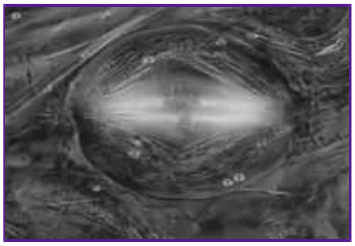
Comment 2: Further Remarks on Polarized Light Microscopy

New approaches in microscopy enable further in vivo analysis of mitotic mechanisms directly in living cells. Among others, they include the use of RNAi to selectively eliminate production of specific proteins; diverse modes of fluorescence microscopy, including localized photo-activation of fluorochromes (Mitchison 1989); two-photon microscopy (Denk et al. 1990); and fluorescent speckle microscopy (Waterman-Storer & Salmon 1998).

Approaches developed recently in our MBL Program include: LC-Pol microscopy, a highly sensitive method which concurrently measures very weak birefringence and maps slow axis orientation over the whole field of view independent of specimen orientation (Oldenbourg 1996, 2007; Fig. 1; Movie 5 courtesy of Jim LaFountain); the centrifuge polarizing microscope which allows polarization, DIC, and green-light-excited fluorescence microscopy of specimen in centrifugal fields up to 10,500 times Earth's gravitational field (Inoué et al. 2001a, b; Fig. 2); and combined orientation-independent DIC and LC-Pol microscopy which allows concurrent measurement of organelle dry mass and birefringence without staining or other chemical treatment (Shribak & Inoué 2006; Shribak et al. 2007; Fig. 3).

Movie



MOVIE 5. Birefringence of crane fly spermatocytes in meiosis-I recorded with LC-PolScope (Oldenbourg 1996). Distribution and activities of the birefringent spindle microtubules and other organelles are captured at high resolution. In the un-flattened cell shown in the first sequence, the optical section in focus shows two pairs of autosomes and the two late-separating sex chromosomes together with their chromosomal spindle fibers. In the flattened cell shown in the second sequence, all four autosomes and their chromosomal spindle fibers are visible, but the spindle becomes distorted in anaphase due to extreme compression of the cell. As anaphase progresses, strongly birefringent mitochondria form a sheath around the spindle. Scattered, lipid-containing "dyctiosomes" are also strongly birefringent (cf. text Fig. 6). (Unpublished movie courtesy of Jim LaFountain of the University at Buffalo.)

FIGURES

Figure 1. Crane fly spermatocyte undergoing meiosis-I, viewed with the LC-PolScope. Note how the birefringent spindles all appear equally bright regardless of orientation. Image brightness measures birefringence retardance;

distribution of slow axis orientations can be superimposed. (Unpublished figure courtesy of Rudolf Oldenbourg, MBL, and Jim LaFountain, University at Buffalo.)

