New Frontiers in Polarized Light Microscopy for Live Cell Imaging

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The Mantis Shrimp can detect linear and circular polarization, and has 12 different types of photoreceptors for color vision from the UV to the infrared (Wikipedia)

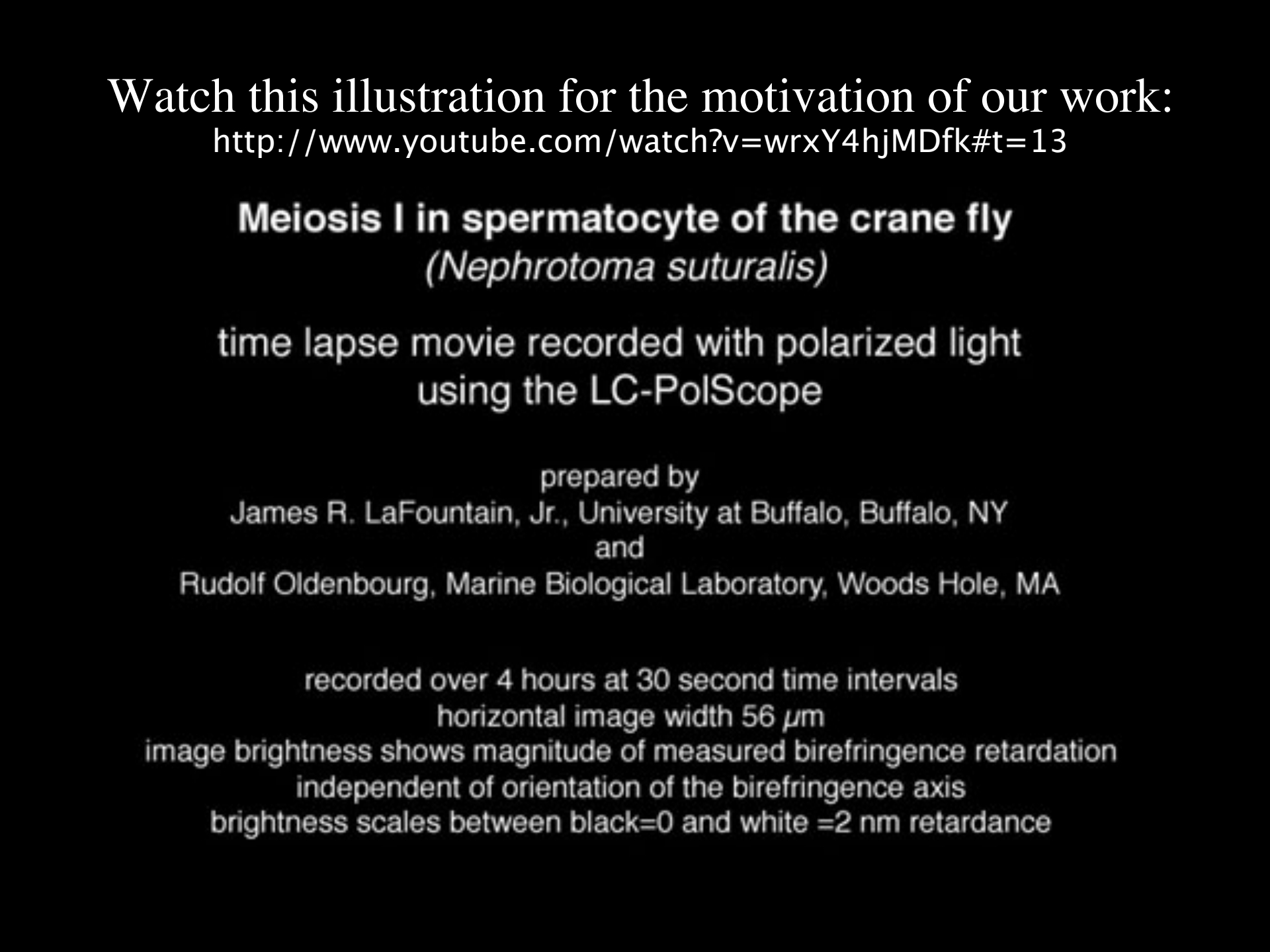
Polarization sensitive eyes

Cuttlefish

Monarch butterfly

but, alas, not the human eye

Watch this illustration for the motivation of our work:
http://www.youtube.com/watch?v=wrxY4hjMDfk#t=13



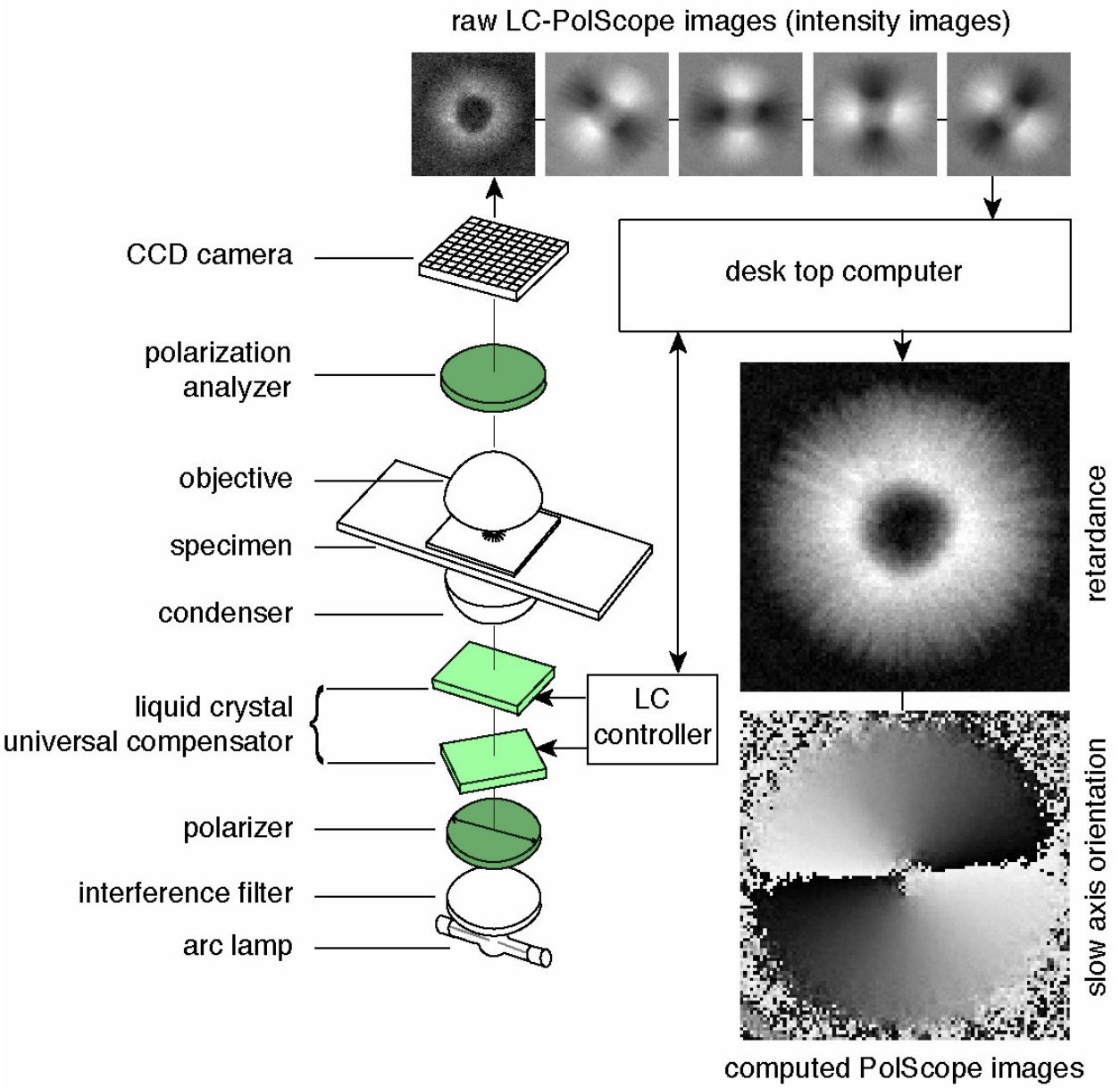
Measuring/Imaging Material Properties

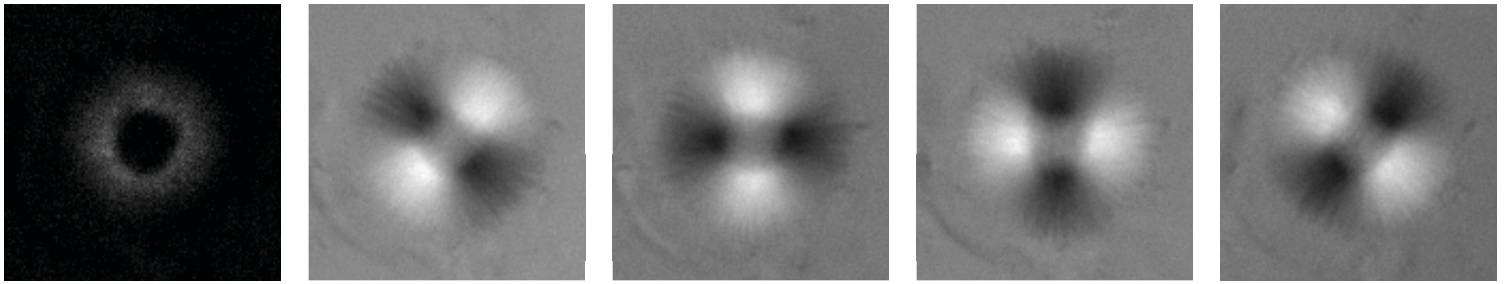
- birefringence (discussed here)

also:

