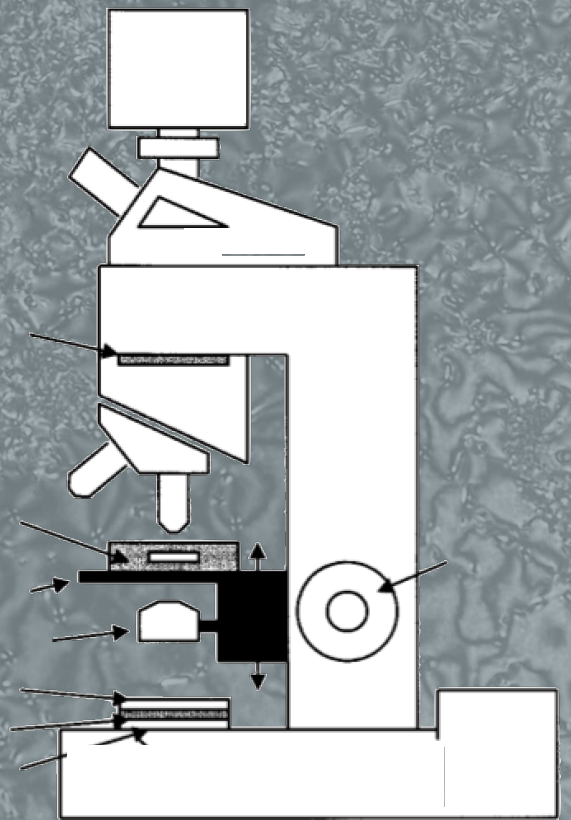
AXIO OBSERVER.A1 GUIDE



A Microscope Bootcamp

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Osuji Group

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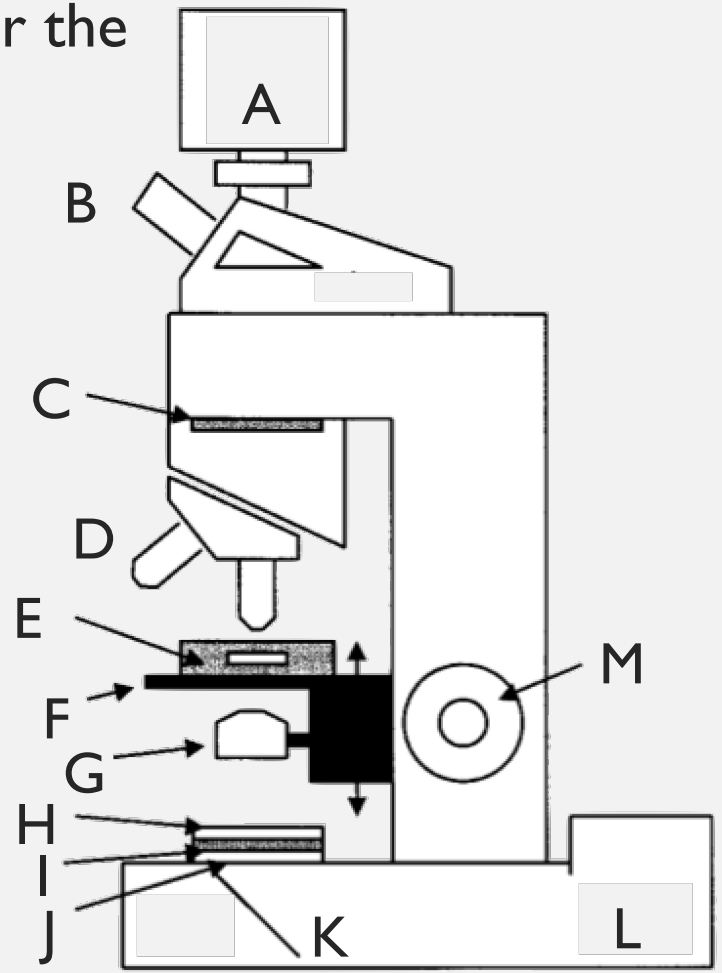
GOALS OF THIS TUTORIAL

- Familiarize group with microscope capabilities
  - Conoscopic Microscopy
  - Fluorescent Microscopy
  - Cubes, waveplates, etc.
- Improve overall image quality for research (Kohler)
- Develop an understanding of POM and LC optics
- Form a growing teaching document that can be added to and edited
  - Can be found on the Osuji Group Website

