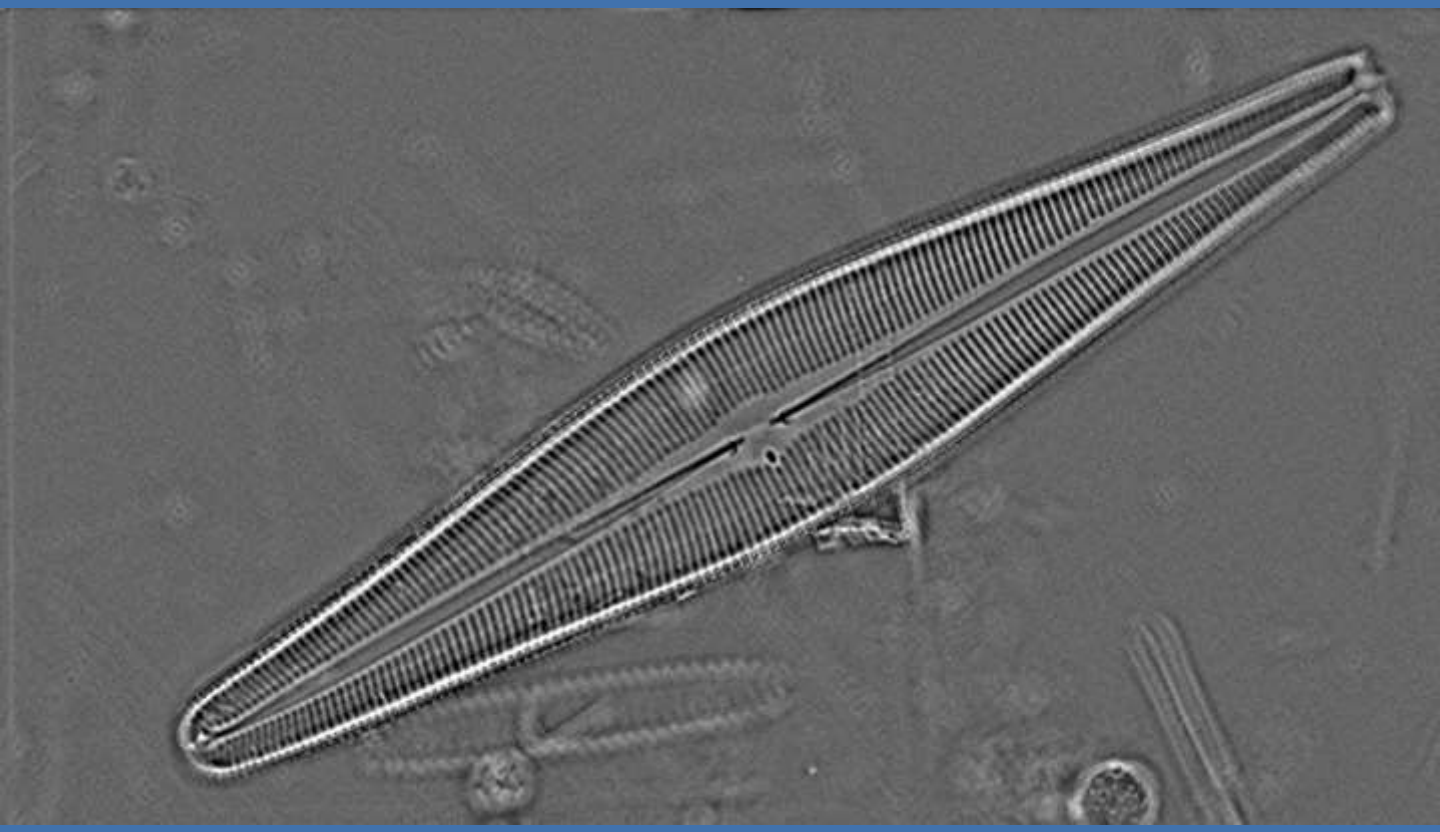
leiða TECHNOLOGIES

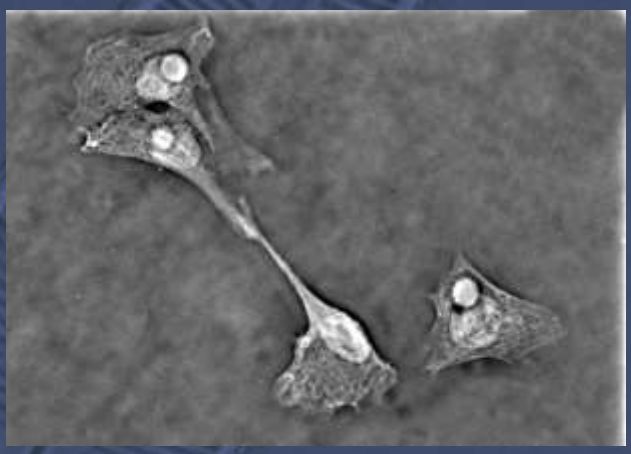
Phase Imaging



leida Technologies is reinventing microscopy

PHASE IMAGING

Phase imaging unveils delays that the light waves are subjected to when passing through samples. This techniques allows the observation of transparent objects without any staining needed (dye, fluorochrome, metalization)



Brightfield observation (left) and phase image obtained by numerical reconstruction (right) of mice myoblasts. This sample was kindly prepared by the LMGP lab in Grenoble.

Firstly used for the microscopic observation of sample, phase imaging is now useful for the measure of refractive index and thickness, as well as for the analysis of the modifications than an object (cell, bacteria) can undergo depending on time or environmental changes.

There are several phase imaging techniques, such as Zernicke phase contrast, differential phase contrast (DIC or Nomarski), Hoffman modulation contrast, Dodt gradient contrast… These techniques have both benefits and drawbacks (specific and expensive equipment, complex settings, loss of resolution and brightness, incompatibility with some plastics, complexity of the switch from a traditional technique to a phase technique…)

By associating the lightning structuring abilities of our µLight sources with powerful image processing algorithms, we offer a set of phase imaging methods within a single system.

