Nuclear dynamics in metastatic cells studied by quantitative phase imaging

Silvia Ceballos[1,3,] Mikhail Kandel[1], Shamira Sridharan[1,2], Freddy Monroy[3], and Gabriel Popescu[1*]

[1]Quantitative Light Imaging Laboratory, Department of Electrical and Computer Engineering, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801,USA.
[2]Department of Bioengineering, University of Illinois at Urbana Champaign, 1304 Springfield Ave., Urbana, IL 61801, USA.
[3]Department of Physics, National University of Colombia, Bogotá, Colombia.

ABSTRACT

We used a new quantitative high spatiotemporal resolution phase imaging tool to explore the nuclear structure and dynamics of individual cells. We used a novel analysis tool to quantify the diffusion outside and inside the nucleus of live cells. We also obtained information about the nuclear spatio temporal mass density in metastatic cells. The results indicate that in the cytoplasm, the intracellular transport is mainly active (direct, deterministic), while inside the nucleus it is both active and passive (diffusive, random). We calculated the standard deviation of velocities in active transport and the diffusion coefficient for passive transport.

Keywords: Quantitative phase imaging, microscopy, biomedical optics, cell dynamics, intracellular transport, diffusion

1. INTRODUCTION

It is known that to understand the behavior of human diseases, one needs to study at cell level. Nowadays scientists from different research fields are converging to unveil the relation between the structural and functional design of cells, how cells respond to environmental challenges, normal processes, such as cell cycle and differentiation, and abnormal processes like neoplastic transformations. However, the interior of a living cell is a complex environment, which is not easy to study[1].

In this regard, some mechanical models have been developed to characterize mechanical responses of living cells[2], and it is possible to observe some relation between cell metabolism and human diseases[3].

It has been shown that cells response to stress, through sensors inside the nucleus[4]. In addition, it is possible to use the morphological changes of cells, to classify neoplastic transformations related with specific diseases[5-6] and it has been established how cell metabolism play an important role in brain function and development[7].

The nucleus is particularly dynamic[8], and in the case of nucleolus, its regulation has a spatio temporal dependence[9]. Therefore, it is necessary to develop new technologies in order to understand the role of intracellular transport in the inner function of the cell[10-14]. Regarding to the internal intracellular transport, some studies had measured the velocity of intracellular transport related with the metabolism in the cytoplasm[15], as well as some characteristics implied in growth and dynamics[12-13,16]. In addition, tomographic techniques have been developed to study biological material[16-17].

*gpopescu@illinois.edu

Quantitative Phase Imaging, edited by Gabriel Popescu, YongKeun Park, Proc. of SPIE Vol. 9336, 93361Q · © 2015 SPIE · CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2183296

Some models of intracellular transport have been developed to research into this topic[18], and it is possible to classify this transport inside cells, in passive diffusion and active transport, which requires chemical energy[19-20].

This study proposes a new technique based on Quantitative Phase Imaging (QPI) to investigate the intracellular transport (active and passive) in living cells. For this, we measured the changes in the refractive index, which are related to the in-plane mass transport of the living cell, without the necessity of tracking individual particles for passive transport[12-13,21].

This method is based on the idea that the measured pathlength fluctuations report on the dry mass transport within the cell[22]. Hence, it is possible to have access to the dry mass density ρ(x,y) which is proportional to the refractive index; this technique is called Dispersion relation Phase Spectroscopy (DPS)[19].

2. METHODOLOGY

To obtain the quantitative phase images we use spatial light interference microscopy (SLIM), a recent optical microscopy technique, capable of measuring nanoscale structures and dynamics in live cells via interferometry[21-23]. SLIM combines two classic ideas in light imaging: Zernike's *phase contrast microscopy*[24-25], which renders high contrast intensity images of transparent specimens, and Gabor's *holography* [26], where the phase information from the object is recorded. Thus, SLIM reveals the intrinsic contrast of cell structures and, in addition, renders quantitative optical path-length maps across the sample. SLIM is implemented as an add-on module to an existing phase contrast microscope[13].

We imaged HeLa cells in culture medium under physiological conditions, 37°C and 5% CO_2 controls. Figure 1a shows an example of such quantitative phase imaging.

