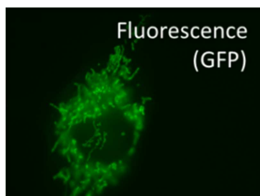
SID4bio

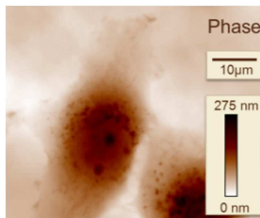
QUANTITATIVE PHASE & FLUORESCENCE COUPLING

LYSOSOME IDENTIFICATION

Phasics offers a new **quantitative phase imaging** technique for optical microscopy to observe **living cells without marking** and to run **accurate statistical analysis** on their evolution (migration, growth, intracellular processes …). This simple **plug-and-play** camera relies on a patented technology, the quadri-wave lateral shearing interferometry[1], which measures directly the phase of the light transmitted through any biological specimen.

The fundamental advantage of this technique is to provide a **great contrast enhancement** in conventional optical microscopy that enables cells observation. Furthermore the Phasics technique offers **quantitative information** about the specimen by directly measuring how one of the light properties, the phase, is changed when going through the specimen.





1.Fluoresence image of mitochondria (GFP)[2]
2. Phasics Image[2]

As compared to fluorescence technique (figure 1), the Phasics technique (figure 2) has the valuable advantage of **not using any marker**. Consequently there is no need for a specific specimen preparation, which makes this technique easy to use and **non-invasive**. In addition, the provided information is **reliable and quantitative** because it is based on the specimen intrinsic properties and not on the marker ability to stain and emit. Finally, the Phasics technique gives a **complete** view of the cells: all the structures appear even though not stained, which leads to a better understanding of the specimen and its interactions.

However, in some cases, the combination with fluorescence images can be of interest. In this study, it was used for locating and measuring the specific phase signature of lysosomes. The study was realized on a wild type COS-7 cell line. The Phasics device was plugged on a conventional bright-filed microscope equipped with its halogen source. A near-infrared filter was placed just after the source to make possible the coupling between phase and fluorescence techniques.

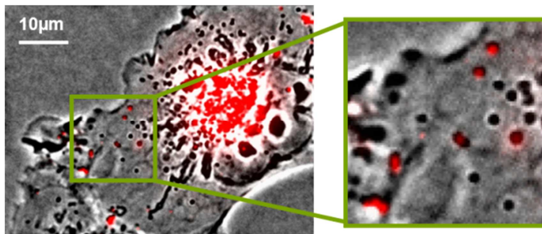
"LYSOSOME PHASE SIGNATURE IS ASSESSED BY COUPLING PHASE AND FLUORESCENCE"

With the **enhanced contrast** provided by the Phasics technology all the elements of the cell are distinguishable. Unfortunately, as most vesicles have a round shape, the lysosomes

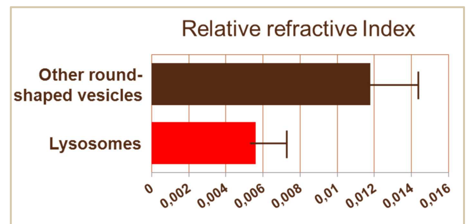
cannot be discriminated from the other vesicles by a simple observation. Further analysis need to be carried on, based on the measured phase value that is an indication of the vesicles density. More precisely, the phase value measured with the Phasics technology is proportional to the thickness of the element and to its refractive index relative to the cytoplasm index[1]. When assuming that all the round-shaped vesicles are spheres, it is possible to retrieve the relative index of these elements.

In order to investigate the relative refractive index value that relates to lysosomes, the Phasics image is combined to an usual fluorescence image in which only the lysosomes are marked by a specific fluorescent labelling (figure 3). **Coupling** those two techniques is feasible as the Phasics sensor is a **plug-and-play** camera that does not involve any change in the light path of the microscope. Also, since the technique is **achromatic**, it works with any filtered light. Consequently it adapts easily to a wavelength that will **not disturb the fluorescence** image. A simultaneous acquisition is then possible.

The statistical analysis[3] of the coupled images showed that the relative refractive index is different for the lysosomes as compared to other vesicles of the same shape (figure 4). Therefore **the lysosomes are differentiated** from the other vesicles **thanks to the quantitative information contained in the Phasics image**.



3. On the coupled phase-fluorescence image[2], the lysosomes appear in red (RFP marking) when the other vesicles stay gray.



4. The relative refractive index of lysosome is different from the round shaped vesicles[2].

"THE PHASE IMAGE WITH NO MARKER BECOMES SUFFICIENT"

By overlaying the fluorescence image and the Phasics image, it was proved that lysosomes introduce a **specific quantitative value** that can be measured with the Phasics technique. Thus **the phase image is enough to observe lysosomes.** With this very simple marker-free technique, lysosomes are studied in the most authentic conditions. Furthermore, it enables **time lapse imaging of lysosomes** in these same conditions preventing a photo-bleaching issue.

REFERENCES

[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] On the courtesy of J. Savatier, S. Monneret and P. Bon from Institut Fresnel – CNRS UMR 7249

[3] J. Savatier et al, "Correlative microscopy of living cells between fluorescence and quantitative phase imaging with a high resolution wavefront sensor", Biophysical Society 55th Annual Meeting, Baltimore: USA (2011)