- diattenuation/dichroism
- polarized fluorescence

Birefringence Imaging with the LC-PolScope

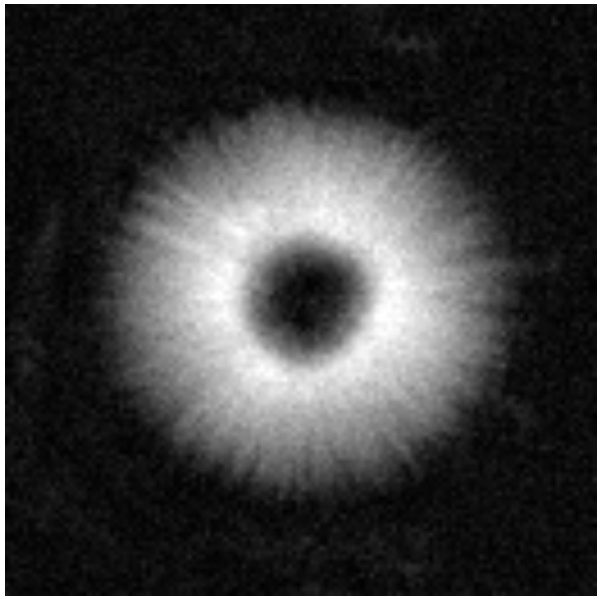




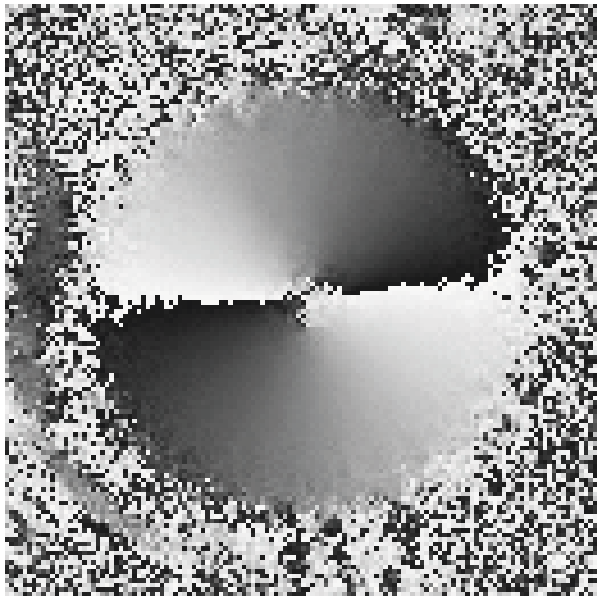
I_1 I_2 I_3 I_4 I_5

$$\sqrt{\frac{(I_4 - I_3)^2 + (I_5 - I_2)^2}{(I_1 + I_2 + I_3 + I_4 - 4I_0)^2}}$$