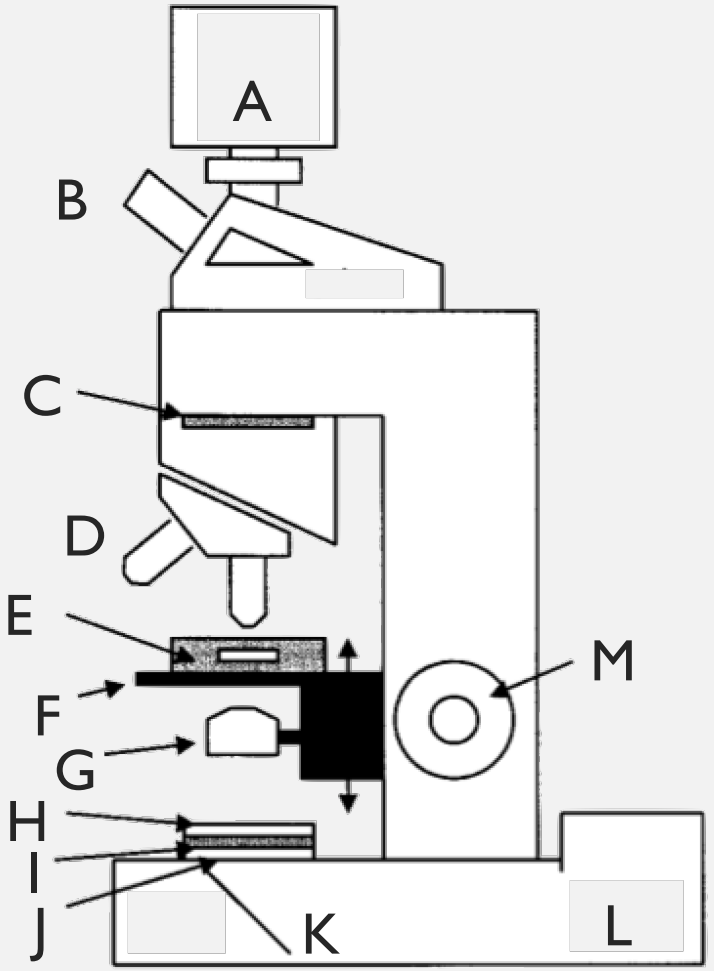
WHAT IS POM?

- Polarizing Optical Microscopy is one of the essential tools for the characterization of mesogenic material
- Provides information on *phase transition* T and p*hase type*
- Microscope parts:
  - A: Video Camera
  - B: Ocular
  - C: Analyzer
  - D: Objectives
  - E: Hot Stage
  - F: Rotation Stage
  - G: Condenser
  - H: Waveplate
  - I: Polarizer
  - J: Lens
  - K: Mirror
  - L: Light Source
  - M: Focus



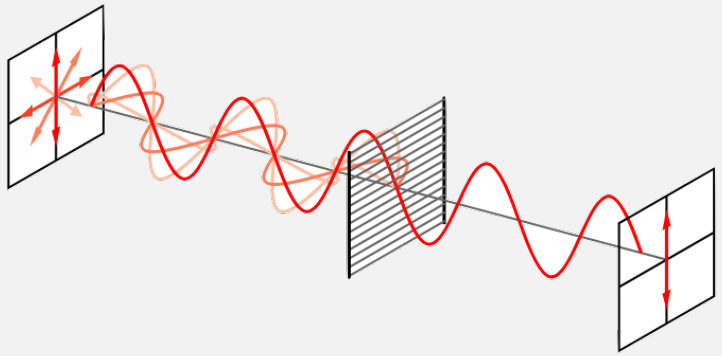
WHAT IS POM?

