IMAGING BIREFRINGENCE IN REAL TIME

Michael Shribak[@], Grant B. Harris[@], Rudolf Oldenbourg[@], and Cliff Hoyt*
[@]Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA
***Cambridge Research and Instrumentation, Inc., Woburn, MA 01801, USA**

KEY WORDS: Birefringence, retardance, polarization, liquid crystal, live cell imaging

Building on the high-sensitivity, orientation-independent birefringence imaging technology of the Pol-Scope [1] (commercially available as the LC-PolScope, http://www.cri-inc.com), we are developing two new systems to provide retardance and orientation images of a specimen in real-time. One system uses a 2-frame, sequential acquisition approach, using fast liquid crystal (LC) retarders; the other system uses a multi-path, concurrent acquisition approach.

The currently available PolScope system sequentially acquires four or five image frames with different elliptical polarization states and processes them to obtain retardance and orientation images at a rate of about 2 frames per second. To speed up the acquisition and processing time we reduced to 2 the number of sequential images required and developed a computational technique based on the last two frames acquired [2]. The display rate of retardance images depends on the exposure time and image size, and is limited by the speed with which the LCs can change state. Our current system can produce a 'real-time' stream of retardance images at approx. 20 frames per second for an 8-bit, 640 x 512 pixel image. The picture on the left shows a retardance image of three protists *Vorticella* with their contracting and expanding stalks captured with this 2-frame approach.

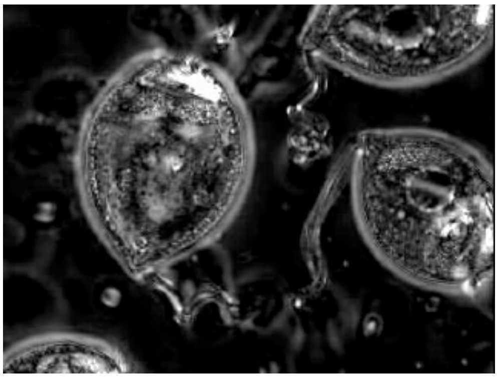

Fig.1: Retardance image of *Vorticella* recorded with 2-frame technique

The second system uses a multi-path approach. Using a set of beam splitters, the image beam is split into multiple paths, each of which passes through a corresponding elliptical (or circular) analyzer. Each analyzer can consist of a linear analyzer and a waveplate with fixed retardation or a liquid crystal variable retarder, where the azimuths of the waveplates and linear polarizers can be adjusted in order to change parameters of the eigenpolarizations. All of the paths are concurrently imaged with one or more cameras. Using the multi-path approach with pulsed illumination, the measurement time would equal the pulse duration, which can be as low as few nanoseconds. We have developed special algorithms for image calculation and corrections for polarization imperfections of the device.

[1] R.Oldenbourg, "A new view on polarization microscopy", *Nature*, **381**, 811-812 (1996).

[2] M.Shribak, R.Oldenbourg, "Techniques for fast and sensitive measurements of two-dimensional birefringence distributions", *Applied Optics*, **42**, 3009-3017 (2003).