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Quantitative imaging of yeast cells using transport of intensity equation

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Abstract— In biology most microscopy specimens, in particular living cells are transparent. In cell imaging, it is hard to create an image of a cell which is transparent with a very small refractive index change with respect to the surrounding media. Various techniques like addition of staining and contrast agents, markers have been applied in the past for creating contrast. Many of the staining agents or markers are not applicable to live cell imaging as they are toxic. In this paper, we report theoretical and experimental results from quantitative phase imaging of yeast cells with a commercial bright field microscope.

We reconstruct the phase of cells non-interferometrically based on the transport of intensity equations (TIE). This technique estimates the axial derivative from positive through-focus intensity measurements. This technique allows phase imaging using a regular microscope with white light illumination. We demonstrate nano-metric depth sensitivity in imaging live yeast cells using this technique. Experimental results will be shown in the paper demonstrating the capability of the technique in 3-D volume estimation of living cells. This real-time imaging technique would be highly promising in real-time digital pathology applications, screening of pathogens and staging of diseases like malaria as it does not need any preprocessing of samples.

Keywords— *Axial derivative;Non-Interferometric imaging; Quantitative phase imaging;Transport of intensity equation.*

I. INTRODUCTION

Rapid phase retrieval is a fundamental problem in biology as phase carries important information about the object structure and other properties. However, measuring the phase of an optical field is a challenging issue. Even the fastest detectors available now a days have integration times that are several orders of magnitude larger than the temporal period of light oscillations. It follows that only the intensity of the field is directly accessible.

Non-destructive techniques that allow accurate and quantitative measurements of phase of an object have had a tremendous impact in many fields ranging from biology to physics and engineering. These techniques are usually divided into interferometric and non-interferometric methods.

Interferometry techniques can quantitatively measure the phase of the wave field. But it requires the uniform irradiance distribution (laser) with stable setup, and other computational issues like phase wrapping are associate with it, and at the same it is very difficult to convert this imaging platform clinical side as the setups are quite complex and costly. However it is now apparent that non-interferometric techniques can allow phase to be measured quantitatively and offering more stable and less technically demanding approaches in comparison with the interferometric techniques.

Non-interferometric quantitative phase imaging technique is realized by transport-of-intensity equation (TIE), which was derived in 1983 by Teague [1-2] with the idea of relating measured intensities for phase quantification. It uses an elliptic partial differential equation called Transport of Intensity Equation (TIE). The main idea is that when a phase object (biological cell) is illuminated by a light from the light source (partially coherent light) having amplitude and phase, and then recording the wave fronts at different distances which are being modulated by the object with a CCD camera, we can reconstruct the phase of the object, which is lost during recording process.

TIE imaging have been increasingly investigated during recent years due to its unique advantages over interferometric techniques [3-5], TIE is a non-interferometric imaging modality, and works with partially coherent light[6], computationally simple [7-8], it does not require phase unwrapping [9], and a complicated optical system [10-12] like in DHM. Also TIE can be used for a wide range of illuminating frequencies including x-rays [13], imaging flow cytometry [14] optical microscopy [15], electron microscopy [16] and X-Ray imaging, and the most important thing is it requires a simple setup and which can be translated to the bedside very easily with cost effectively.

Despite of these merits offered by TIE, This technique did not gained much attention and widespread applications as like interferometric techniques in the field of quantitative phase microscopy. But recently TIE have been demonstrated for dynamic process of macrophage phagocytosis with single shot quantitative phase imaging [17] and for the characterization of micro optics [18], which realizes the importance of TIE in the field of Quantitative phase imaging with nanometric precision with high SNR. In this work we have extended the application of TIE to render the three dimensional *yeast cells* from the commercially available microscope.

II. Methodology: Setup of TIE

In the Paraxial regime waves have inherent property by which we can retrieve phase of the wave field from the captured intensities between two defocused planes which intern proportional to the phase contrast. A simple way to obtain the TIE is the use of the parabolic equation [1-2]:

$$\left(i\frac{\partial}{\partial z}+\frac{\Delta_{\perp}}{2k}+k\right)U=0 \qquad (1)$$

Where k is the wavenumber, U is the optical field and $\Delta_{\perp}$ is the transverse Laplacian. The wave amplitude U can be written in terms of intensity and phase as:

$$U(x,y,z)=\sqrt{I(x,y,z)}e^{i\varphi(x,y,z)} \qquad (2)$$

Substituting equation (2) into (1) and taking the imaginary part results in TIE equation as

$$\vec{\nabla}_{\perp}.[I(r_{\perp},z)].\vec{\nabla}_{\perp}\varphi(r_{\perp},z)=-k\frac{\partial I(r_{\perp},z)}{\partial z} \qquad (3)$$

Where $\nabla_{\perp}=\nabla_{x,y}=\nabla$ is the 2D gradient operator over the transverse direction $r_{\perp}=(x,y)$, where is the position vector representing the transverse spatial coordinates. Where $k=\frac{2\pi}{\lambda}$ is the propagation constant.

