mudanyali, and Aydogan Ozcan, "Imaging without lenses: achievements and remaining challenges of wide-field on-chip microscopy," *Nature Methods*, vol. 9, no. 9, pp. 889–895, 2012.

[3] Lei Tian and Laura Waller, "Quantitative differential phase contrast imaging in an LED array microscope," *Opt. Express*, vol. 23, no. 9, pp. 11394–11403, May 2015.

[4] Christian J. Schwarz, Yuliya Kuznetsova, and S. R. J. Brueck, "Imaging interferometric microscopy," *Opt. Lett.*, vol. 28, no. 16, pp. 1424–1426, Aug 2003.

[5] Sergey A Alexandrov, Timothy R Hillman, Thomas Gutzler, and David D Sampson, "Synthetic aperture fourier holographic optical microscopy," *Physical Review Letters*, vol. 97, no. 16, pp. 168102, 2006.

[6] John M Rodenburg and Helen ML Faulkner, "A phase retrieval algorithm for shifting illumination," *Applied Physics Letters*, vol. 85, no. 20, pp. 4795–4797, 2004.

[7] Gregory R Brady, Manuel Guizar-Sicairos, and James R Fienup, "Optical wavefront measurement using phase retrieval with transverse translation diversity," *Optics express*, vol. 17, no. 2, pp. 624–639, 2009.

[8] J.M. Rodenburg, "Ptychography and related diffractive imaging methods," vol. 150 of *Advances in Imaging and Electron Physics*, pp. 87–184. Elsevier, 2008.

[9] Lei Tian, Xiao Li, Kannan Ramchandran, and Laura Waller, "Multiplexed coded illumination for Fourier ptychography with an LED array microscope," *Biomed. Opt. Express*, vol. 5, no. 7, pp. 2376–2389, Jul 2014.

[10] Li-Hao Yeh, Jonathan Dong, Jingshan Zhong, Lei Tian, Michael Chen, Gongguo Tang, Mahdi Soltanolkotabi, and Laura Waller, "Experimental robustness of Fourier ptychography phase retrieval algorithms," *Opt. Express*, vol. 23, no. 26, pp. 33214–33240, Dec 2015.

[11] Lei Tian and Laura Waller, "3D intensity and phase imaging from light field measurements in an LED array microscope," *Optica*, vol. 2, no. 2, pp. 104–111, Feb 2015.

[12] Michael Chen, Lei Tian, and Laura Waller, "3D differential phase contrast microscopy," *Biomed. Opt. Express*, vol. 7, no. 10, pp. 3940–3950, Oct 2016.

[13] M. Levoy, R. Ng, A. Adams, M. Footer, and M. Horowitz, "Light field microscopy," in *SIGGRAPH '06*, 2006, pp. 924–934.

[14] J. R. Fienup, "Phase retrieval algorithms: a comparison," *Appl. Opt.*, vol. 21, no. 15, pp. 2758–2769, 1982.

[15] J. R. Fienup and C. C. Wackerman, "Phase-retrieval stagnation problems and solutions," *J. Opt. Soc. Am. A*, vol. 3, no. 11, pp. 1897–1907, 1986.

[16] Emmanuel J Candès, Xiaodong Li, and Mahdi Soltanolkotabi, "Phase retrieval via wirtinger flow: Theory and algorithms," *arXiv:1407.1065*, July 2014.

[17] Regina Eckert, Lei Tian, and Laura Waller, "Algorithmic self-calibration of illumination angles in fourier ptychographic microscopy," in *Imaging and Applied Optics 2016*. 2016, p. CT2D.3, Optical Society of America.