$$\arctan\left(\frac{I_4 - I_3}{I_5 - I_2}\right)$$

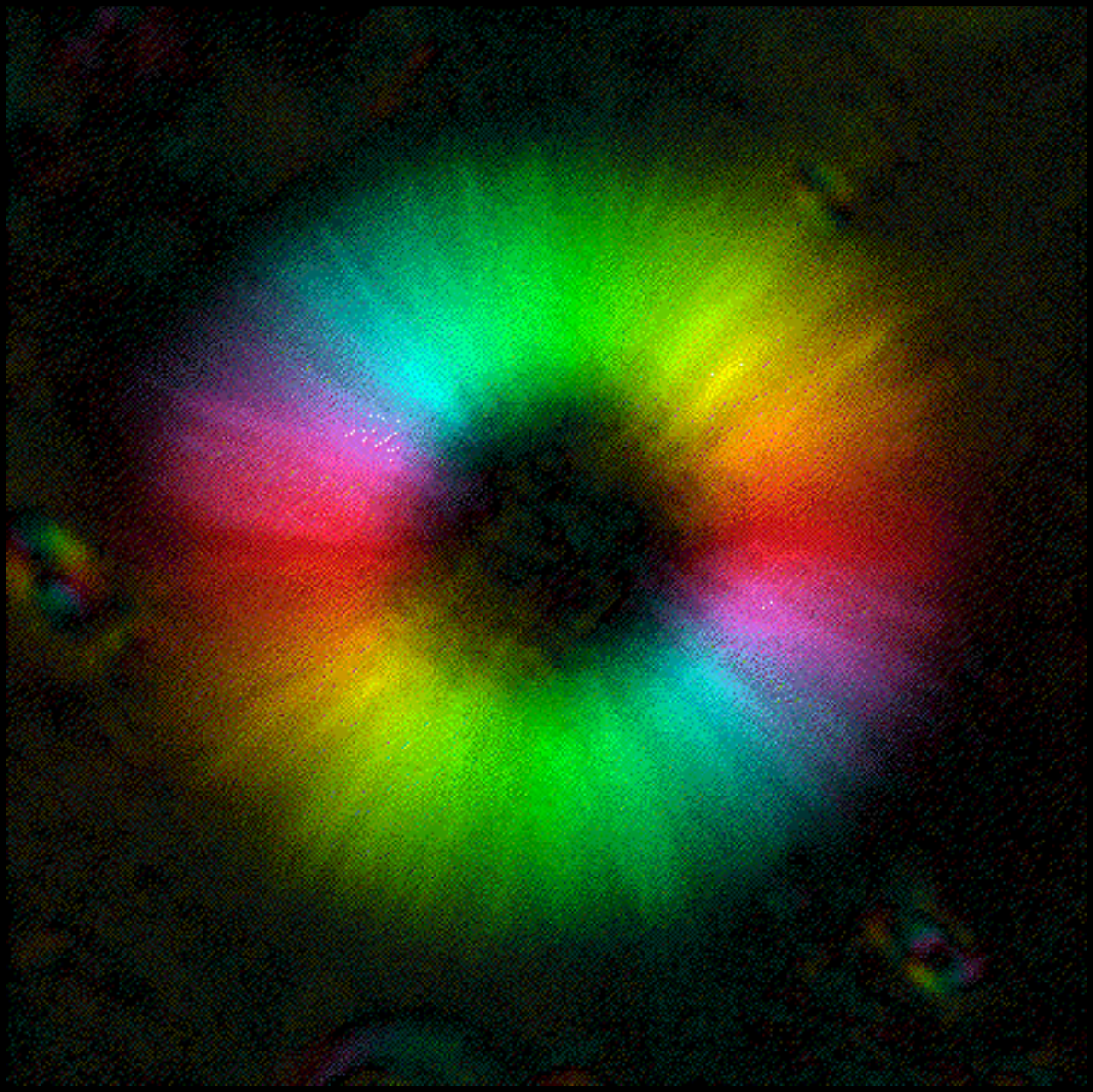


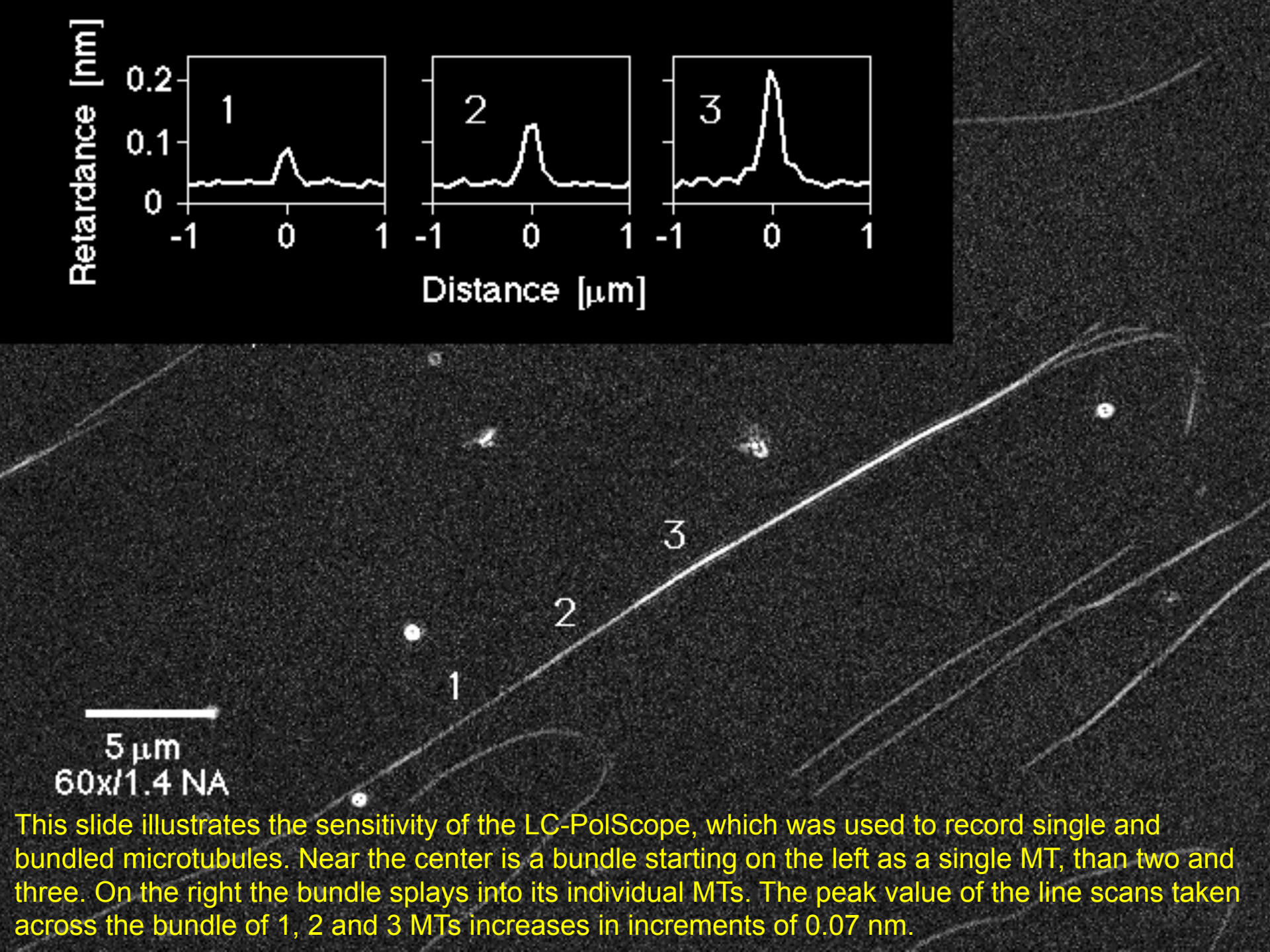
retardance



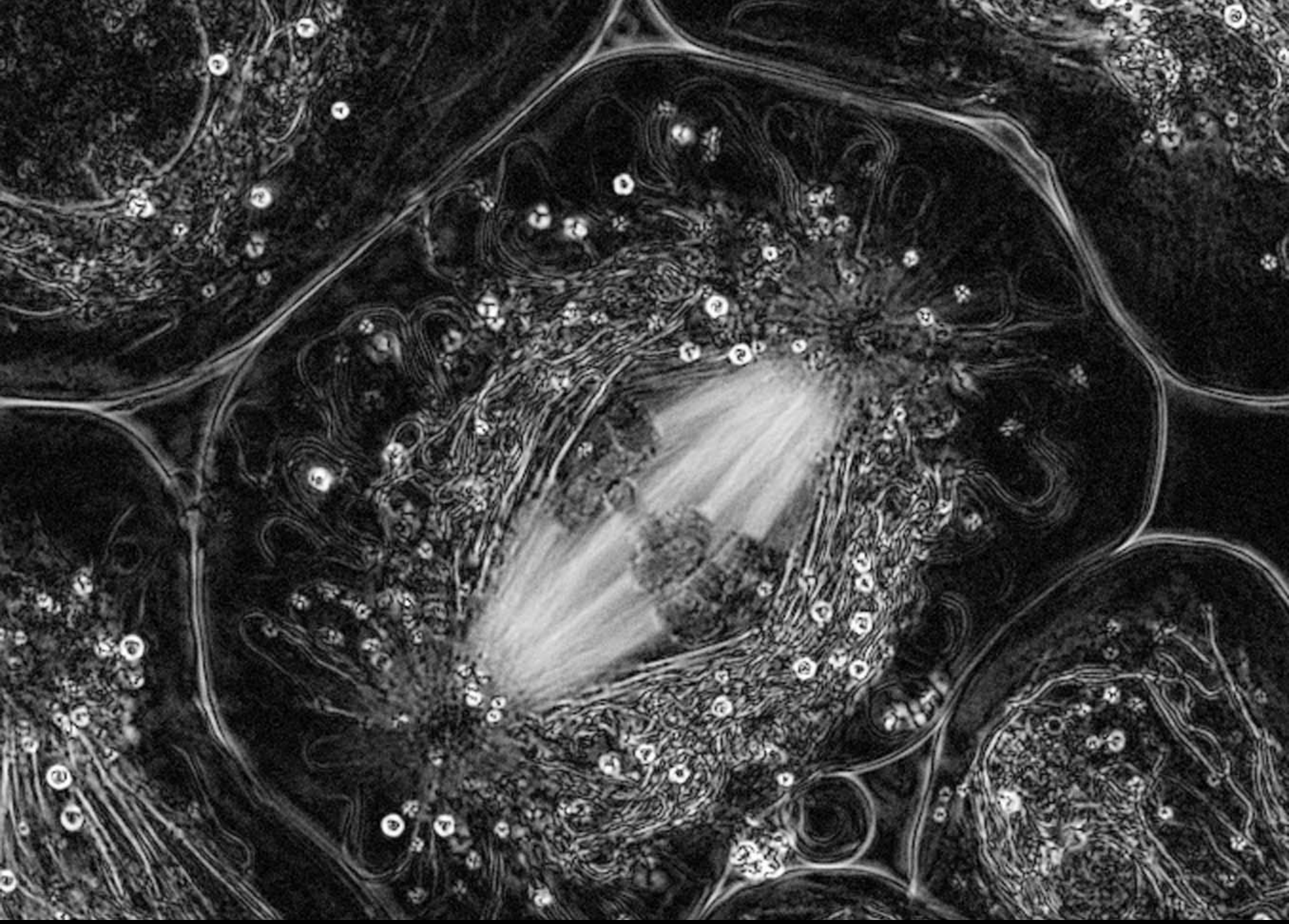
orientation

Aster, retardance and slow axes color encoded

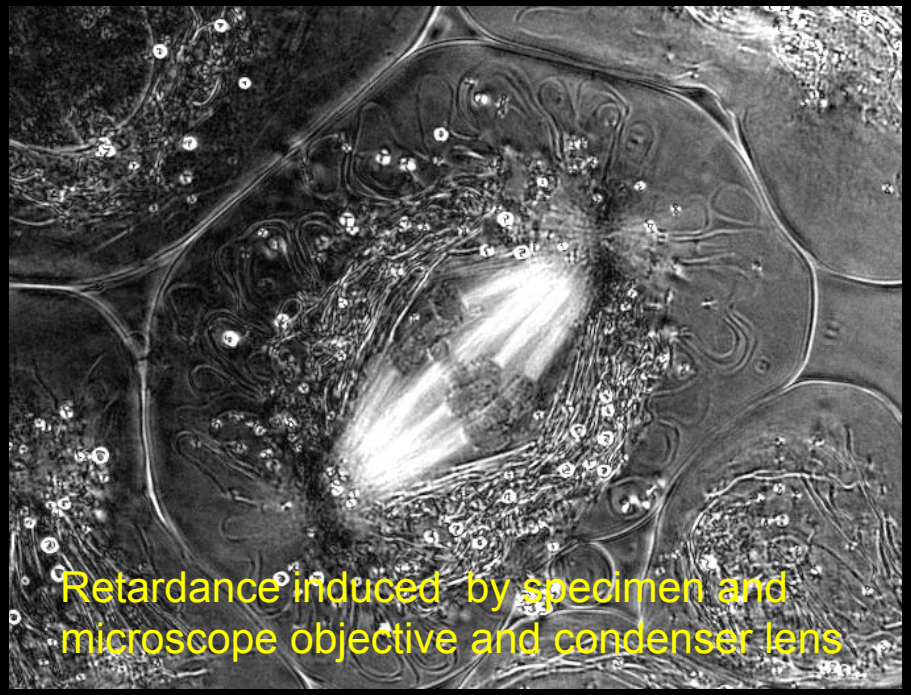


