Nomarski Microscopy

HC70AL
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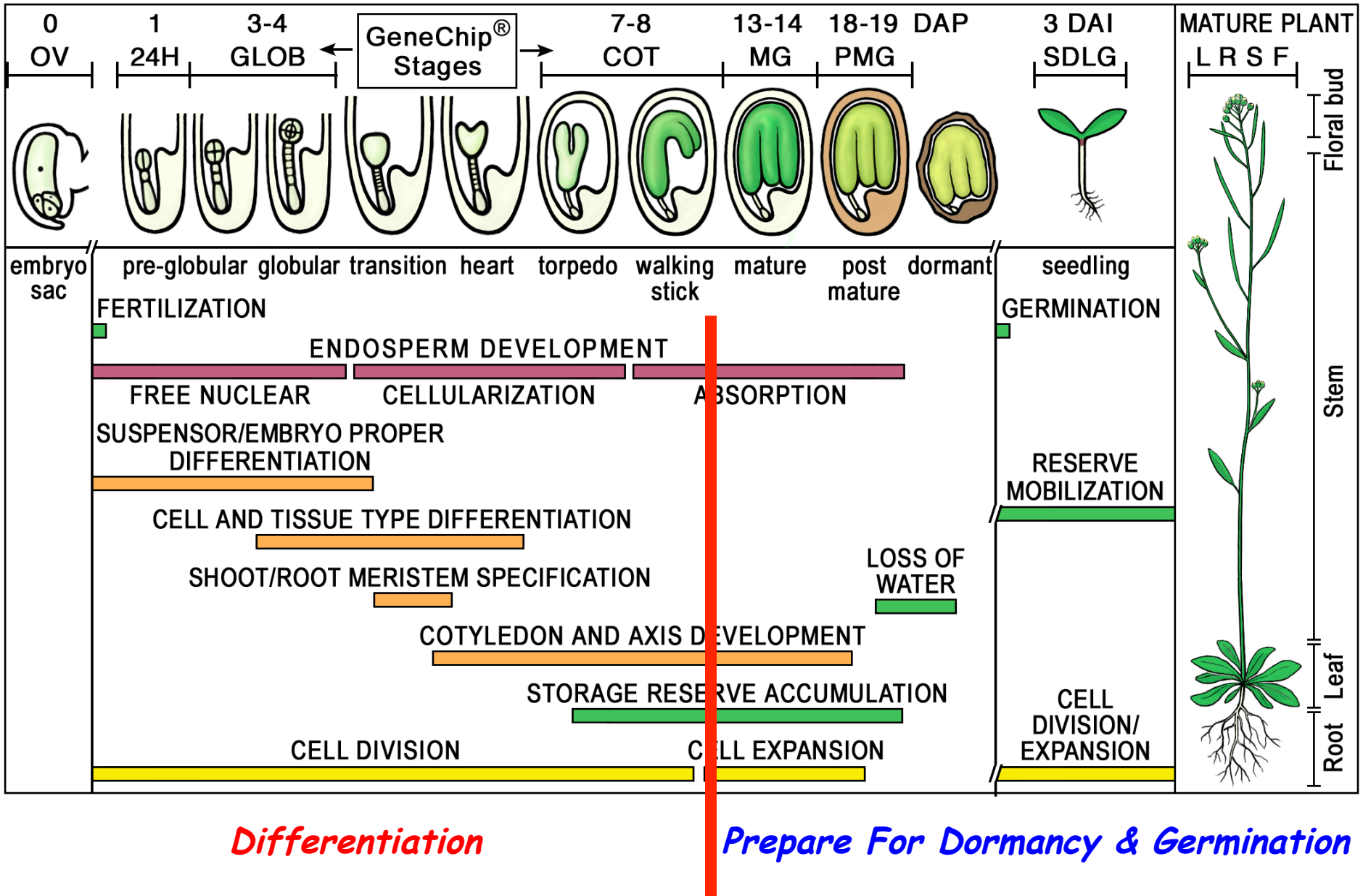
