SIMULTANEOUS IMAGING OF PHASE AND BIREFRINGENT SPECIMEN WITH NEW PHASE/POL CONTRAST MODALITY

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KEY WORDS: interference, polarization, interference colors, Newton colors, vector interference colors, phase microscopy, polarized light microscopy, polychromatic light

Interference of two combined white light beams produces Newton colors if one of the beams is retarded relative to the other by from 400 nm to 2000 nm. If the retardance is below 400nm the classical two-beam interference produces grey shades only. Therefore, cells and tissues under a regular polarization microscope are seen as grey image, which contrast disappears at certain orientations. Recently we proposed a polychromatic polarization microscope, which employs vector interference of polarized light [1, 2]. The polychromatic polscope generates the full spectrum colors at retardance of several nanometers, which was not possible before. The image hue is determined by orientation of the birefringent structure. The previously colorless birefringent images of organelles, cells, and tissues become vividly colored.

The combined phase/pol contrast microscope can be created by a simple modification of the polychromatic polscope. The new setup employs polychromatic polarization optics, a phase contrast objective in the imaging path and annulus in the illumination path. This combination allows to see the cell morphology (phase) and structural anisotropy (birefringence) in one image, simultaneously. The image brightness shows the dry mass distribution and the color depicts the molecular orientation.

Polarized light microscopy reveals malarial pigment, hemozoin, which is a crystalline product of the digestion of hemoglobin by the parasites. By imaging hemozoin directly, it does not require exogenous contrast agents to diagnose malaria. We examined blood smears of Plasmodium yoelii infected mice with and without phase contrast. As one can see (Fig. 1), the colored brilliantly birefringent granules of the hemozoin are clear visible. The small hemozoin granules are surrounded by large brownish red blood cells. In a fresh sample, the granules freely rotate and change the color according to their optical axis orientation. The hemozoin appears as appealing Christmas lights that are blinking in changing colors.

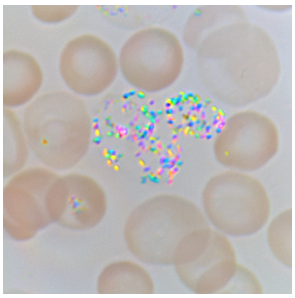
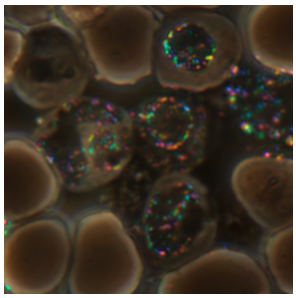
 (a)  (b)

Figure 1: Images of blood smears of Plasmodium yoelii infected mice with polychromatic polscope (a) and with phase/pol contrast microscope (b). The image size is 26μm x 26μm.

[1] M. Shribak, "Polychromatic polarization microscope: bringing colors to a colorless world", *Nature/ Scientific Reports*, **5**, 17340 (2015).
[2] NIH Director's Blog: https://directorsblog.nih.gov/2017/04/27/snapshots-of-life-neurons-in-a-new-light/