Figure 1b illustrates the procedure developed to retrieve the dispersion relation associated with intracellular transport, which is the relationship between the decay rate, Γ, and wave number, q. From the SLIM phase maps, we calculated the dispersion relation, $\Gamma(q_x, q_y)$. Thus, we first perform the spatial Fourier transform of each frame, then we calculate the temporal bandwidth, Γ, at each spatial frequency (q_x, q_y) via the temporal Fourier transform. Then, we azimuthally averaged to obtain the radial function $\Gamma(q)$ (Fig. 1c).

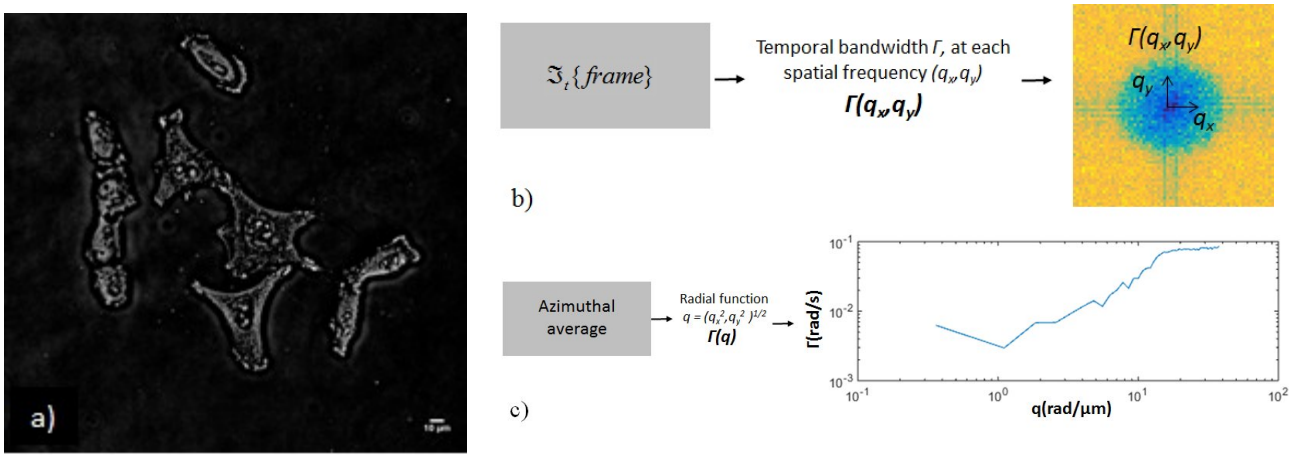

Figure 1. a) Ten HeLa cells studied over 5 minutes. b) Experimental procedure to obtain the dispersion relation $\Gamma(q_x, q_y)$. c) Azimuthal average of $\Gamma(q_x, q_y)$ to obtain $\Gamma(q)$, plotted in log-log axis. By fitting this experimental curve with a q^1 function, it is possible to measure the standard deviation velocity in active transport. Fitting with a q^2 function, gives information about the diffusion coefficient in passive transport.

3. RESULTS

To study the dynamics of intracellular transport in HeLa cells, we acquired SLIM images for a period of 5 minutes, with an acquisition rate of 1 frame every 5 s and a magnification of 40X. We measured DPS over ten HeLa cells and obtained values of velocity for active transport in the cytoplasm and the nucleus. Specifically, the measurements in the cytoplasm show only active transport. The transport in the nucleus shows passive or diffusive transport at high values of wave number, q (fig 2 right). Table 1 shows the standard deviation of velocity and the average diffusion coefficient for ten cells.