Better see the invisible to better understand

µLight for phase imaging

µLight is a light source that can allows the definition of illumination patterns (colour, intensity position). By applying a specific lightning structure, we can choose to highlight some optical properties of an object. Numerical processing are then applied to take into account this structure, to make the best use of all the available information and provide high quality images

µLight source

Differential phase contrast observation of a marrow pollen.
J.P. Fayol – Laboratoire de Contrôle des Pollens – 38 St. Lattier

Possible phase techniques with µLight are :

- Zernicke phase contrast (in association with objectives with phase rings)
- anomalous phase contrast (phase contrast with brightfield objectives)
- relief contrast
- differential phase contrast
- phase imaging
- inline digital holography

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Methods combination

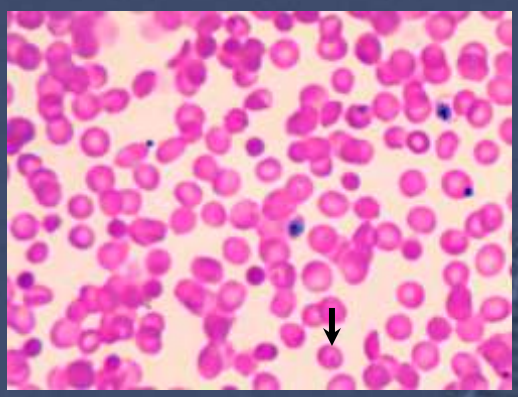
Switching from a microscopy observation method to another can lead to faults :

- defocus
- shift of the observed field
- brightness change

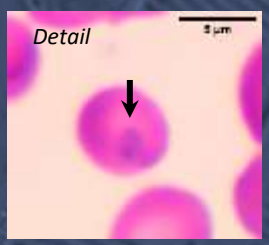
With µLight, a switch between methods does not involve any optical or mechanical component movement: no vibration, possibility to use preset settings, automatic transitions.