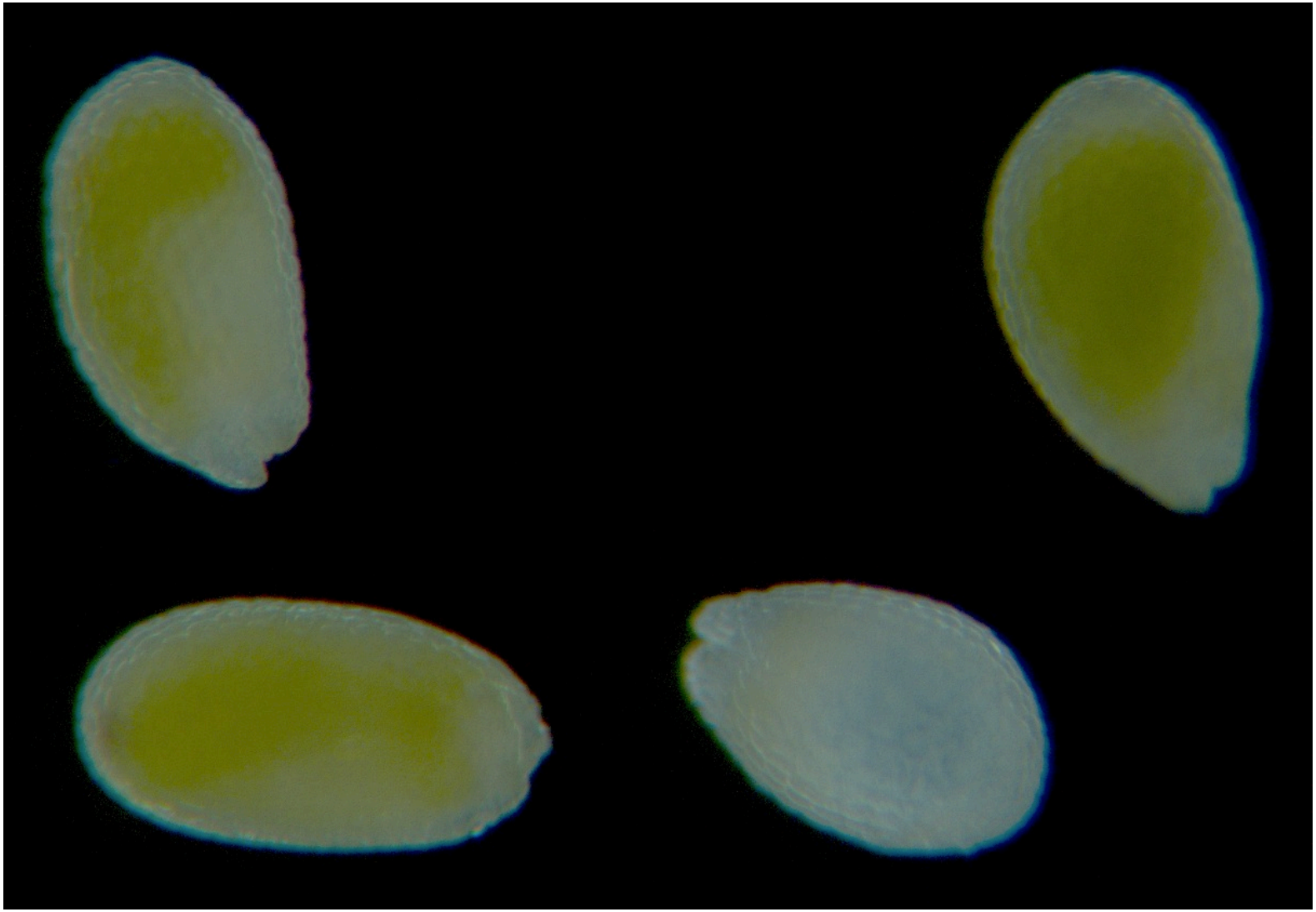
Stages of Arabidopsis Seed Development



What Stages of Embryos Are in These Arabidopsis Seeds?