One fundamental assumption of the TIE is that complex amplitude varies slowly along the propagation path, it means

$$\frac{\partial^2 U}{\partial z^2}\approx 0, \qquad (4)$$

And therefore $U(x,y,z=z_0)=U(x,y,z_0)e^{-ikz_0}$ (5)

In practice, the terms $\partial_z I$ and I_0 are obtained from a series of measurements, and a unique solution for φ exists, as long as $I_0(x,y)>0$ everywhere in the image plane. One major source of error is the accuracy in determining the axial derivative, usually axial derivative is computed by finite difference method by selective defocused image planes.it is very important to select optimum [26] defocused planes as if we select the small defocus images we will get noise corrupted phase reconstruction and if we select the large defocused images we will get the nonlinearity corrupted phase reconstruction, So we need to balance between these two constraints.

The TIE is a Poisson type equation which relates a modified Laplacian of the phase of the wave to the intensity variation along the optical axis in absence of singularities in image plane. The main problem to solve TIE equation is unknown boundary conditions. Which was bypassed by Fast Fourier Transform (FFT) based method by solving the TIE non-iteratively in the frequency domain with implicit periodic boundary conditions due to the periodic properties of the Fourier Transform [11].

The experimental configuration used for the present study is commercial transmission light microscope as shown in figure1. We used a commercial inverted bright-field microscope (Carl Zeiss Axio-observer A1). The illumination is from a halogen lamp having central wavelength of 632nm. We used microscope objective of 40x with NA=0.65 for imaging.

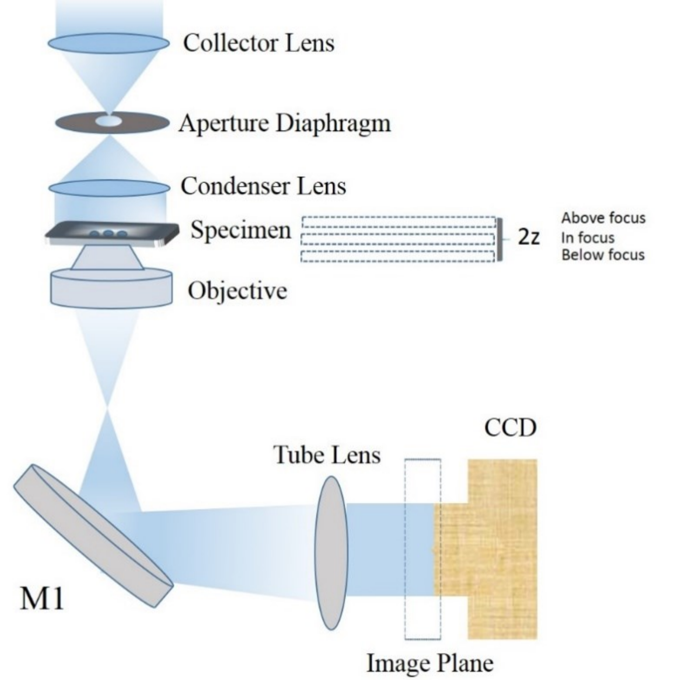

Figure: 1 configuration Bright field microscopy

TIE uses only object field intensities at axially sampled planes without any interference, we have recorded through focus intensities of *yeast cells* in the microscope. Then solved TIE for determining the object-plane phase from the first derivative of intensity in the near Fresnel region [19-20]. Equation.3 is a useful approximation of paraxial light propagation as it can be inverted to recover the phase using intensity measurements in the axial direction, which is solved for the phase starting from intensity measurements. Implementing the TIE formalism in practice has been difficult and results into phase errors.

Numerous different solutions for eq. (3) have been suggested [6, 9, 21-24] to accurately estimate the phase. Some of these algorithms works well only when zero image intensity is assumed outside the image area, which may not reflect all real experimental situations, or will require the use of special apertures, otherwise results into boundary artifacts. Paganin and Nugent [6] proposed an alternative definition of phase via the poynting theorem and showed that TIE can be used with some limitations for general case, including electromagnetic fields. With the assumption of $I\vec{\nabla}\varphi=\vec{\nabla}\psi$, a formal solution to TIE eq. (3) is given by.

$$\varphi(r_\perp, z) = -k\nabla_\perp^{-2}.\{\nabla_\perp[\frac{\nabla_\perp \nabla_\perp^{-2} \partial_z I}{I}]\},\ I \neq 0 \qquad (6)$$

Where $\nabla_\perp^{-2}$ the inverse Laplacian operator is calculated by appropriate method [11].