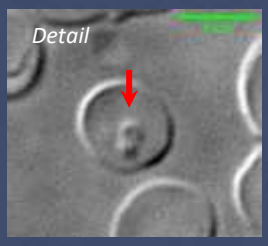
The combination of information from different observation methods is thus facilitated.

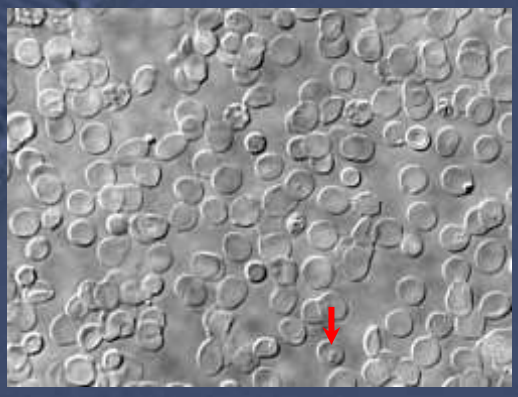
Example: images of red blood cells, some of which contain malaria parasite. On the color image, some parasite are difficult to spot (arrow). Phase images allow the easy detection of parasites, which is confirmed by the brightfield image.



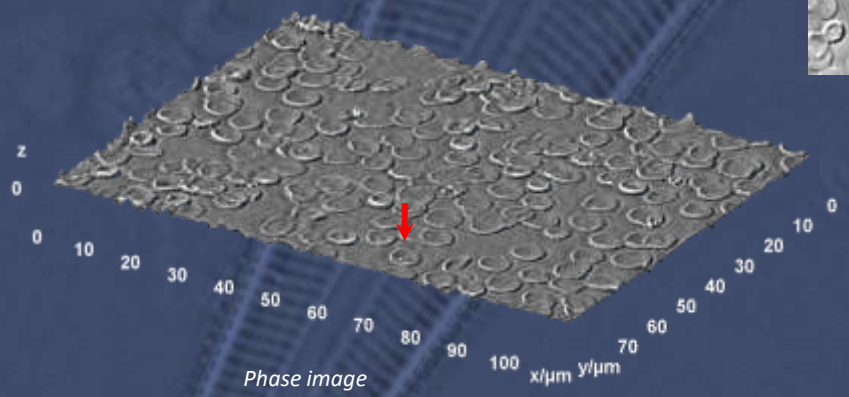
Color brightfield image



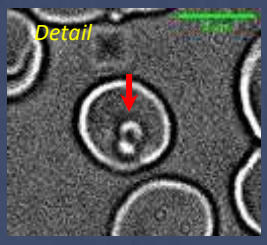




Differential phase contrast



Phase image



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Differential phase contrast

Differential Phase Contrast microscopy is an old technique but complex to implement with traditional microscopes, it becomes straightforward combining a µLight source, a digital camera and the OQaPI software.

Composite color image of an Althea pollen in differential phase contrast. Detail : brightfield image.

Obtained images are equivalent to those provided by the Differential Interference phase Contrast (DIC), with the following advantages:

- ❖ Economical solution
- ❖ Very few settings
- ❖ no directional effects
- ❖ compatible with PMMA plates

SE-DPC (Single Exposure Differential Phase Contrast)

- ❖ Possible for weakly absorbing objects, this mode allows the tracking of fast temporal variations.

Better see the details to better understand

Image of the phase

Bump or hollow ?

Differential phase contrast (Fig. 1), as the DIC, provides an image which is likened to the derivative of the phase (see Fig. 2). Without knowledge about the object structure, one can not know if we observe a bump or a hollow (ex : a hole or a speck of dust).