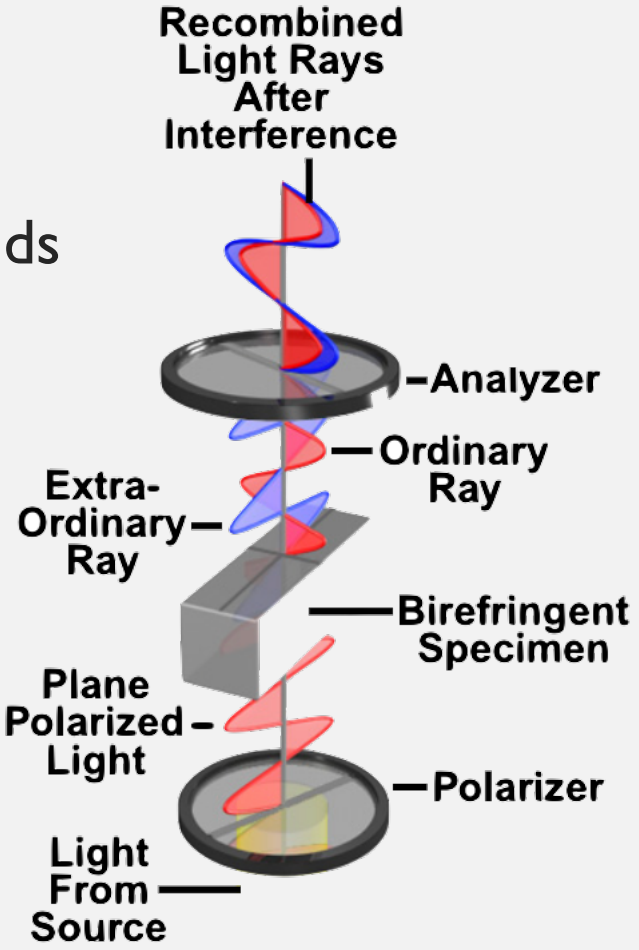
- Polarizer / Analyzer: Optical filter that lets light waves of a specific polarization pass through
- Waveplates are optical devices that alters the polarization state of a light wave
  - Half-waveplate: shifts the polarization direction of linearly polarized light
  - Quarter-waveplate: converts linearly polarized light into circularly polarized light and vice versa
- Condenser: collects light from source and assures uniform illumination of samples
- Transmission and reflection POM follow this light path



BASIC LIQUID CRYSTAL OPTICS

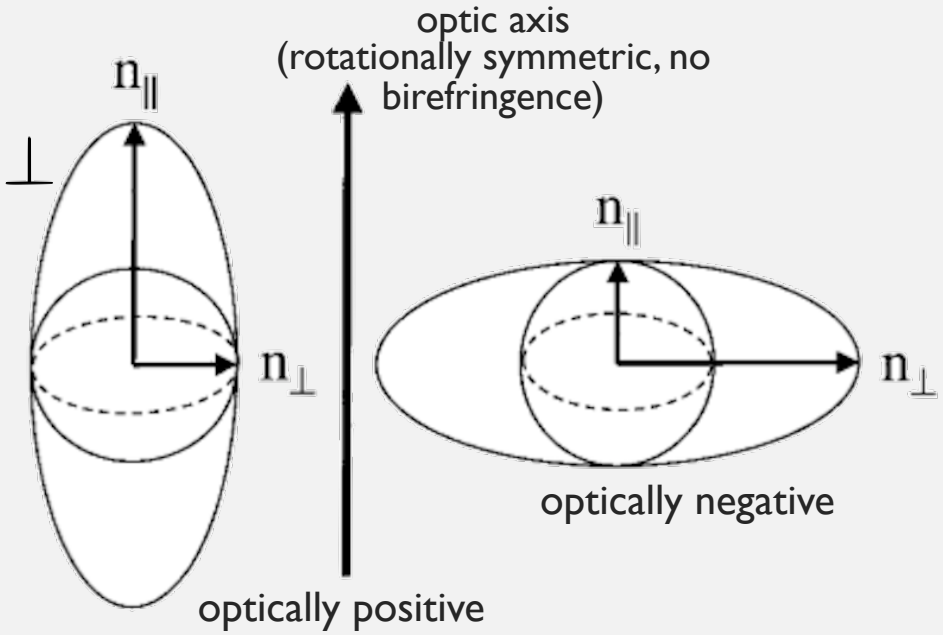
- Liquids are isotropic: index of refraction is independent of the incident light beam
- Crystalline materials are anisotropic: index of refraction depends on the direction of propagation of light (exhibit birefringence)
- Birefringent materials produce two individual wave components when one wave passes through
- Ordinary ray: follows Snell's law
- Extraordinary ray: perpendicular to ordinary ray





