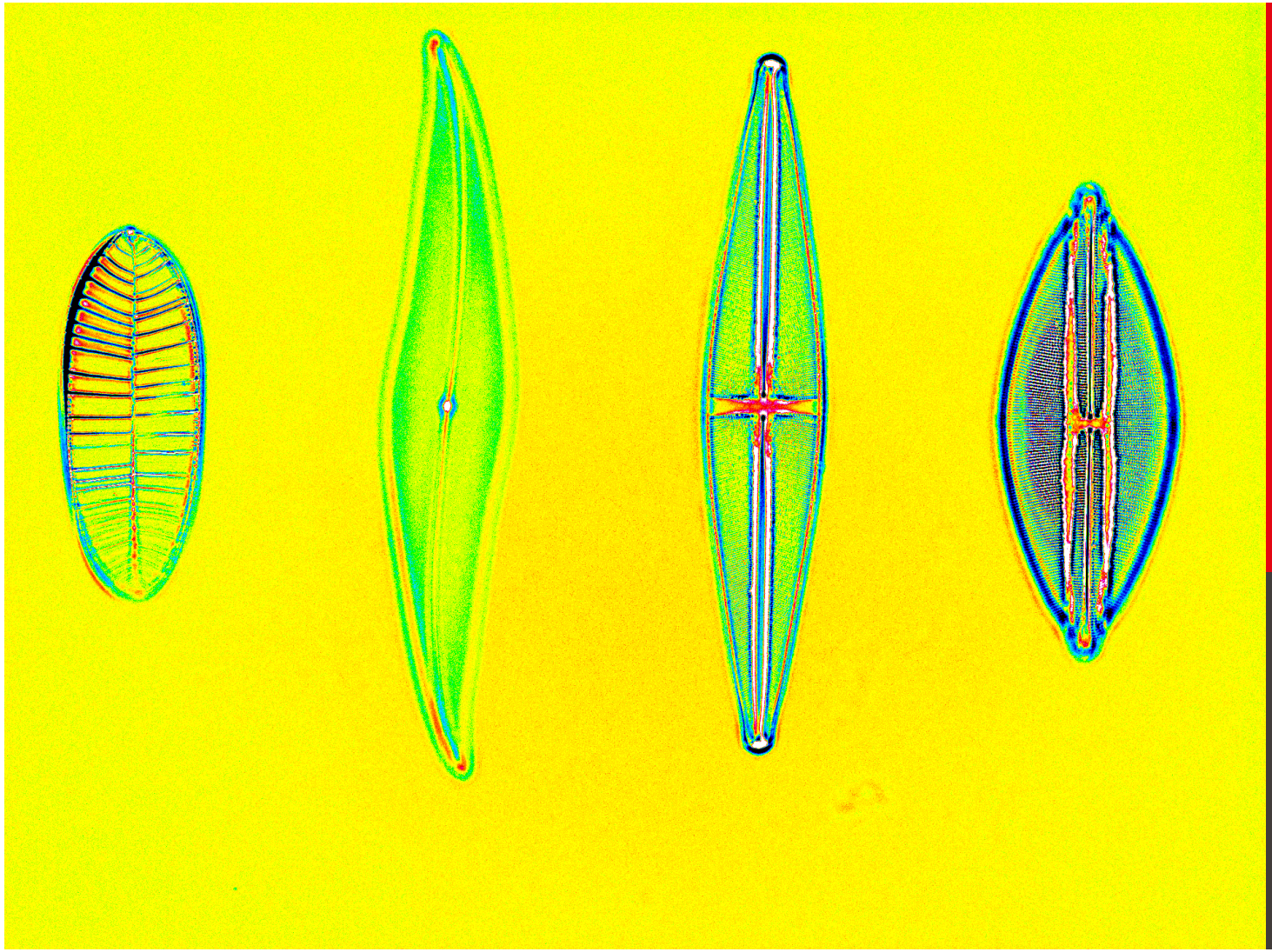

Biomicroscopy II

Transmission Techniques

École polytechnique fédérale de Lausanne

Spring Semester, 2021

How to set up Koehler illumination

Arne Seitz, BIOP, EPFL

Transmission

- Focus on the specimen
- Close field diaphragm
- Focus condenser until field diaphragm is seen sharp
- Center field diaphragm
- Open/close field diaphragm up to 80 –90 %
- Remove eyepiece, look down to the aperture diaphragm
- Center (if possible) aperture diaphragm
- Open aperture diaphragm up to 60 –70 %
- Start with low magnification objective. Repeat for every objective used