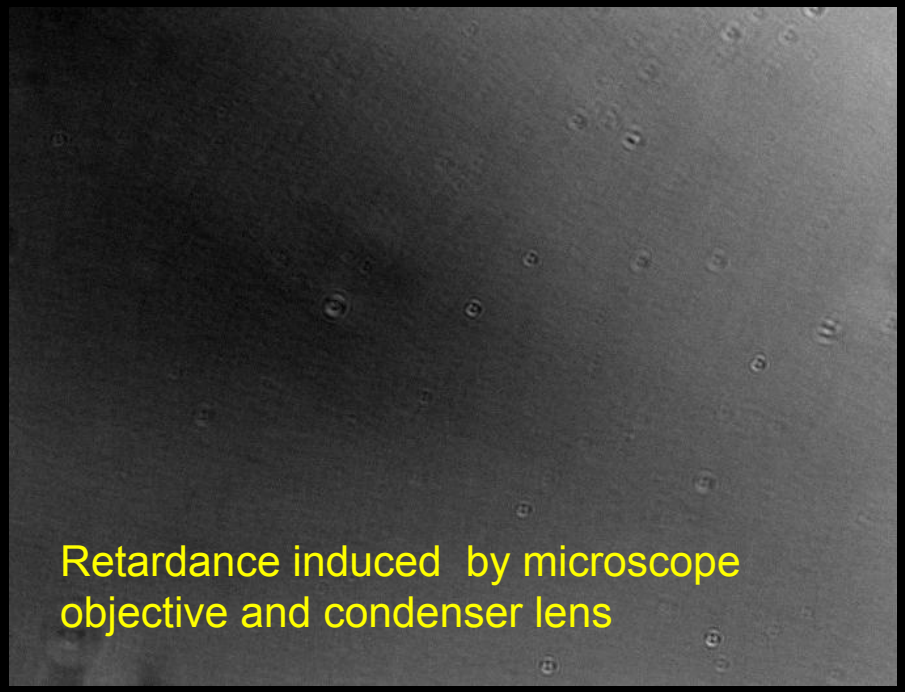


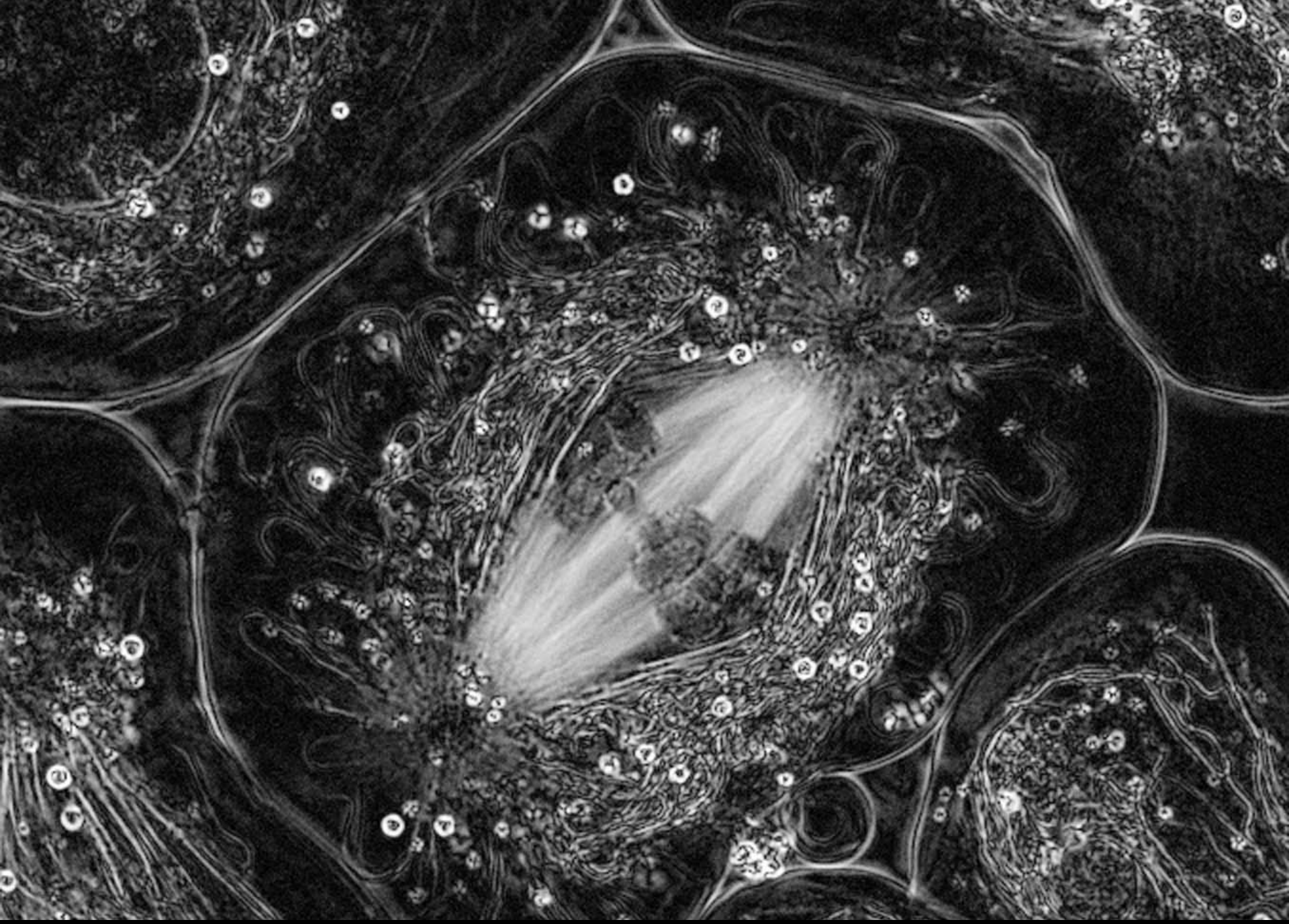
This slide illustrates the sensitivity of the LC-PolScope, which was used to record single and bundled microtubules. Near the center is a bundle starting on the left as a single MT, than two and