Cotyledon and Mature Green Stage Embryos

Embryos were hand dissected from seeds using syringe needles

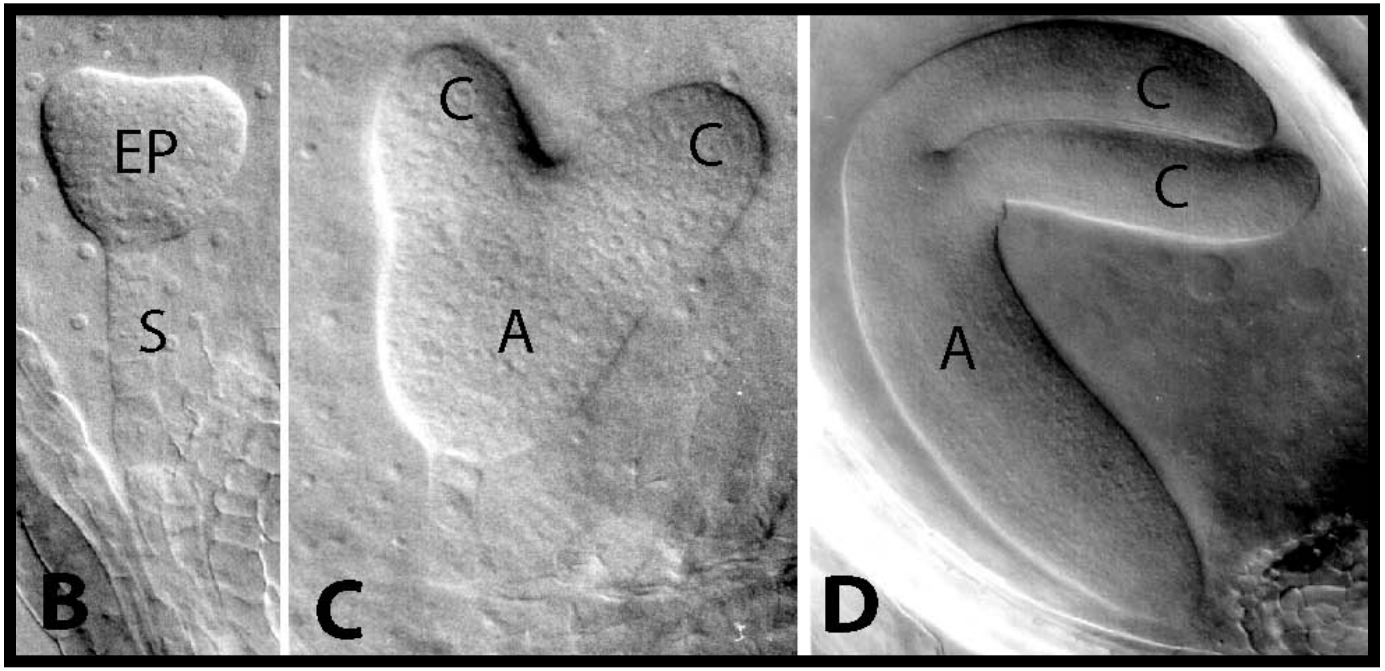
Globular Stage Embryo and Heart Stage Embryo

Globular

Heart

- ***Is it easy to microdissect these intact embryos from their seeds?***
- ***Is there a better way to determine embryo stage in a high-throughput approach?***

Use Differential Interference Contrast (DIC or Nomarski) Microscopy to Observe Arabidopsis Embryos within the Seed!



Early Heart **Cotyledon** **Torpedo**

- **Excellent resolution and contrast**
- **Can focus on a thin plane section of a thick specimen without confusing images from above or below the plane**
- **Images produced by a DIC microscope are relief-like and seem to have a shadow cast**

What Is the Difference between Bright Field and Differential Interference Contrast Microscopy?