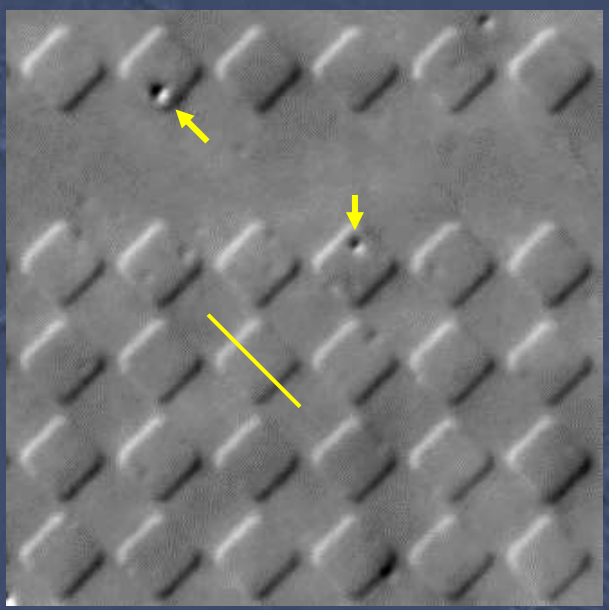

Fig. 1 : DPC of a lattice of engraved glass.

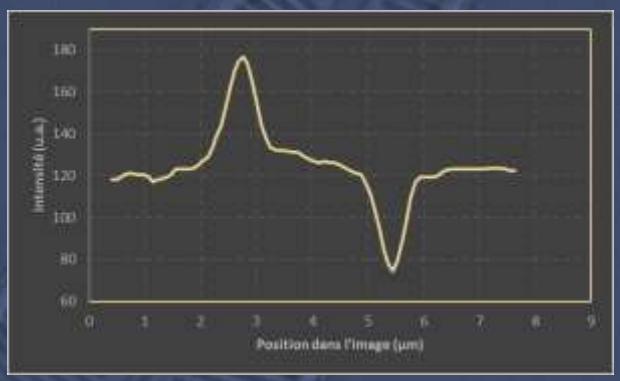

Fig. 2 : Profile taken from Fig. 1.

By numerical process done with the OQaPI software, we get the image which is faithful to the exact structure of the object. (Fig. 3).

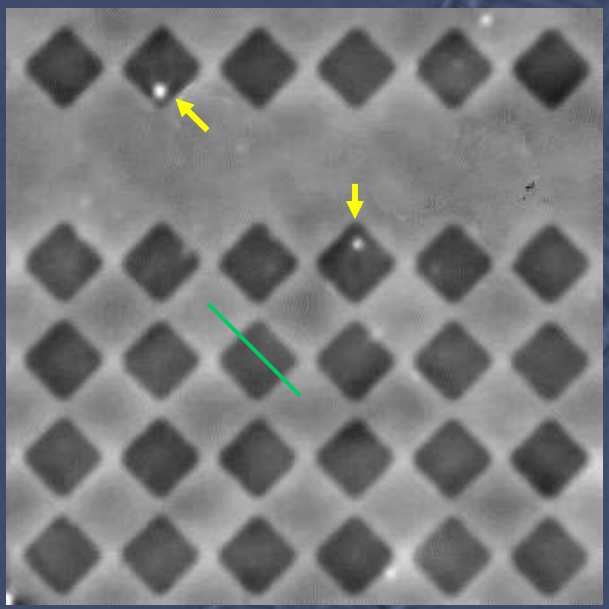

Fig. 3 : Phase image of the lattice.

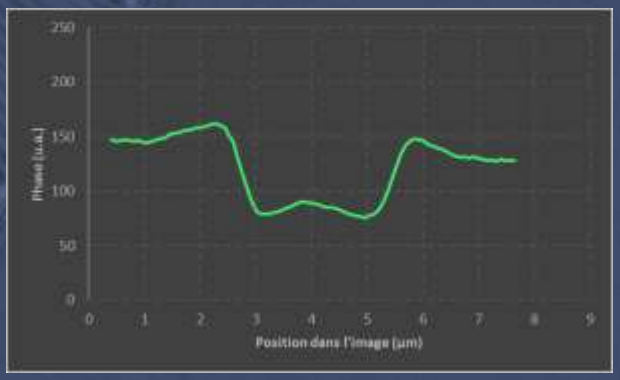

Fig. 4 : Profile taken from Fig.3.