three. On the right the bundle splays into its individual MTs. The peak value of the line scans taken across the bundle of 1, 2 and 3 MTs increases in increments of 0.07 nm.



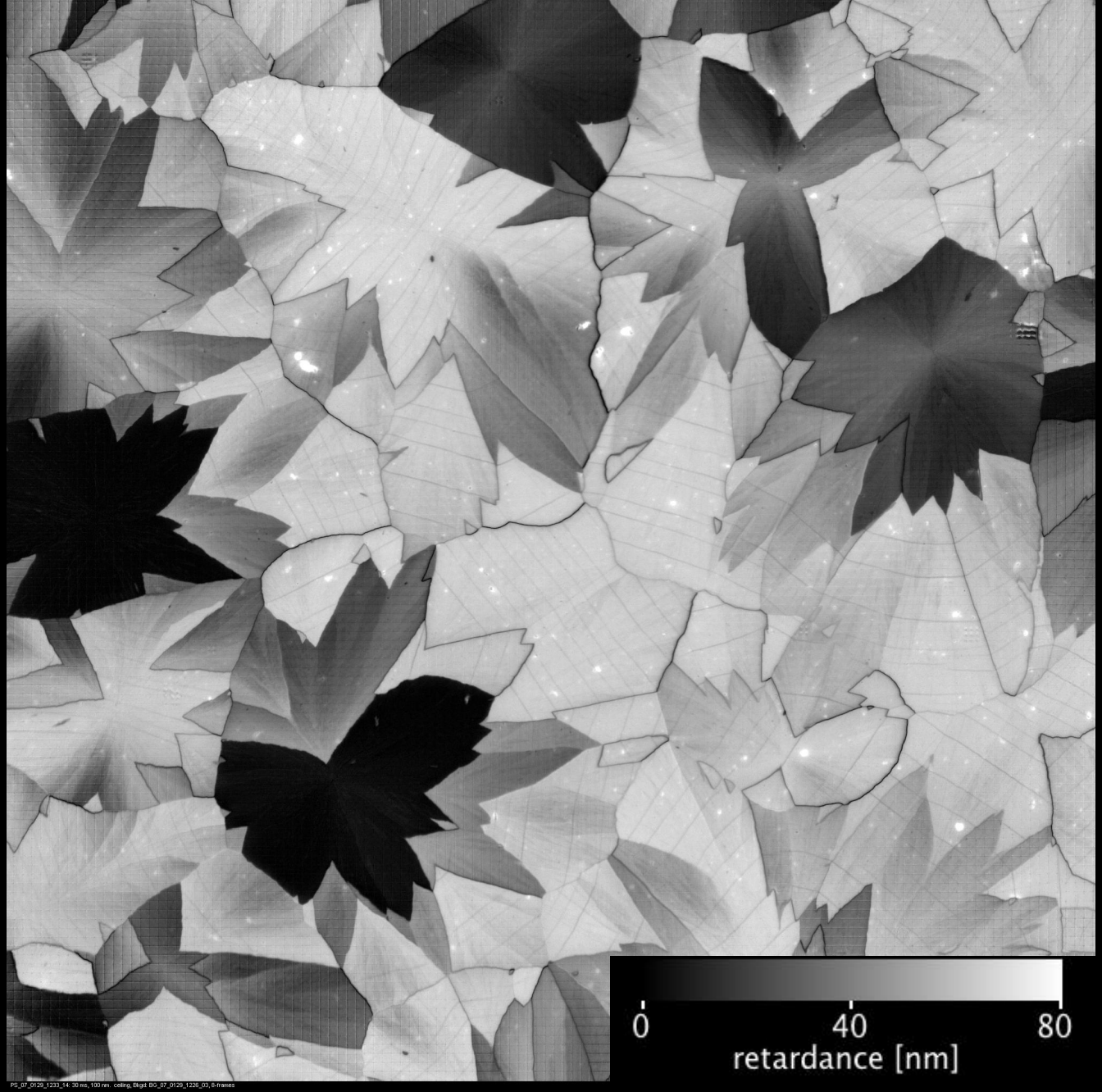
Spermatocyte, LC-PolScope, by James LaFountain and Rudolf Oldenbourg