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How to set up Koehler illumination

Fluorescence

- Focus on the specimen
- Swing in focusing aid (if available)
- Focus image of arc sharply
- Swing out focusing aid
- Close field diaphragm
- Center field diaphragm

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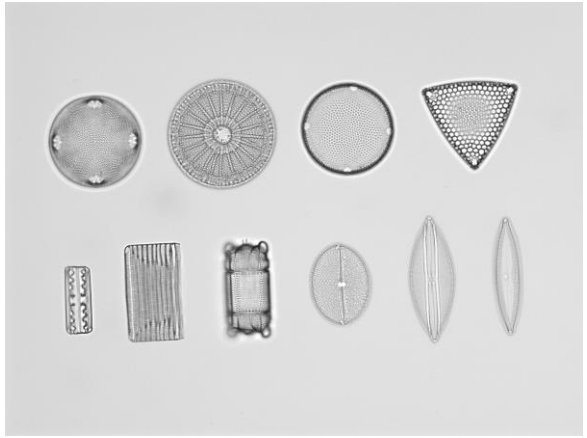
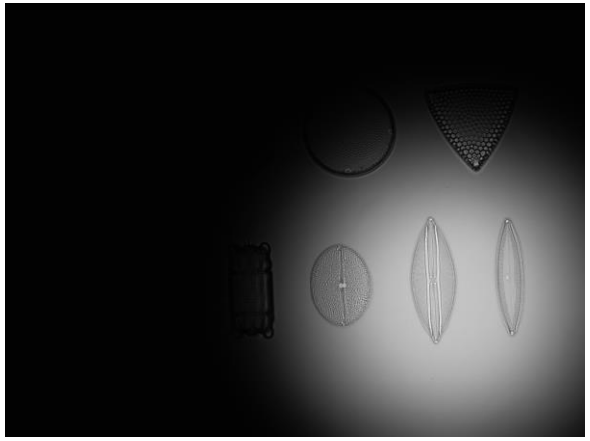
Adjustment for Koehler illumination

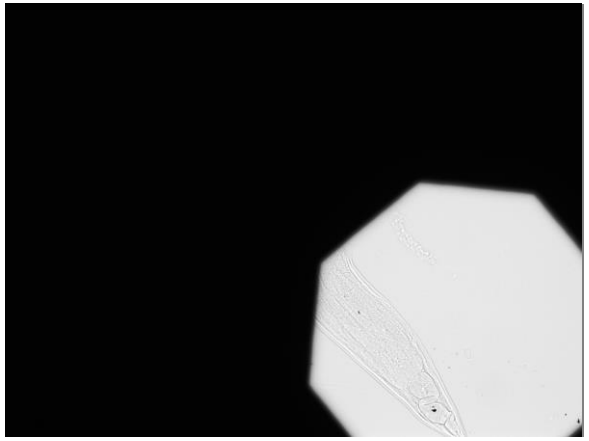
1. Focus on the sample



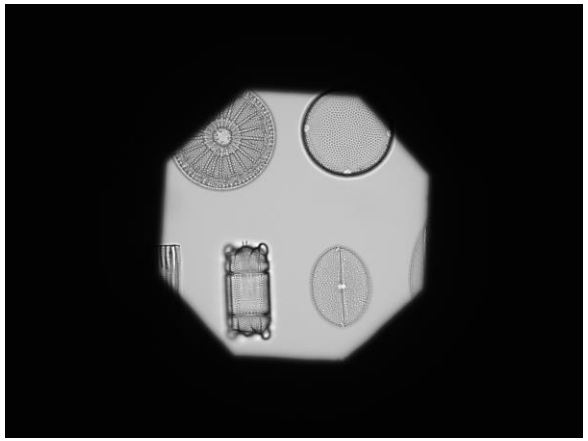
2. Close the field diaphragm



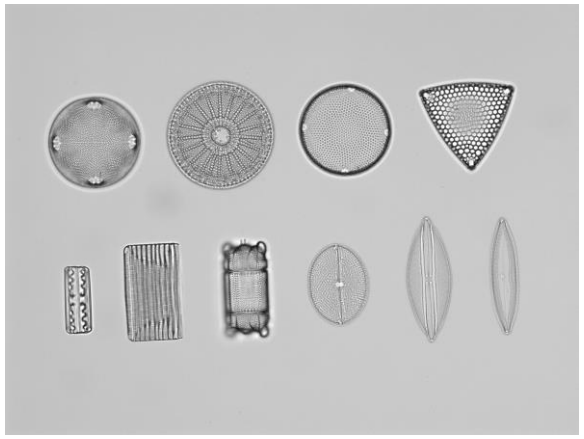
3. Set the condenser height in order to obtain a sharp spot



