

NANOMETRIC RESOLUTION INTRACELLULAR DYNAMICS STUDY USING QUADRIWAVE LATERAL SHEARING INTERFEROMETRY

Roman Zinchuk^{1,2}, Julien Savatier², Serge Monneret² and Benoit Wattellier¹

¹PHASICS S.A., Espace Technologique, Route de l'Orme des Merisiers, Saint Aubin, France

²Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France
Email: bw@phasics.fr

KEY WORDS: quantitative phase imaging, vesicle tracking, deep-learning

It is now known that interaction between cells and their environment or between intracellular compartments is based on complex vesicular transport processes. The nature and dynamics of such processes are still a hot topic of scientific study.

Progress about intracellular trafficking is currently essentially made by constant innovation in fluorescence-based techniques, now reaching single molecule resolution in living cells. In this case, fluorophore photobleaching is an issue for long term studies (over a few tens of minutes). Quantitative phase imaging techniques are conventionally used to create long-term high contrast imaging of cells, label-free.

Last year we obtained results having a localization resolution between 40 and 50 nm using a standard wave front sensor based on QWLSI. This was obtained on fixed cells, assumed not to move at all. We upgraded our set-up with our improved wave front sensing technique called QWLSI-HD (HD for High Definition), where we could multiply by 9 the number of measurement points per image and therefore decrease the phase image pixel size from $19.5 \mu\text{m}$ down to $6.5 \mu\text{m}$ [1,2]. Using this technique, we expected to reach the 1-nm resolution limit, like it was reached by other techniques such as interferometric scattering microscopy (iSCAT [3]). In addition, we worked on adding specificity to quantitative phase imaging. We trained Artificial Intelligence algorithms (based both on deep and machine learning) to recognize intracellular vesicles: mitochondria, lysosomes and endosomes. We used fluorescence imaging to train the AI algorithms. We compared performances between the algorithms and also between fixed and living samples. We conclude that training made on fixed samples does not efficiently apply to living samples.

[1] P. Bon, G. Maucort, B. Wattellier, S. Monneret, "Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells," *Optics Express* 17 (15), 13080-13094 (2009).

[2] P. Bon, J. Savatier, M. Merlin, B. Wattellier, S. Monneret, "Optical detection and measurement of living cell morphometric features with single-shot quantitative phase microscopy," *J. Biomed. Opt.* 17 (7), 076004 (2012).

[3] Kukura, Philipp; Ewers, Helge; Müller, Christian; Renn, Alois; Helenius, Ari; Sandoghdar, Vahid (2009-11-01). "High-speed nanoscopic tracking of the position and orientation of a single virus". *Nature Methods*. 6 (12): 923–927. doi:10.1038/nmeth.1395. ISSN 1548-7091. PMID 19881510