Differential Interference Contrast

Fig. 1a: Amobeba Proteus, DIC

Bright Field

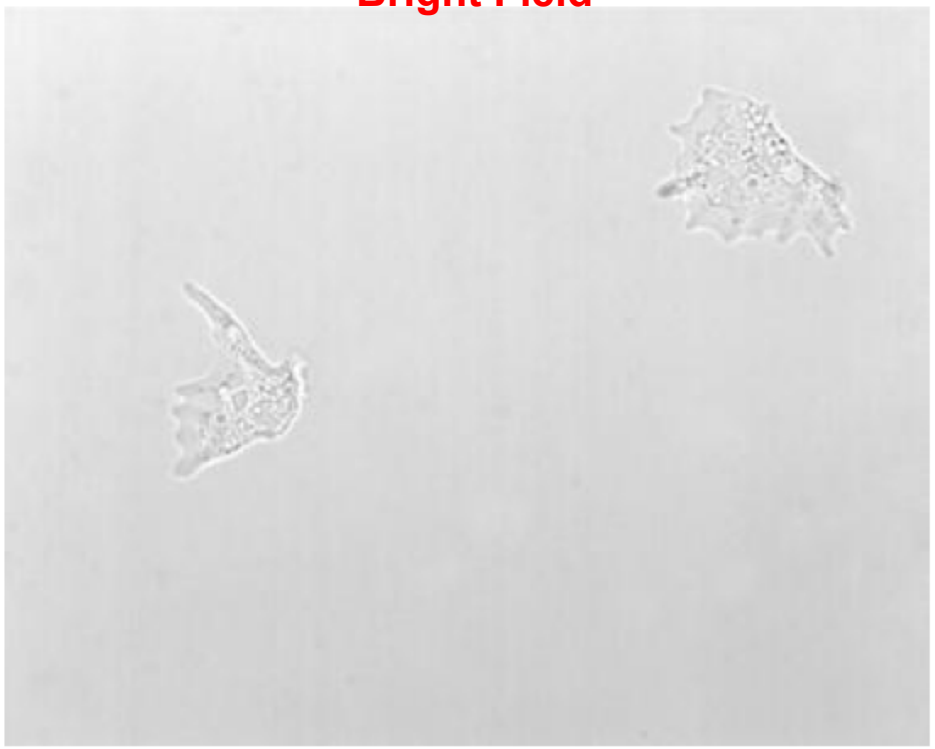


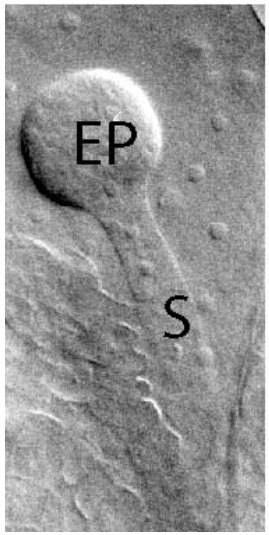
Fig. 1b: Amobeba Proteus, bright field

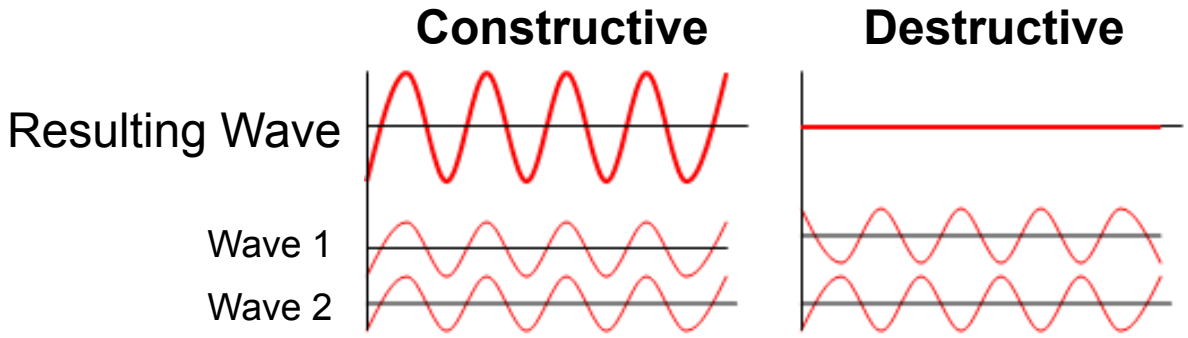
Differential interference contrast (DIC) microscopy is a good alternative to brightfield microscopy for gaining proper images of unstained specimens that often only provide a weak image in brightfield.