Retardance induced by specimen and
microscope objective and condenser lens


Retardance induced by microscope
objective and condenser lens



Retardance of specimen after removing the retardance induced by objective and condenser lens



Retardance image of polycrystalline calcite film

Biomineralization

Morphosynthesis of Nacre-Type Laminated $CaCO_3$ Thin Films and Coatings**

Dirk Volkmer, Marc Harms, Laurie Gower, and Andreas Ziegler*
University of Ulm
Angewandte Chemie, 2005, Vol. 44: 639-44.

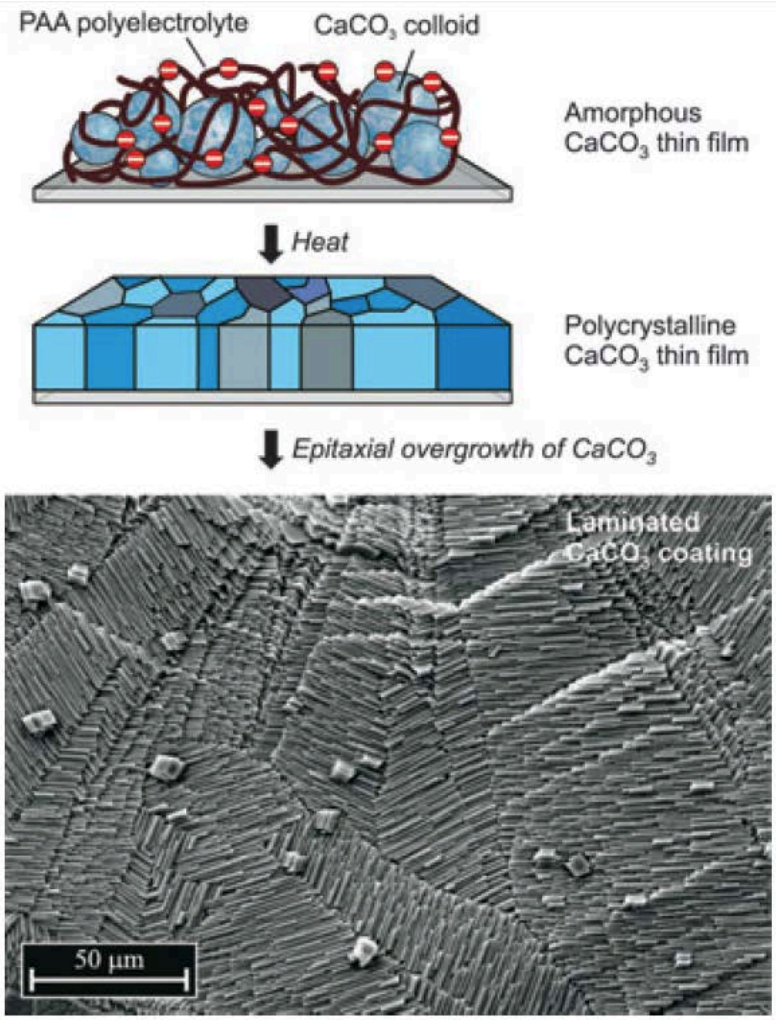
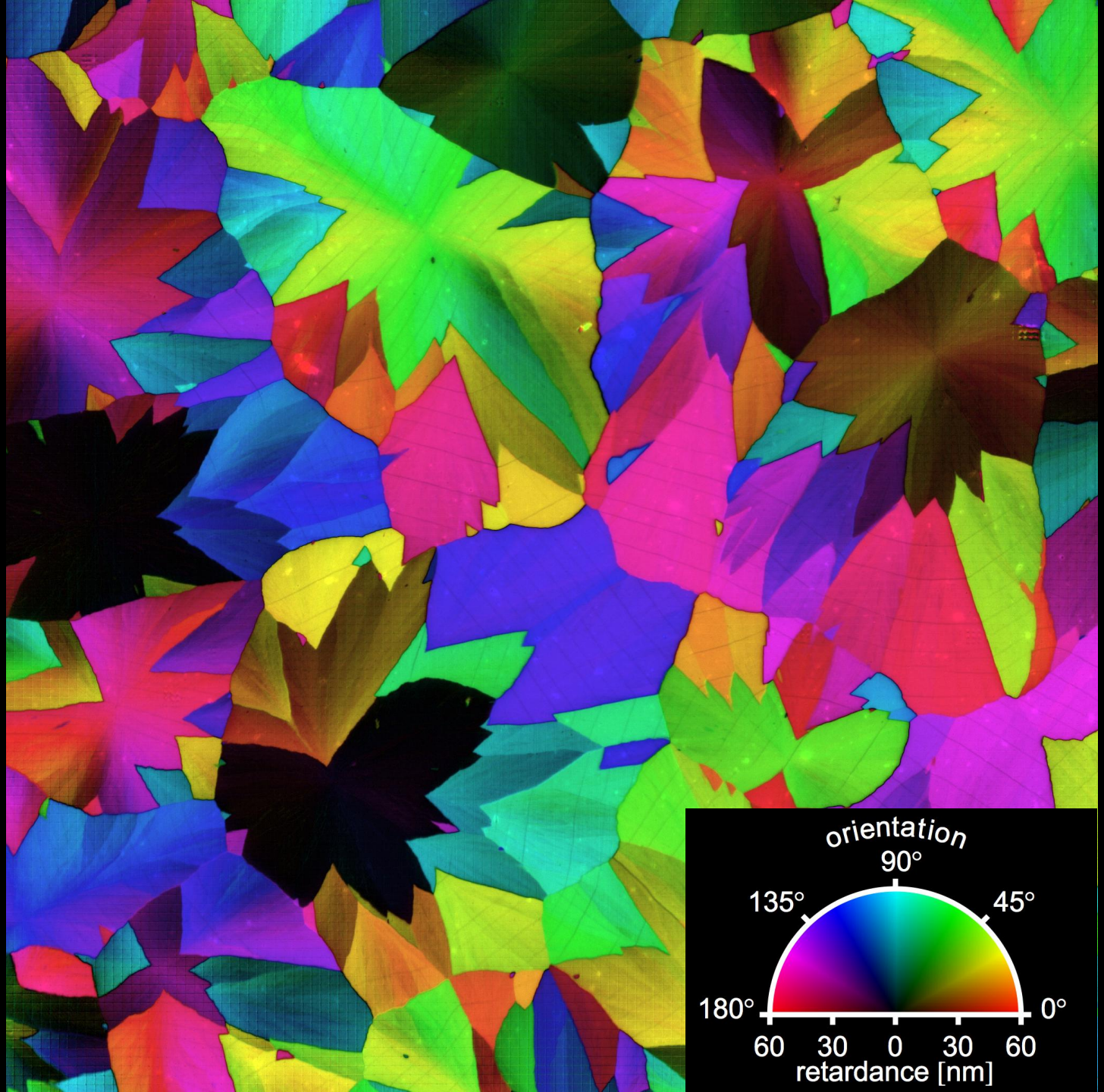
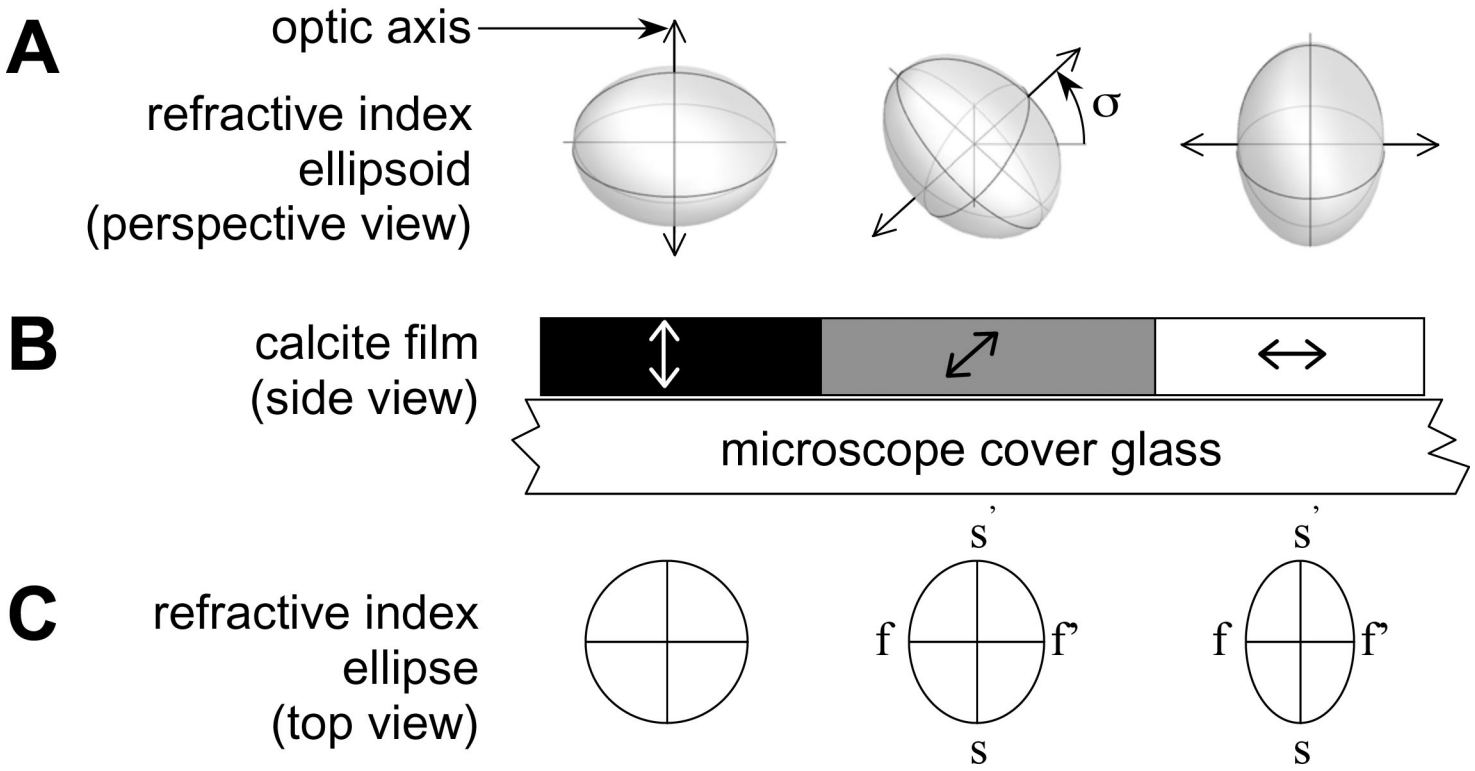
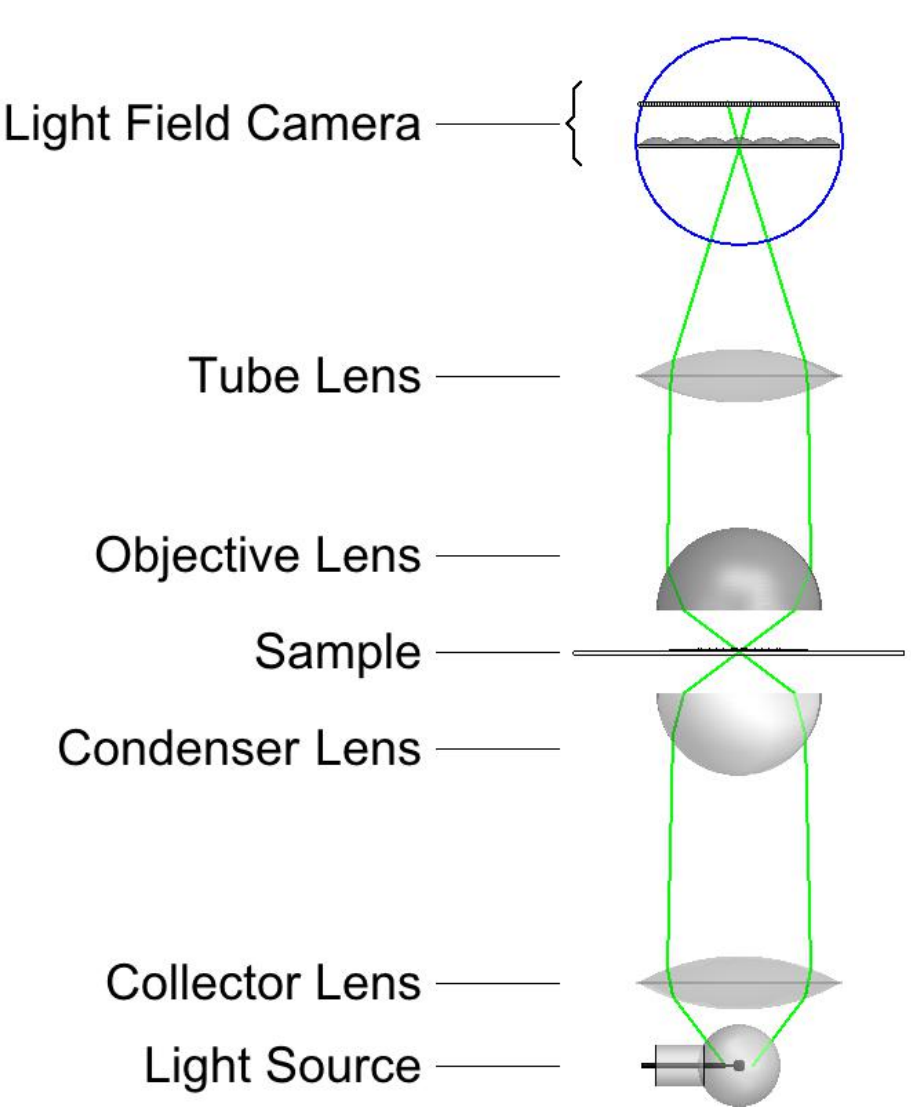


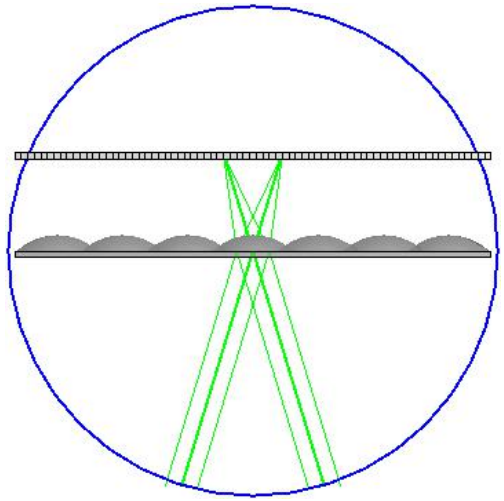
Figure 1. Scheme of a three-step procedure for the morphosynthesis of nacre-type laminated $CaCO_3$ coatings. In the first step an amorphous highly hydrated $CaCO_3$ thin film is deposited on a glass substrate. Upon heating this precursor film is transformed into a polycrystalline thin film consisting of a mosaic of flat single-crystalline calcite domains. In the last step highly oriented single and multiple layers of calcite crystals are grown epitaxially on the underlying polycrystalline thin film.


orientation
90°
135°
45°
180°
0°
60
30
0
30
60
retardance [nm]