4. Center the spot with condenser centering screws



5. Open the field diaphragm



6. Remove eyepiece and set aperture diaphragme

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Contrasting techniques

Dark field: Fine structural features at, and even below, the resolution limit of a light microscope. Highly suitable for metallographic and crystallographic examinations with reflected light.

Phase contrast: Used for visualizing very fine structural features in tissues and single cells contained in very thin (< 5 µm), non-stained specimens.

DIC: Method shows optical path differences in the specimen in a relief-like fashion. The method is excellently suited for thick, non-stained specimens (> 5 µm). Can be used for optical sectioning.

PlasDIC: The same specimen as conventional DIC but in plastic dishes.

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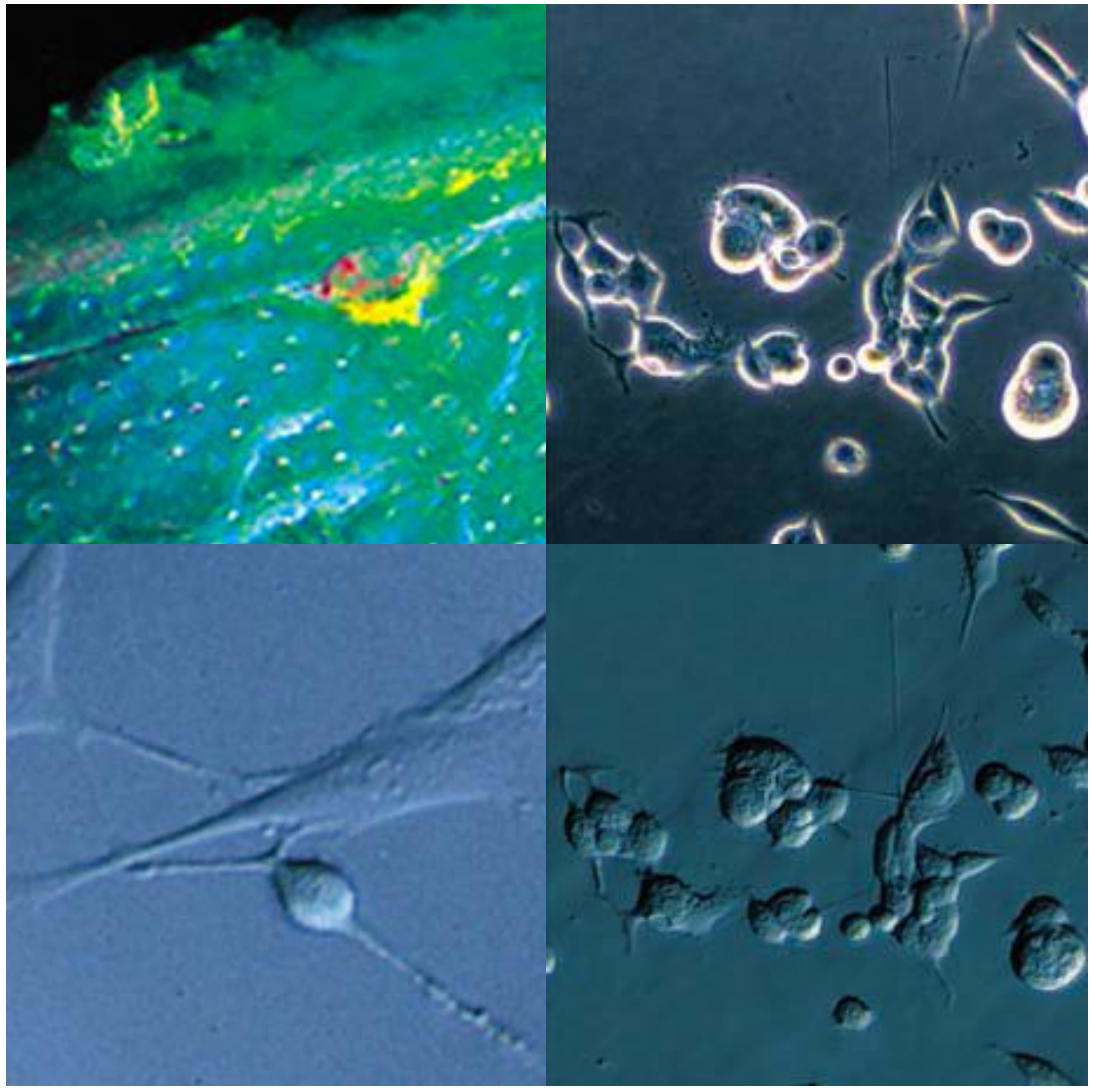
Examples of contrasting methods