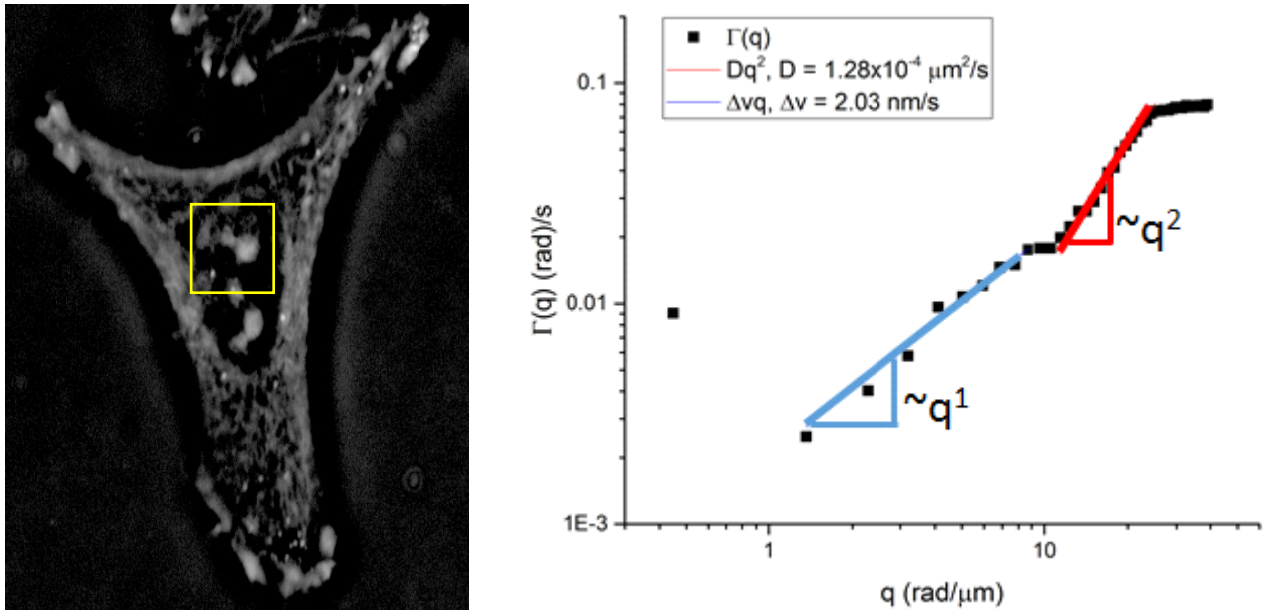

Figure 2. Left: quantitative phase image of HeLa cell (metastatic). Rectangle shows the region of interest chosen to do the measurements of diffusion inside the nucleus. Right: The dispersion relation associated with intracellular transport, represented by the relationship between the decay rate Γ and its wave number q. Linear fitting (low values of q) is related with the velocity distribution of active transport, $\Delta v = 2.03 \pm \pm 2.00\text{x}10^{-1}$ nm/s. Square fitting is related with passive or diffusive transport, the diffusion coefficient measured was $D = 1.28\text{x}10^{-4} \pm 5.80\text{x}10^{-5}$ μm²/s.

Table 1. Average velocity and average diffusion coefficient for ten cells. Cytoplasm shows only active transport. Nucleus shows active and passive transport.

	Cytoplasm	Nucleus	
	v (nm/s)	v (nm/s)	D (μm²/s)
Average in ten cells	$2.33 \pm 2.00\text{x}10^{-1}$	$2.21 \pm 6.82\text{x}10^{-1}$	$1.40\text{x}10^{-04} \pm 5.80\text{x}10^{-5}$

4. CONCLUSIONS

Using SLIM to image live cell dynamics and DPS for analysis, we found that the intracellular transport in the cytoplasm is due mainly to active transport. Inside the nucleus, our results revealed a combination of both active and passive transport. The standard deviation velocity related to active transport, is slightly smaller in the nucleus ($2.21 \pm 6.82\text{x}10^{-1}$ nm/s) than in the cytoplasm ($2.33 \pm 2.00\text{x}10^{-1}$ nm/s). The technique used in this work shows that it is possible to study intracellular transport in living cells without dyes. We anticipate that such studies will reveal new understanding about cell function and disease.

Acknowledgements: Silvia Ceballos is grateful to the Administrative Department of Science, Technology and Innovation of the Government of Colombia (Colciencias, http://www.colciencias.gov.co/) and the National University of Colombia at Bogotá for their support along this study. This research was supported by the National Science Foundation (grants CBET 08-46660 CAREER, CBET-1040462 MRI), Agilent Laboratories and Phi Optic, Inc. For more information, visit http://light.ece.uiuc.edu/.

Competing Interests: Gabriel Popescu has financial interest in Phi Optics, Inc., a company developing quantitative phase imaging technology for materials and life science applications

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