BASIC LIQUID CRYSTAL OPTICS

- Most liquid crystal mesogens have rotational symmetry around an optical axis
  - These comprise uniaxial phases (no rotational symmetry – biaxial)
- We can study the indicatrix of an LC phase to understand it's birefringence
  - We note the index of refraction parallel and perpendicular to the optical axis of sample
    - Sample can be optically positive or optically negative depending on the sign of $\triangle n = n_{\parallel} - n_{\perp}$
      - Positive dn > 0
        - LC phases: N, SmA
          (optic axis coincides with director)
      - Negative dn < 0
        - LC phases: Cholesteric
          (optic axis coincides with helix axis)



BASIC LIQUID CRYSTAL OPTICS

- An incoming beam splits into two rays in material
- These propagate at different velocities because they experience different refractive indices n_o and n_e which leads to a total phase difference of waves:

$$\delta = \frac{2\pi}{\lambda}(n_e - n_o)d$$

  - Depends on vacuum wavelength and distance travelled
  - Indices of refraction are related to principal indices by

$$n_o = n_\perp \qquad n_e = \frac{n_\parallel n_\perp}{\sqrt{n_\parallel^2 \cos^2\phi + n_\perp^2 \sin^2\phi}}$$

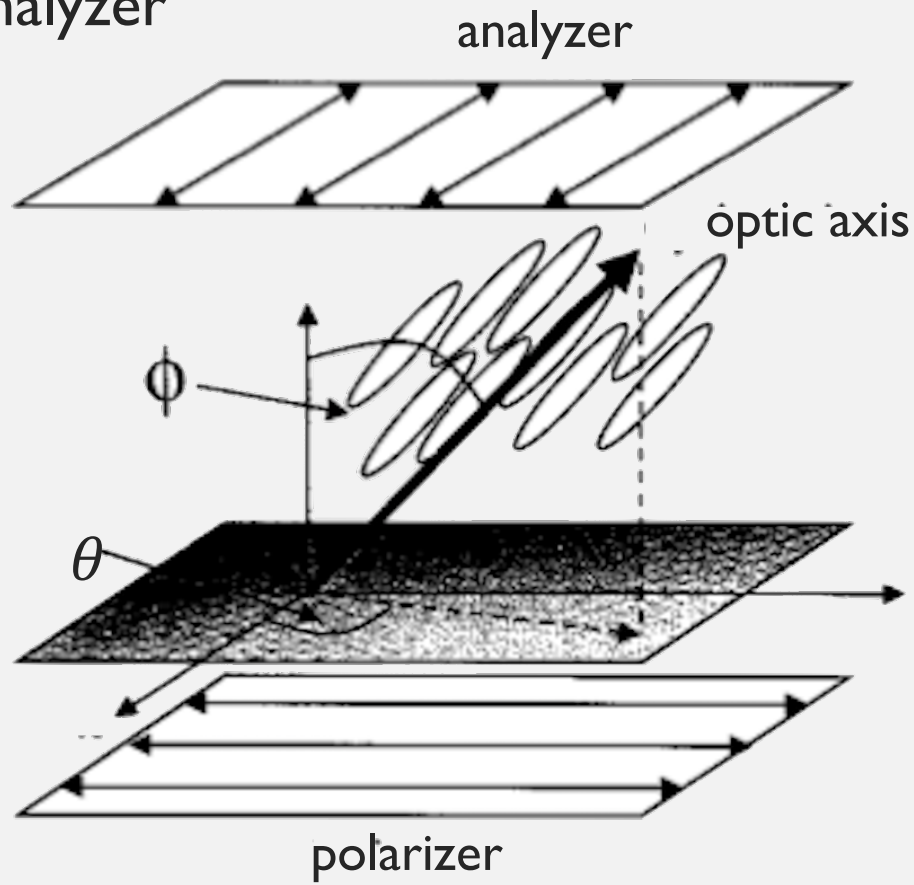
  - Depends on angle between the optic axis and the direction of light propagation

BASIC LIQUID CRYSTAL OPTICS

- In general, linearly polarized light → elliptically polarized light by crystal with a component that can pass through the crossed analyzer
- Transmitted light intensity is given by