Dark field:
Bone thin section



Phase contrast:
HEK cells

DIC:
Neurons

PlasDIC:
HEK cells

Arne Seitz, BIOP, EPFL

Adjustment for DIC for an inverted microscope

- Set up Koehler illumination in transmission
- Move specimen out of the light path
- Insert polarizer, analyzer and lower Wollaston prism
- Shift Wollaston prism to place dark line in the center
- Turn polarizer until the line is seen mostly dark
- Insert eyepiece back into eyetube
- Insert upper Wollaston prism (in condenser)
- Move the specimen back into the light path
- Move lower Wollaston prism to get required contrast

- Options (if available)
- Rotate the stage to highlight desired area in the sample
- Insert the lambda plate if color staining is required

- Repeat procedure for each objective being used

Adjustment for DIC

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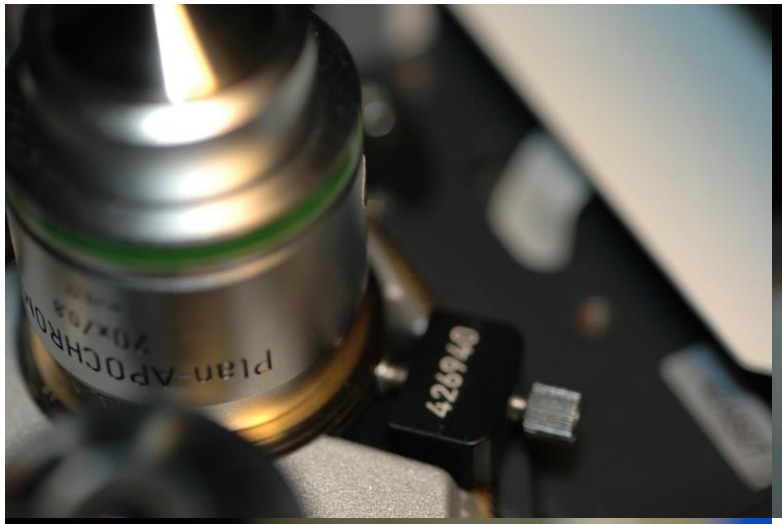
1 Lower prism

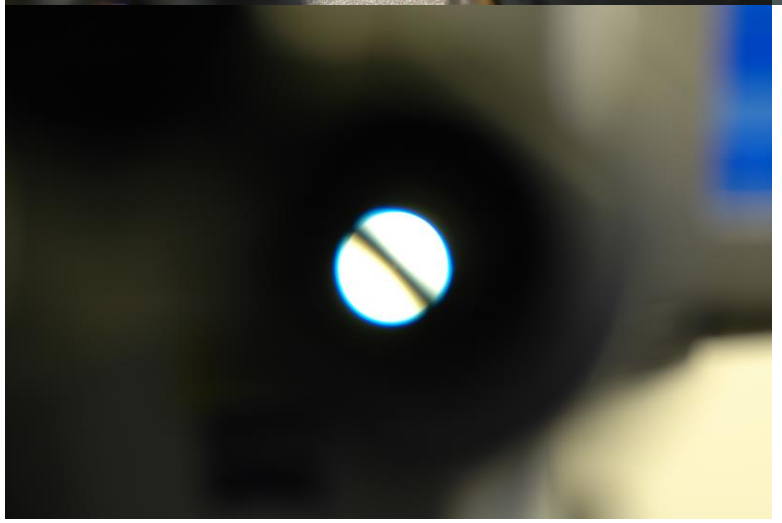
2 Polariser

3 Upper prism

4 Analyser

Adjustment for DIC





Insert polarizer, analyzer and lower Wollaston prism

Shift Wollaston prism to place dark line in the center

Turn polarizer until the line is seen mostly dark