http://www.leica-microsystems.com/science-lab/differential-interference-contrast-dic/

Differential Interference Contrast Microscopy

- **How it works**
  - polarized light is separated into two beams, which take slightly different paths through a sample depending on its thickness and/or refractive index. The different paths, cause a change in phase between the two beams. When the beams are recombined, their interference can be visualized as change in darkness.
- **What is interference?**
  - If two waves of the same wavelength and frequency are in phase, both the wave crests and wave troughs align. This results in constructive interference.
  - Alternatively, if the two waves of the same wavelength and frequency are out of phase, then the wave crests will align with wave troughs and vice-versa. This results in destructive interference





Phase difference is converted to amplitude difference, which can be visualized as improved contrast

How Does Light Travel through the Microscope When Using DIC?

8. light travels through the analyzer

7. second prism combines light paths; interference pattern occurs
   interferes with an adjacent point with different phase

6. light travels through the objective → focused by second prism

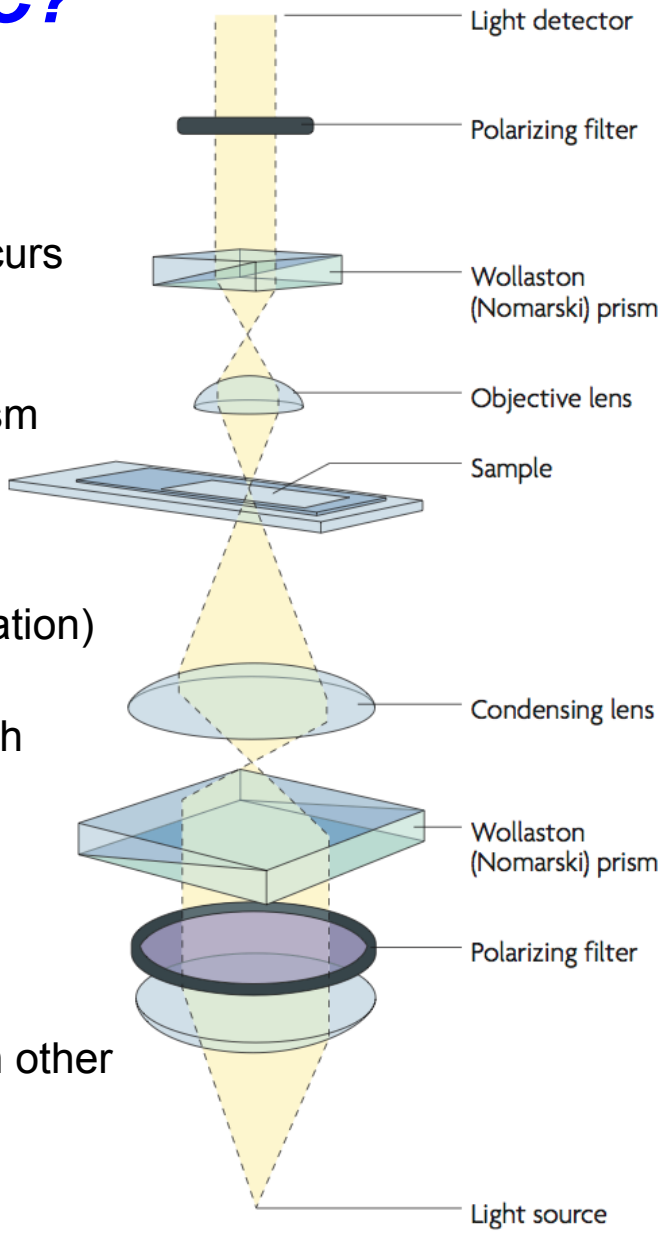
5. light travels differently through different sample thicknesses
   two different waves, two “brightfield” images
   (images do not interfere with each other because of polarization)