Here, the squared pattern is composed of hollows, and the bright spots are specks of dust.

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Mastered phase for enhanced imaging …

Usually, one separate absorption images provided by brightfield microscopy from phase images. But optical instruments are not perfect and a bit of the information from one mix with the other.

Using the algorithms we develop, we go get this lost information to replace it in their image of origin.

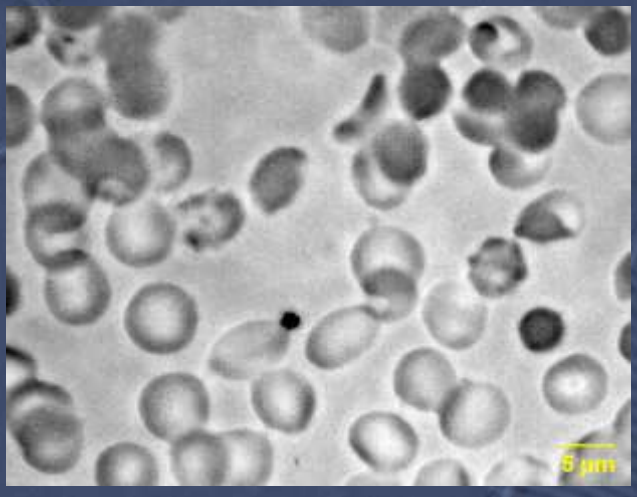

Fig. 2: Direct brightfield image.

The quality of the brighfield image of a smear is enhanced (bottom) and reveal details very hard to discern in a direct image (left).

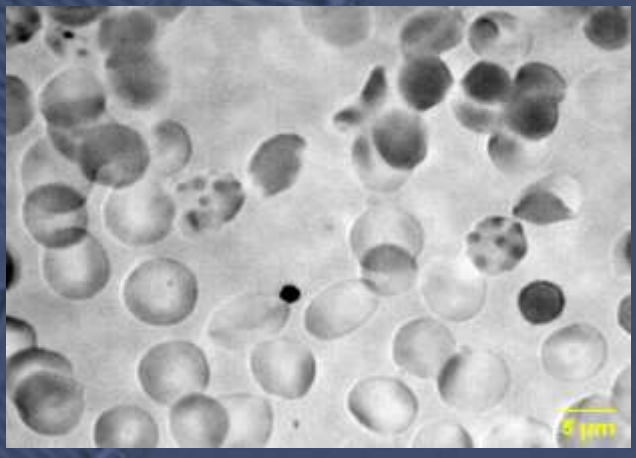

Fig. 1: Corrected brightfield image.

The interest of this method of image enhancement is illustrated below:

- The enhancement of the images quality after correction allows to identify a parasite in a red blood cell with a better reliability. The visual analysis in images is confirmed by measure (plot)

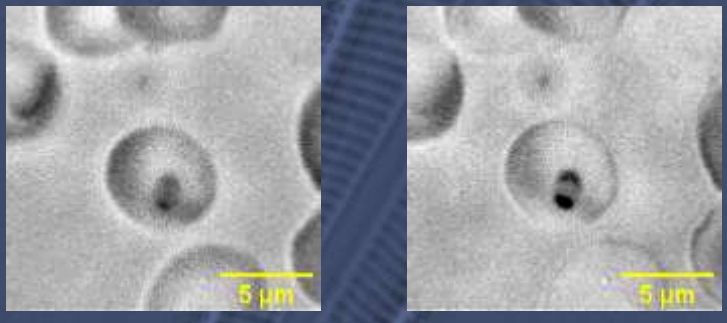

Fig. 3: Red blood cell parasitized by Malaria. Direct brightfield image (left) and corrected image (right).