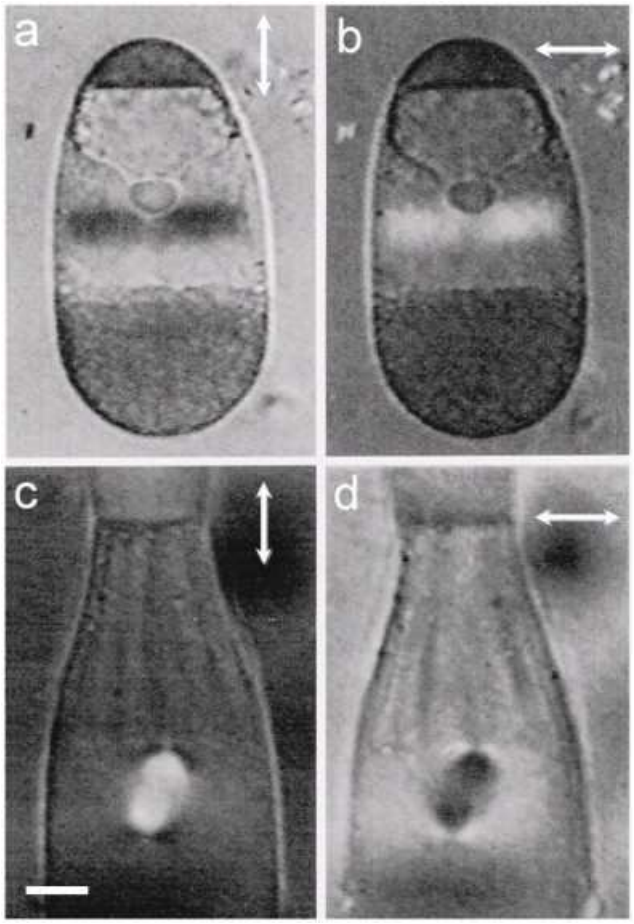


Figure 2. *Chaetopterus* oocyte observed during centrifugation with the centrifuge polarizing microscope. Top panels: stratified oocyte in calcium-free seawater before activation. Within the cell, from top (centripetal) to bottom (centrifugal), one sees: oil cap, gigantic germinal vesicle (= nucleus of inactivated oocyte) with heavy nucleolus protruding below, negatively birefringent layer of vertically aligned membranes, clear cytoplasmic zone, discreet mitochondrial layer, and yolk layer. Bottom panels: after activation by exposure to Ca^{2+} in normal seawater. Germinal vesicle and layered membranes have broken down, and positively birefringent meiosis-I spindle has appeared in the clear zone. The cell has become less rigid and more deformed by the centrifugal field. Double arrows indicate slow axis direction of compensator. Scale bar: 20 µm. (Inoué 1999.)

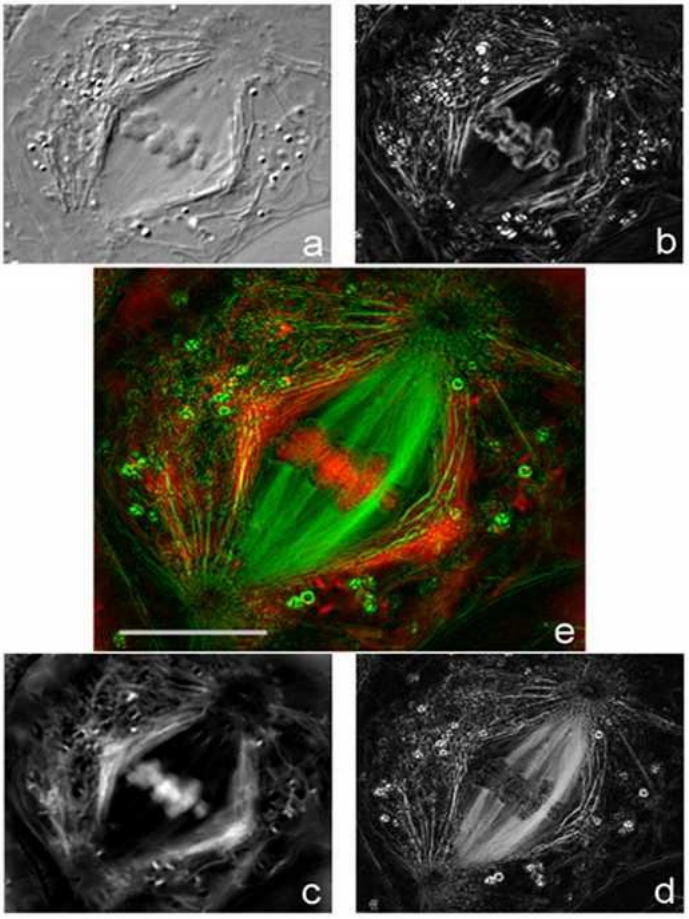


Figure 3. Live, unstained crane fly spermatocyte displayed in different modes with orientation-independent DIC system. (a) conventional DIC, (b) phase gradient image, (c) dry mass distribution, (d) LC-Pol image, (e) red pseudocolored image of c combined with green pseudocolored image of d. Scale bar: 10 µm. (Reprinted from Shribak et al. 2007 courtesy of the Microscopy Society of America and Cambridge University Press.)

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