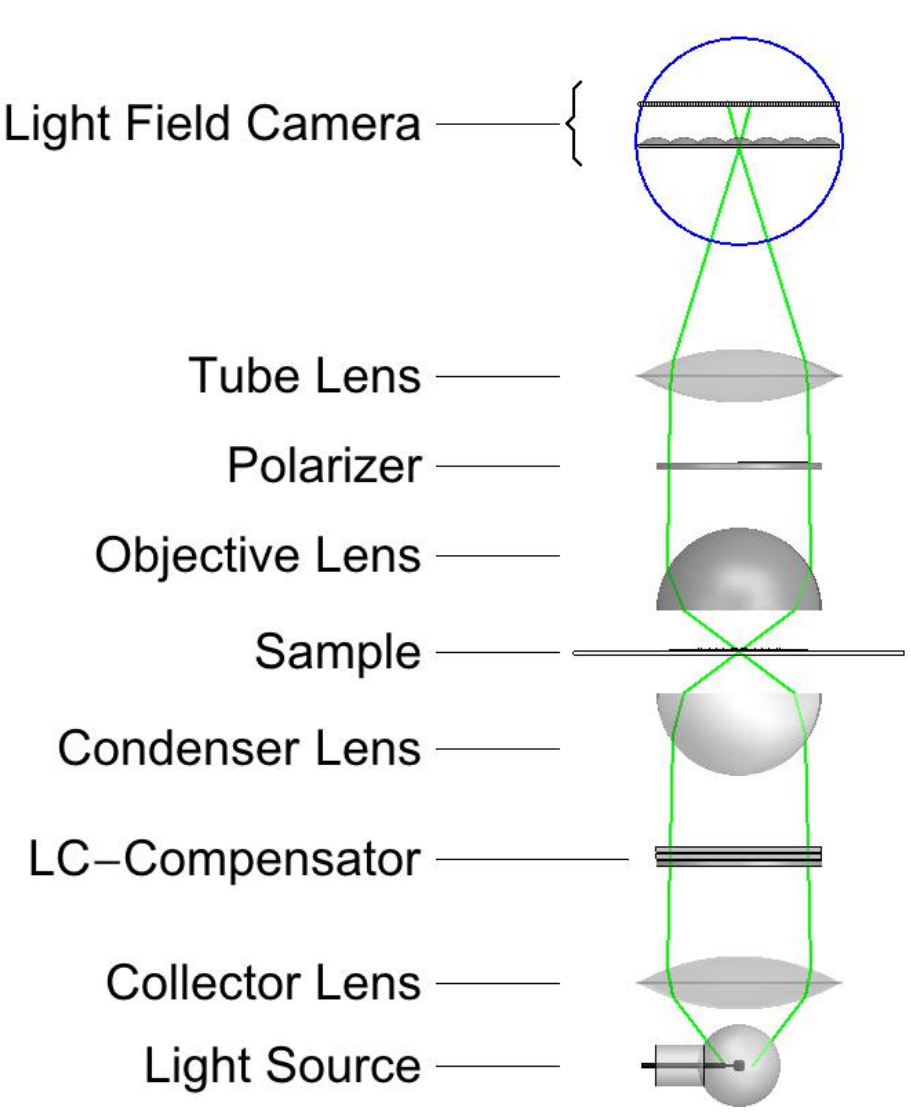

A
optic axis
refractive index
ellipsoid
(perspective view)
σ
B
calcite film
(side view)
microscope cover glass
C
refractive index
ellipse
(top view)
s'
f
f'
s
s'
f
f'
s

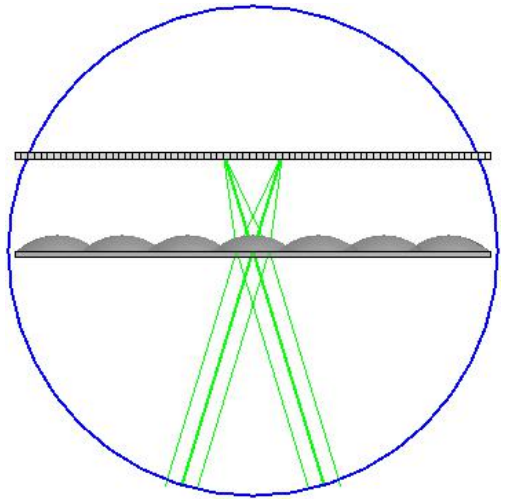
Light field camera consists of microlens array and CCD sensor array

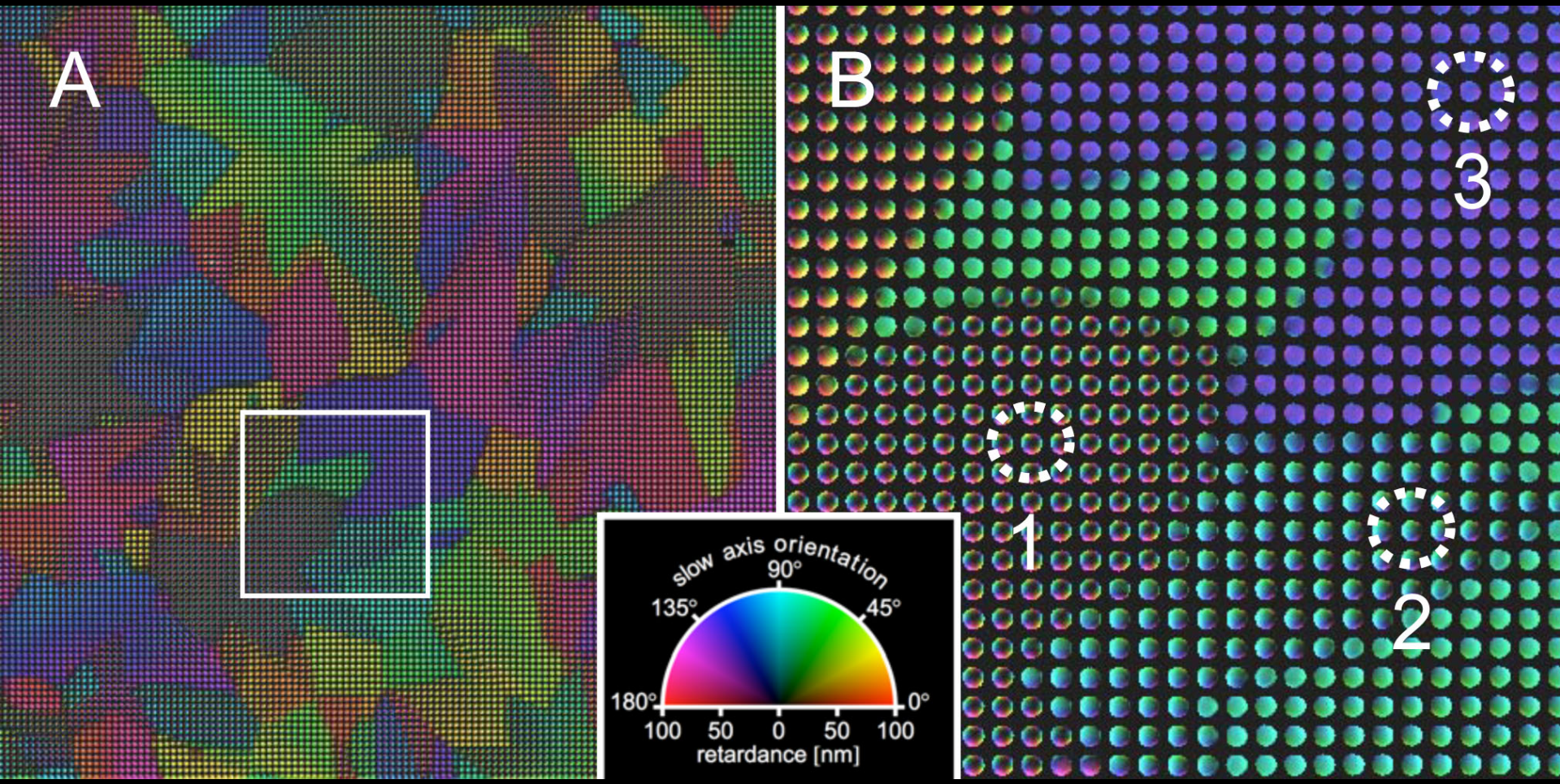




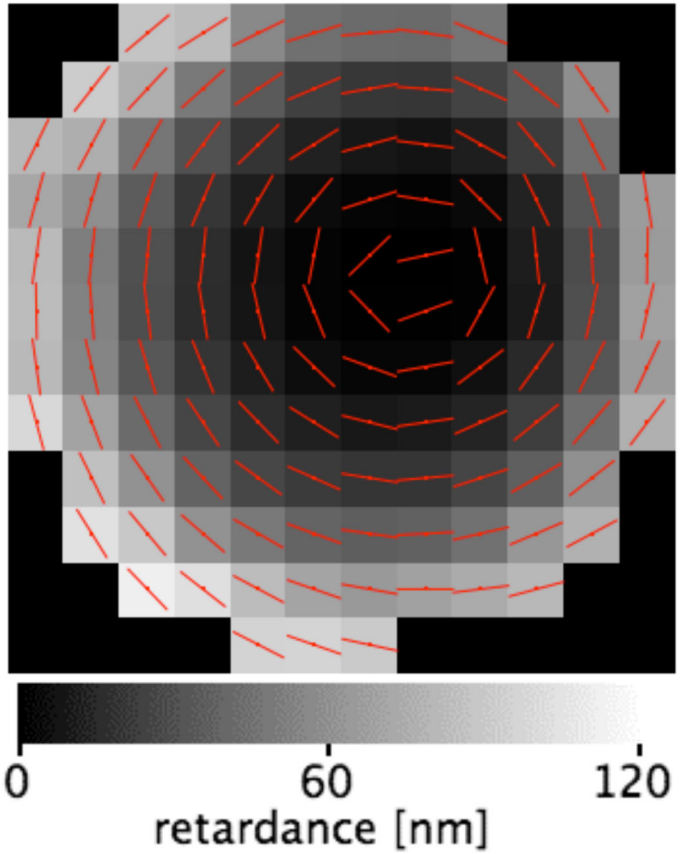
Light field camera and LC-PolScope components added to standard microscope