The assumption $I\vec{\nabla}\varphi = \vec{\nabla}\psi$ is valid, if ψ is conservative, which means that the path integral around a closed path is zero, and the phase φ is single valued and it does not contain any singularities i.e. intensity I is assumed to be strictly positive.

III. Results and discussions

We have registered three intensity images in the bright field microscope along the axial direction with a defocus step of $\pm 5\mu m$ are shown in figure 1a-1c respectively. We solved the transport to intensity equation as per the eqn.6. The reconstructed image consists of phase errors, which are due to incorrect boundary estimation and are corrected with interpolation methods. The resultant reconstructed phase image is shown in figure 2d.

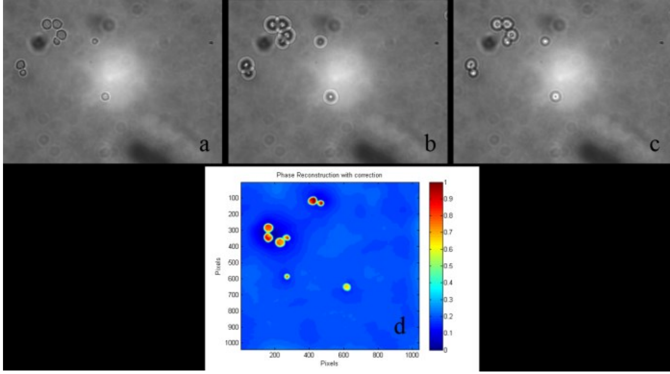

Figure: 2 Bright field images of the *yeast* cells taken from the microscope. a) 5µm defocused above from the in focus image, b) infocus image c) 5µm defocused below from the in focus image, d) Phase reconstruction of *yeast* cells.

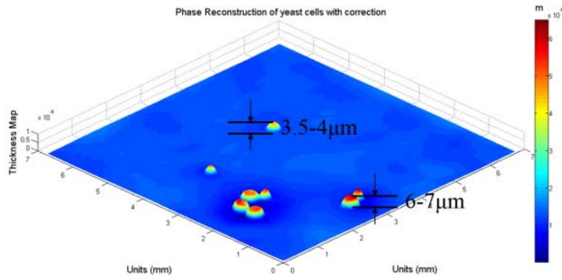

Figure: 3 Phase reconstruction of *yeast cells* in 3D

Once we have the quantitative phase (Δϕ) we can find find the object thickness 'h' through the equation

$$h = \frac{\Delta\phi\lambda}{2\pi(n - n_{medium})} \qquad (7)$$

In fig. 3 we can see enhanced view of the *yeast* cells in 3D. The thickness of the *yeast* cells was found to be around $2 - 7\mu m$.

The knowledge of Δϕ, h and the wavelength λ allows determination of refractive index map (n).

IV. CONCLUSION

We have demonstrated the quantitative phase imaging of the *yeast cells* using the commercially available bright field microscope. TIE provides speckle-free, stable and quantitative phase retrieval with partially coherent light, which allows optical path-length measurements with nanometric sensitivity and high SNR. Since it is noninterferometric it does not require additional reference beam to extract phase. Since the halogen source is partially coherent, it is really difficult to use the standard interferometric techniques to quantify the thickness. So this noninterferometric, quantitative phase imaging technique is the right tool for quantifying three dimensional information from the commercial BFM.

References

[1]. M. R. Teague, "Deterministic phase retrieval: a Green's function solution," J. Opt. Soc. Am. 73, 1434–1441 (1983).

[2]. M.Teague et al., "Image formation in terms of the transport equation," Journal of the Optical Society of America A, vol.2, no.11, pp.2019-2026, 1985

[3].Tishko T, Titar V and Tishko D 2005 Holographic methods of three dimensional visualization of microscopic phase objects J. Opt. Technol. 72(2) 203

[4]. Grilli S, Ferraro P, De Nicola S, Finizio A, Pierattini G, Meucci R 2001 Whole optical wave fields reconstruction by Digital Holography Opt. Exp. 9 294

[5]. Marquet P, Rappaz B, Magistretti P, Cuche E, Emery Y, Colomb T, and Depeursinge C "Digital holographic microscopy: a non-invasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy" Opt. Lett. 30:468, 2005

[6]. D. Paganin and K. Nugent, "Non-interferometric phase imaging with partially coherent light," Phys. Rev. Lett. 80, 2586–2589 (1998).