4. rays are focused by the condenser so that they pass through
   adjacent points in the sample 0.2 microns apart
   two coherent light waves: 0° and 90° polarization

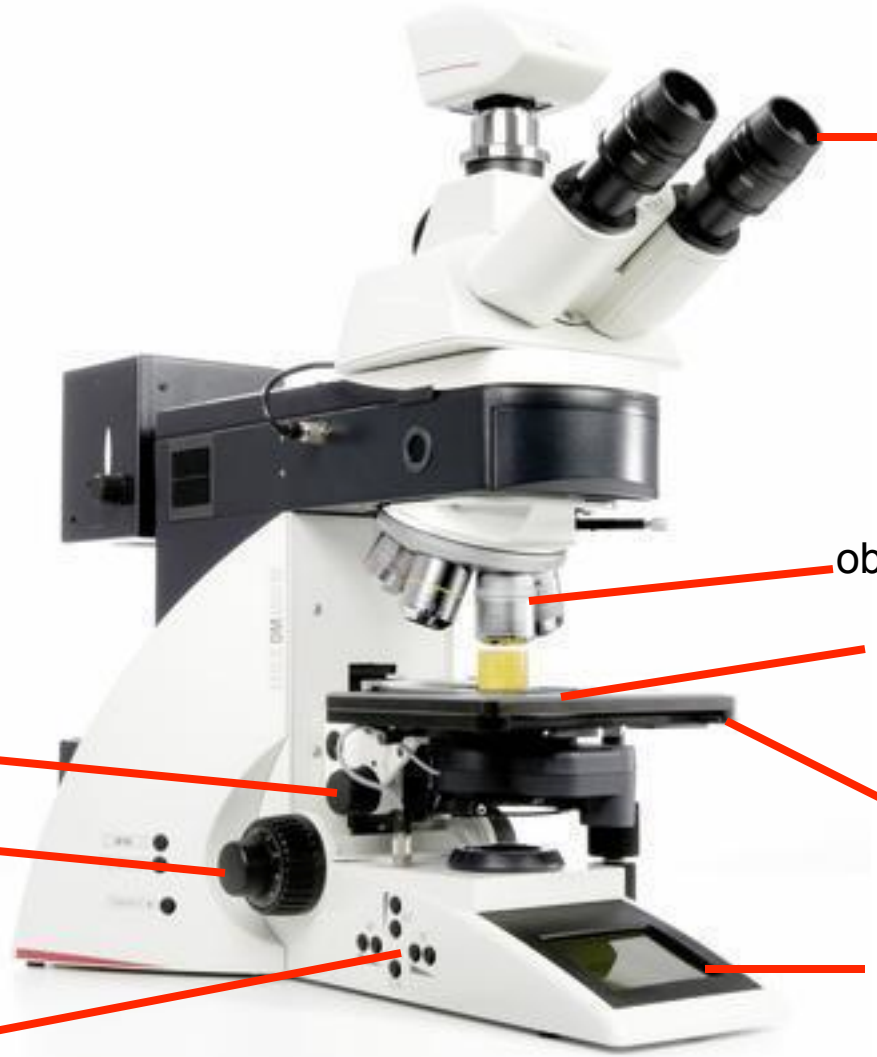
3. light is focused outside the prisms

2. polarized light enters prisms → 2 polarized rays 90° to each other

1. unpolarized light enters microscope → polarized



How to Control the Microscope



eyepiece

objective lens

stage and
specimen holder

condenser

bias

focus

touch screen display

adjust field diaphragm,
aperture, light intensity