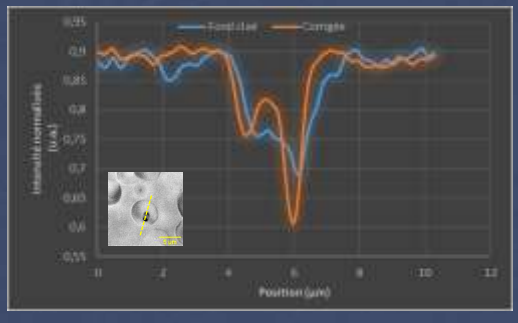

Fig. 4: Intensity profile for both images (see insert).

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OQaPI : integrated software

OQaPI is our software allowing to process numerically the images by using algorithms specially developed.

OQaPI can work by itself but also in association with image processing softwares (for example ImageJ).

The association with ImageJ allows to work in a functional, powerful and widely used environment for microscopy (ImageJ is a free software).

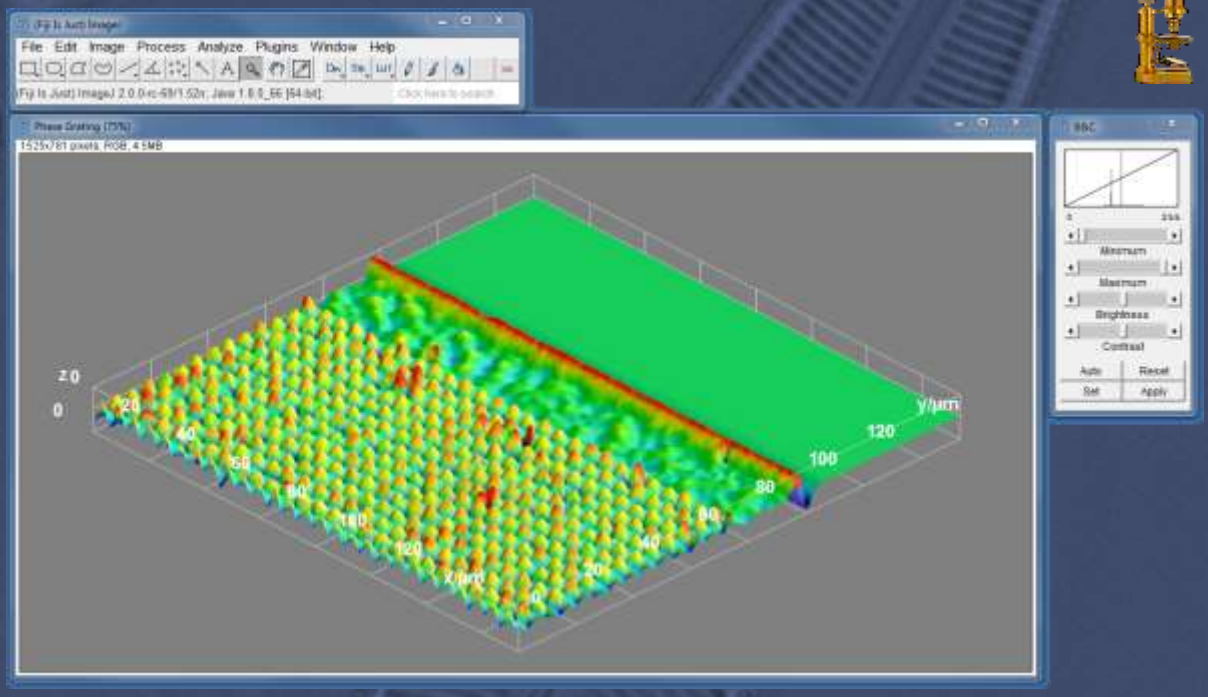

Fig. 1 : Surface image of the phase of a lattice composed of bowls engraved in glass (80 nm deep, 4 x 4 µm side). The phase reconstruction has been performed with OQaPI.