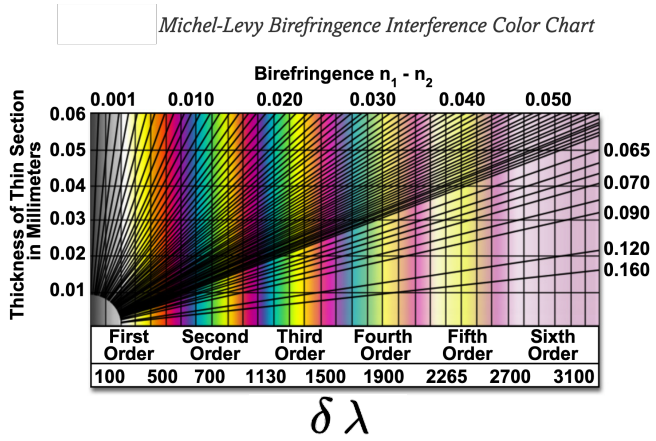
$$I = I_0 \, sin^2 2\theta sin^2 \frac{\delta}{2}$$

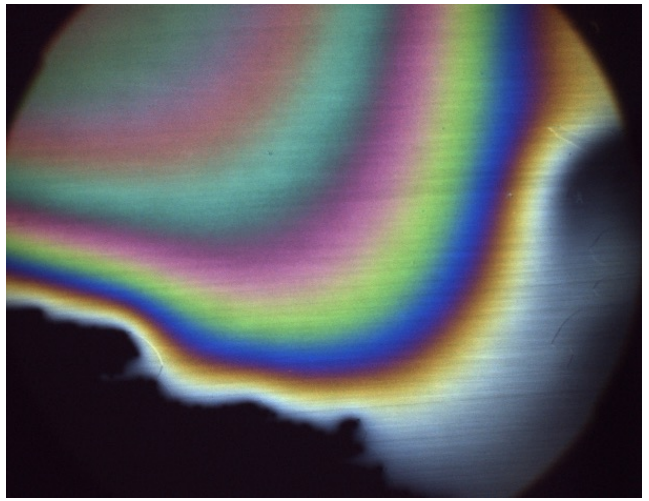
- I_o is the light intensity after the polarizer and θ is the angle between the analyzer and the projection of the optic axis onto the sample plane



THEORY OF BIREFRINGENCE

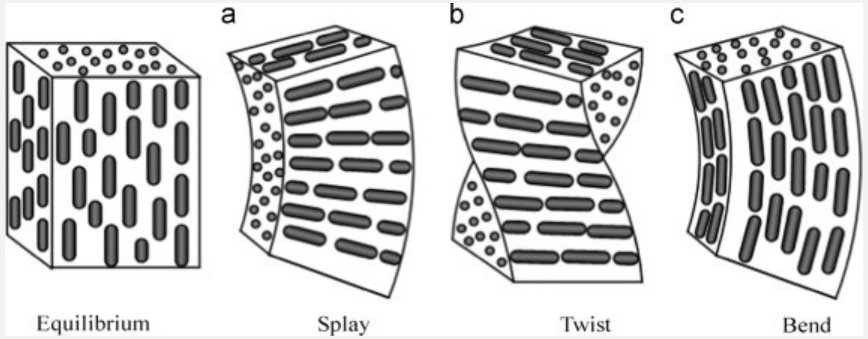
- Birefringence of an anisotropic material is estimated when observed in POM
- A relationship between interference color and phase difference between rays is developed
- This graph plots retardation on the x-axis and specimen thickness on the y-axis
- Birefringence is determined by a family of lines that emanate radially from the origin
- Each line has a measured value of birefringence corresponding to thickness and interference color

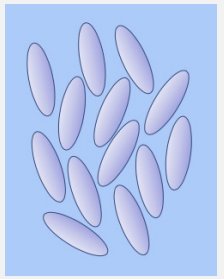




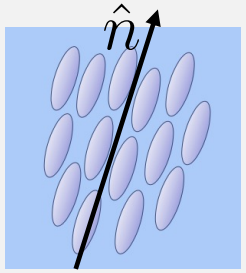
BASIC LIQUID CRYSTAL OPTICS

- Long range order in LC is characterized by director $\hat{n}$
  - Average orientation of the molecules within a small volume of LC
- Topological defects form in LCs when constraints or boundary conditions cannot be satisfied by continuous strain of the LC director via:
  - Bend:
  - Twist:
  - Splay:

