Fast and sensitive screening of biogenic composite materials using polarized light imaging

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Biogenic composite materials increasingly attract research interest because of their extraordinary material properties and their potential for use in engineering and biomedical applications. Natural biomineralization processes are able to deposit manifold and elaborate mineral structures at ambient temperatures, which serve diverse purposes, such as mechanical support, protection and in navigation [1], [2]. Our long-term goal is to non-invasively study the controlled biomineralization in organisms in vivo [3].

Polarized light microscopy provides an excellent technique for analyzing biominerals due to its ability to measure sub-microscopic molecular structures dynamically and non-destructively in samples under their native environmental conditions. Specific structures are contrasted owing to their intrinsic anisotropic properties without any need for labeling agents such as fluorescent markers. Polarized light imaging, which has already been applied for biological specimens by W. Schmidt since 1924 [4], has recently been improved with respect to quantification and image acquisition and is now commercially available with the LC-PolScope (Abrio IM™ Imaging System, [5]).

So far, only few studies address the quantification of retardance and azimuth values relevant to structural molecules of complex specimens [6]. Biological materials reaching from the nanometre to the millimetre scale show a huge variability in their refractive indices. In order to fully exploit the potential of the LC-PolScope technique, we analyzed biominerals (Figure 1) as well as purely organic biogenic structures (Figure 2) from agricultural and model plants with respect to retardance and orientation of their optically active components. We quantified the birefringence of the various biological materials as a function of the refractive indices of various embedding media. Non-biogenically precipitated calcium minerals of variable shape and size are investigated for possible use as a standard reference. Our findings show that, in addition to being suitable for live cell imaging, the LC-PolScope Abrio Imaging System is applicable as a fast and sensitive screening method for biological composite materials. Combined with conventional live cell fluorescence imaging, LC-PolScope microscopy has the potential to make biomineralization processes visible in vivo.

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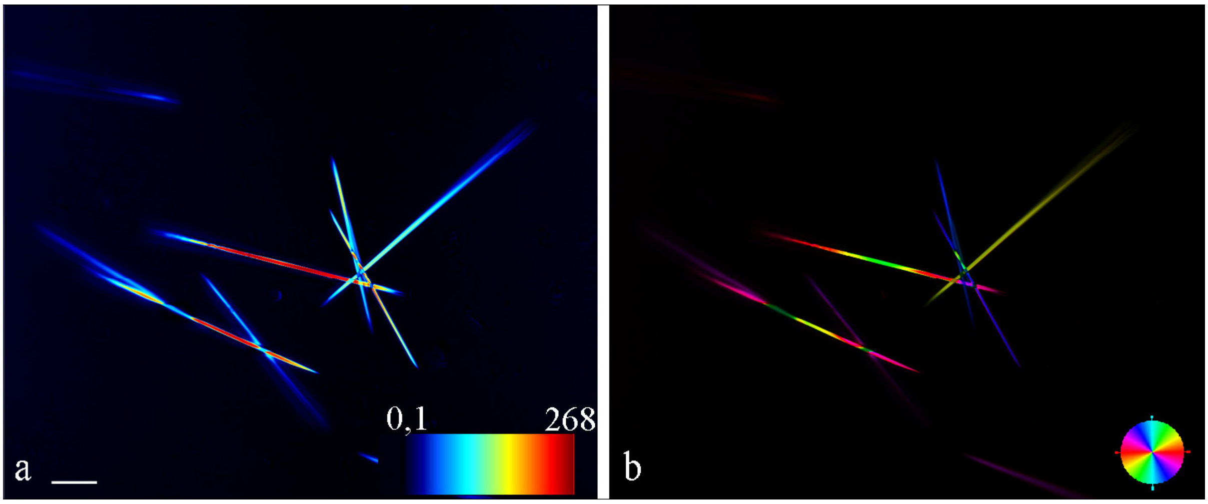

Figure 1. Polarized light micrographs of biogenic calcium oxalate crystals from *Actinidia sp.* (a) Polarized image showing intensity values with the retardance ranging from 268nm (red) to the minimum, i.e. an isotropic background, in black. (b) Orientation of birefringent constituents of an object as encoded by colours. bar: 20µm.

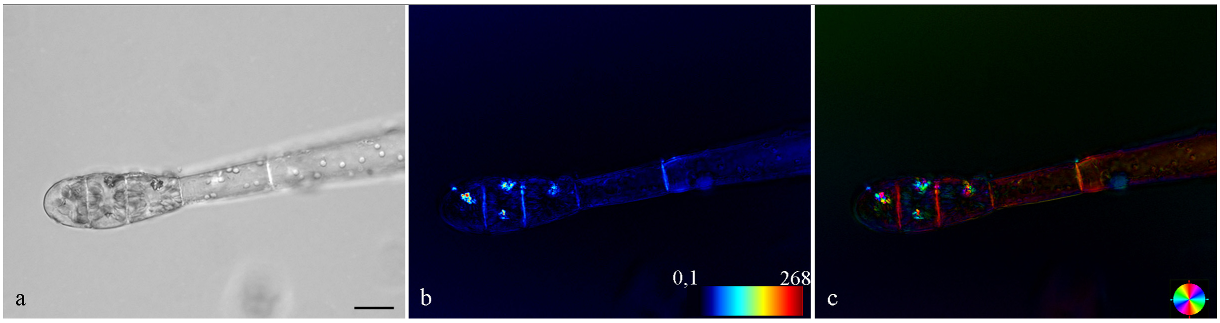

Figure 2. Polarized light micrographs of a *Nicotiana benthamiana* trichome with calcium crystals. (a) Bright field image (b) Polarized image representing the intensity of birefringence. The retardance range was set to 268nm as indicated in red. (c) Orientation of birefringent constituents of the plant tissue as indicated by colours. bar: 20µm.

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