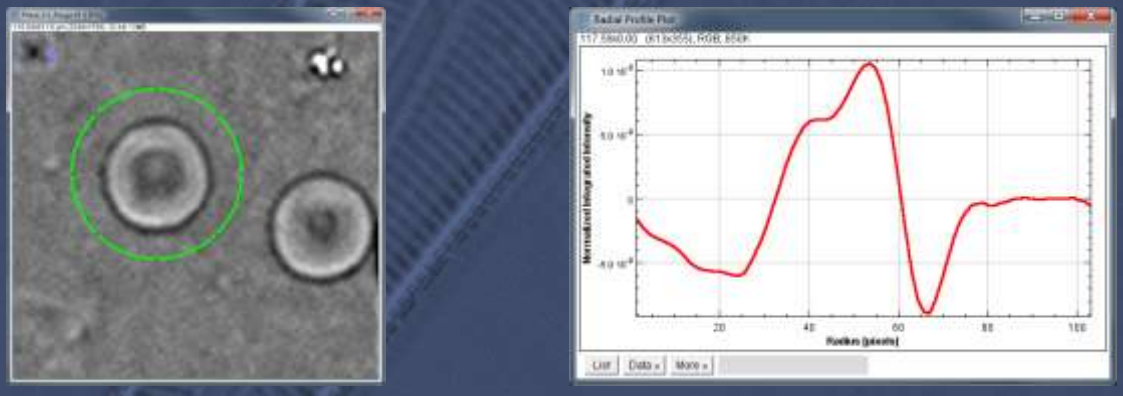

Fig. 2 : Phase image of red blood cells reconstructed with OQaPI (left), and radial intensity profile (right) performed in the ImageJ environment.

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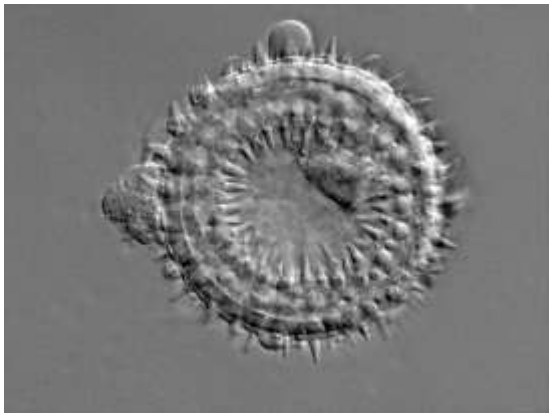
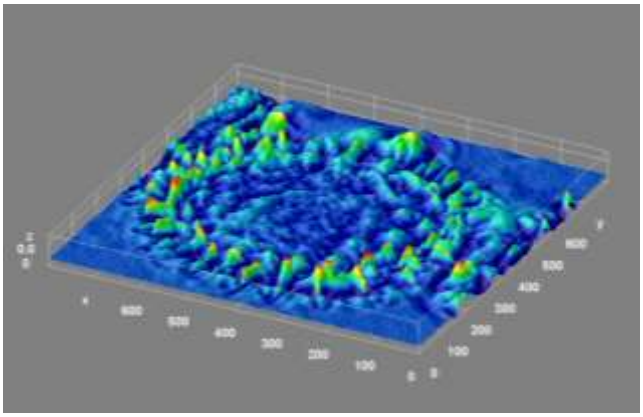
- leiða Technologies is a company based in Isère (France) and developing innovative solutions for microscopy.
- OQaPI is an image processing software which works with images obtained with a structured µLight device. This software provide:
  - Differential phase contrast images (see page 3),
  - Corrected brigthfield images (see page 7),
  - Phase images (see page 2).
- µLight is a light source for transmission microscopy allowing to give structure to the illumination. By shaping patterns controlled in intensity and colour, µLight makes use of the properties of optics providing access to different phase imaging techniques (please contact us for further information).

OQaPI

- Independent use: images are processed by OQaPI and can be loaded in an image processing software.
- Use with ImageJ: OQaPI is ran from ImageJ and the processed images are imported automatically.
- Specifications:
  - Operating system : Windows 7 and 10 (32 et 64 bit)
  - Disk space : 7 Mo.
  - Memory capacity : 2 Go





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