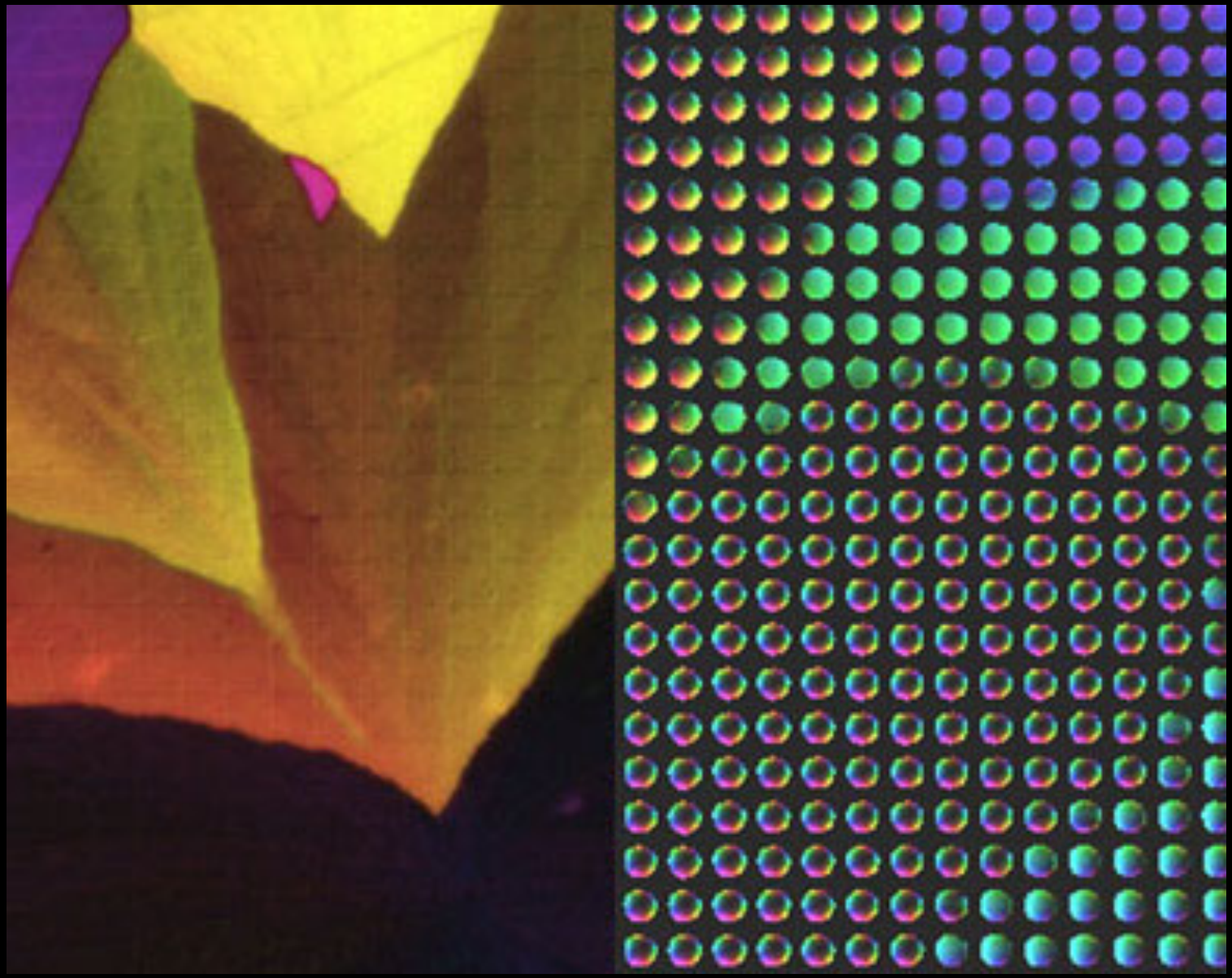




Enlarged area in B shows conoscopic images behind each microlens



Conoscopic image behind a single microlens formed by rays that have passed through a single calcite crystal whose optic axis is slightly tilted from the microscope optical axis.



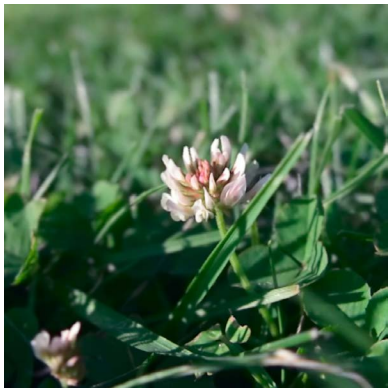
Lytro light field camera

3D image data with a single snapshot

capture a single light field image

many focus planes:

plane 1 plane 2



many perspective views:

view 1 view 2

developed by a group in Computer science and Electrical Engineering at Stanford University.

What's up in polarized light microscopy

- polarized light field processing:
  3D optic axis orientation and birefringence
  for every volume element
- forward modeling of polarization properties of transparent specimens (specimen ➔ image)
- inverse solutions (image ➔ specimen)
- Combination with other techniques: fluorescence
- Another application: stress and cracks in glass
- Education: MBL Microscopy courses

There is plenty of room at the bottom

Richard Feynman

OPS OpenPolScope *Discover the Order in Living Things*

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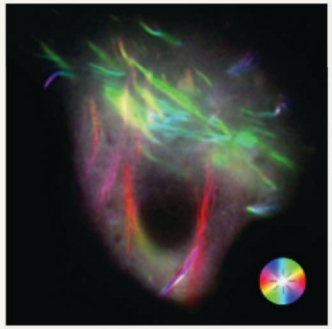
What is OpenPolScope ?

http://openpolscope.org

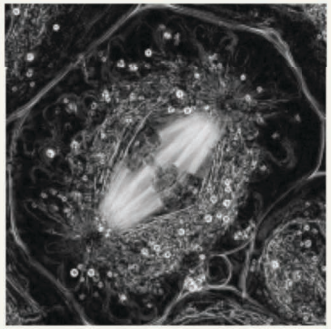
OpenPolScope.org was created for users and developers of polarized light microscopy techniques. It is an open-access platform for the collection and dissemination of knowledge about the technology, its applications and its further development. The website is maintained by members of the Cellular Dynamics Imaging Group and the Laboratory of Rudolf Oldenbourg at the Marine Biological Laboratory in Woods Hole, Massachusetts.

OpenPolScope technology is a set of software and hardware components for the acquisition, processing and analysis of polarized light images formed with microscope and other imaging optics. The **software** is available as plugins for the open source imaging platforms ImageJ and Micro-Manager. We also make available information on the **hardware** needed to implement a polarized light microscope that is compatible with the OpenPolScope software. In combining hardware and software, users are able to acquire polarized light images for measuring the birefringence, diattenuation, and polarized fluorescence in man-made and natural materials at high sensitivity and at high spatial resolution.

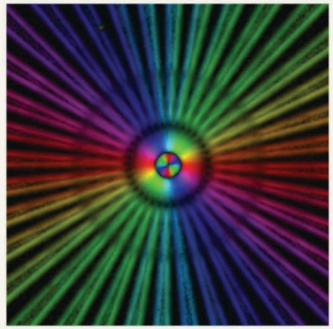
Our goal is to make polarized light microscopy accessible to a wide community of scientists and enthusiasts of polarized light imaging, for stimulating interdisciplinary collaborations in applying the technology and developing it further. In promoting this goal, we also provide services to colleagues who wish to implement and use the technology in their own work. Currently, the OpenPolScope supports three imaging modes: birefringence, fluorescence polarization, and diattenuation. The images below link to information on each mode.



Fluorescence Polarization



Birefringence



Diattenuation

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