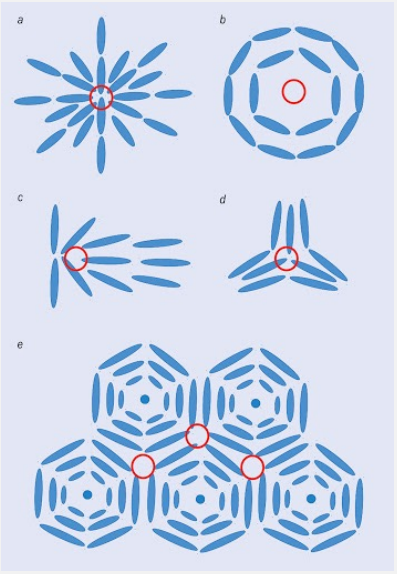




Isotropic Phase
No Orientational Order



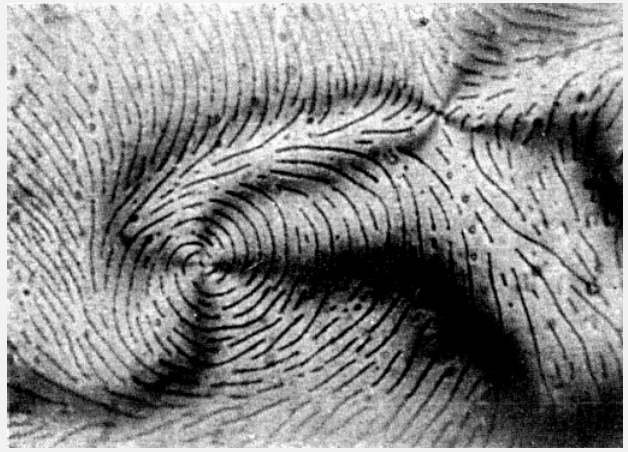
Nematic Phase
Specified Director $\hat{n}$