[7]. N.Streibl, "Phase imaging by the transport equation of intensity," Optical Communications, vol.49, no.1, 1984.

[8]. T. Gureyev, A. Roberts, and K. Nugent, Phase retrieval with the transport-of-intensity Equation: matrix solution with use of Zernike polynomials, J. Opt. Soc. Am. A 12 no. 9 (1995), 1932–1941.

[9]. T. Gureyev, A. Roberts, and K. Nugent, Partially coherent fields, the transport -of-intensity Equation, and phase uniqueness, J. Opt. Soc. Am. A 12 no. 9 (1995), 1942–1946.

[10]. D.VanDyck and W.Coene, "A new procedure for wave function restoration in high resolution electron microscopy," Optik, vol.77, no.3, pp.125-128, 1987.

[11]. V.V. Volkov, Y. Zhu, and M. De Graef. "A new symmetrized solution for phase retrieval using the transport of intensity equation". In: Micron 33.5 (2002), pp. 411-416. ISSN: 09684328.

[12]. A.Barty, K.Nugent, D.Paganin and A.Roberts,"Quantitative optical phase Microscopy, "Optical letters, vol.23, no.11, pp.817-819, 1988.

[13]. S.Mayo, P.Miller, S.Wilkins, T.Davis, D.Gao, T.Gureyev, D.Paganin, D.Parry, A.Pogany, and A.Stevenson,"Quantitative x-ray projection microscopy: Phase contrast and multi-Spectral imaging," Journal of Microscopy, vol.207, pp.79-96, 2002.

[14]. Sai Siva Gorthi and Ethan Schonbrun, "Phase imaging flow cytometry using a focus-stack collecting microscope," Opt. Lett. **37**, 707-709 (2012).

[15]. Shan Kou et al. "Transport-of-intensity approach to differential interference contrast (TI-DIC) microscopy for quantitative phase imaging". In: Opt. Lett. 35.3 (2010), pp. 447-449. doi: 10.1364/OL.35.000447.

[16]. Kazuo Ishizuka and Brendan Allman. "Phase measurement of atomic resolution image using Transport of intensity equation". In: Journal of electron microscopy 54.3 (2005), pp.191-197.

[17]. C. Zuo, Q. Chen, W. Qu, A. Asundi, "Noninterferometric single-shot quantitative phase microscopy," Optics Letters 38, 3538-3541 (2013).
[18]. C. Zuo, Q. Chen, H. Li, W. Qu, and A. Asundi, "Boundary-artifact-free phase retrieval with the transport of intensity equation II: applications to micro lens characterization," Optics Express 22, 18310-18324 (2014).
[19]. F. Roddier, Wave front sensing and irradiance transport equation, Applied Optics, 29 no. 10 (1990), 1402–1403.
[20]. T. Gureyev and K. Nugent, Phase retrieval with the transport of intensity equation II: Orthogonal series solution for nonuniform illumination, J. Opt. Soc. Am. A. 13 no. 8 (1996), 1670–1682.
[21]. Laura Waller, Lei Tian, and George Barbastathis. “Transport of Intensity phase- amplitude imaging with higher order intensity derivatives". In: Opt. Express 18.12 (2010), pp.12552-12561. doi: 10.1364/OE.18.012552.
[22]. Laura Waller, Shan Shan Kou, Colin J. R. Sheppard, and George Barbastathis, "Phase from chromatic aberrations," Opt. Express 18, 22817-22825 (2010).
[23]. L. Waller, Y. Luo, S.-Y. Yang, and G. Barbastathis, “Transport of intensity phase imaging in a volume holographic microscope,” Opt. Lett. 35(17), 2961–2963 (2010).
[24]. Chao Zuo, Qian Chen, and Anand Asundi, "Boundary-artifact-free phase retrieval with the transport of intensity equation: fast solution with use of discrete cosine transform," Opt. Express 22, 9220-9244 (2014).
[25]. Aamod Shanker, Lei Tian, Martin Sczyrba, Brid Connolly, Andrew Neureuther, and Laura Waller, "Transport of intensity phase imaging in the presence of curl effects induced by strongly absorbing photomasks," Appl. Opt.**53**,J1-J6(2014).
[26]. Konstantinos Falaggis, Tomasz Kozacki, and Malgorzata Kujawinska, "Optimum plane Selection criteria for single-beam phase retrieval techniques based on the contrast transfer Function," Opt. Lett. 39, 30-33 (2014)