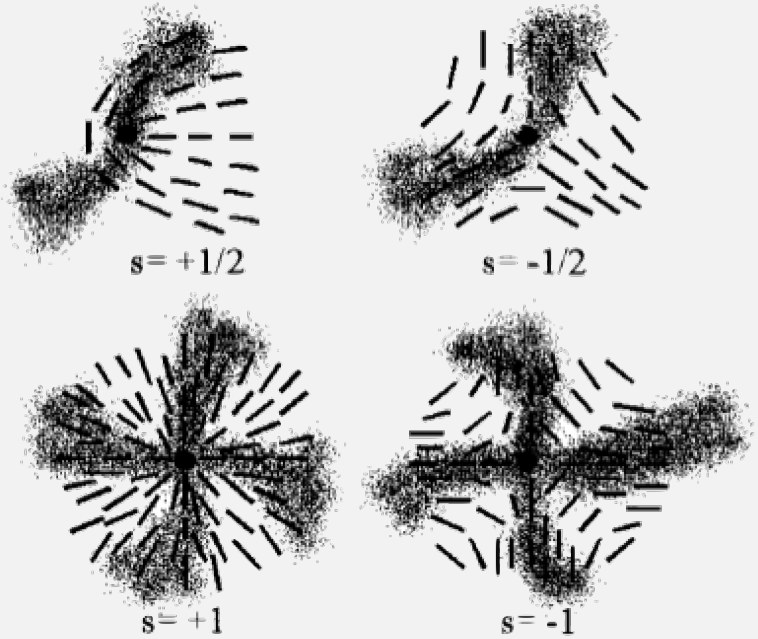


Examples of
Topological Defects

UNAMBIGUOUS RECONSTRUCTION OF THE DIRECTOR AND WINDING NUMBERS OF THE DEFECTS

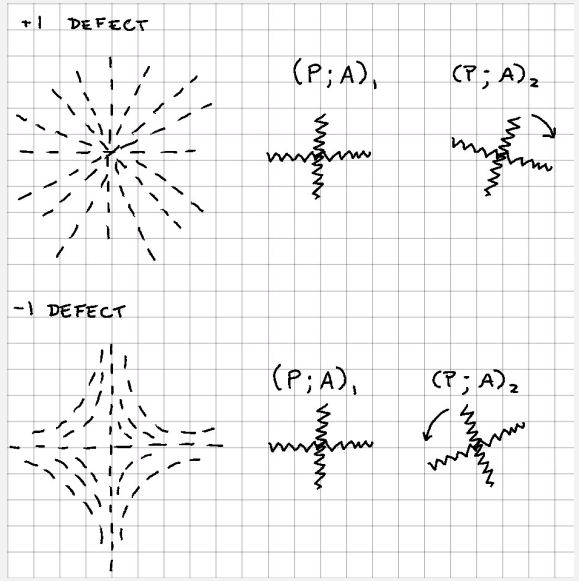
- We study Schlieren textures observed between crossed polarizers
- Around defect, the number of brushes oriented along the polarizer or analyzer direction equals four times the winding number of the defect
  - N = 4s
- Dark brushes in the polarized image mark places where the director lies in the direction of either a polarizer or an analyzer
- Defects are found at intersections of these brushes
- The common pinwheel textures with ±1 winding are recognized by their characteristic four brushes.
- Cholesterics are described elsewhere
- *Textures of Liquid Crystals (Dierking)*

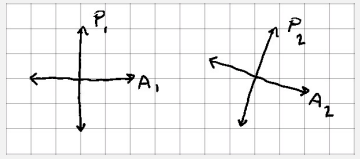




DEFININING DEFECT WINDING NUMBER

- In addition to defining charge, it is possible to define the sign of the charge of a defect using rotation
- When rotating polarizer/analyzer brushes will rotate
  - In the same direction: defect is +
  - In the opposite direction: defect is -





- This can be described mathematically as follows:

$\varphi = n\theta$

$n \rightarrow$ WINDING NUMBER

$\theta_0 \rightarrow$ ANGLE OF P-A

DARK BRUSHES APPEAR WHEN LC LINES UP WITH:

POLARIZER: $\varphi = \theta_0 + m\pi$

ANALYZER: $\varphi = \theta_0 + m\pi + \frac{\pi}{2}$

SO,

$n\theta = \theta_0 + m\pi$

$n\theta = \theta_0 + m\pi + \frac{\pi}{2}$

SOLVING FOR θ;

$\theta = \frac{1}{n}(\theta_0 + m\pi)$

$\theta = \frac{1}{n}(\theta_0 + m\pi + \frac{\pi}{2})$

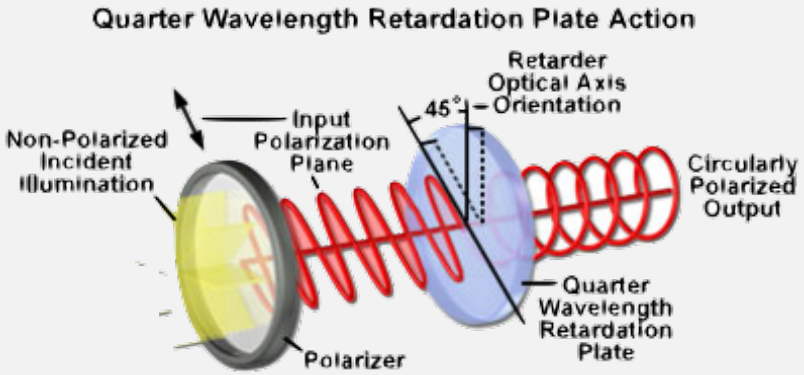
AS θ_0 INCREASES

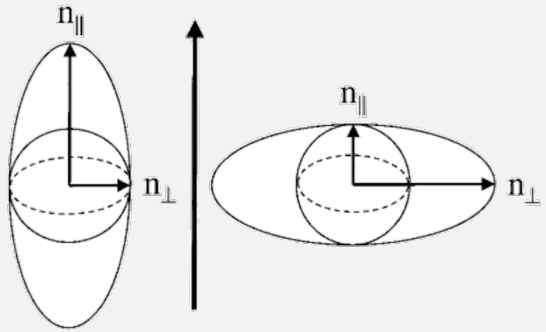
$n > 0$ θ INCREASES

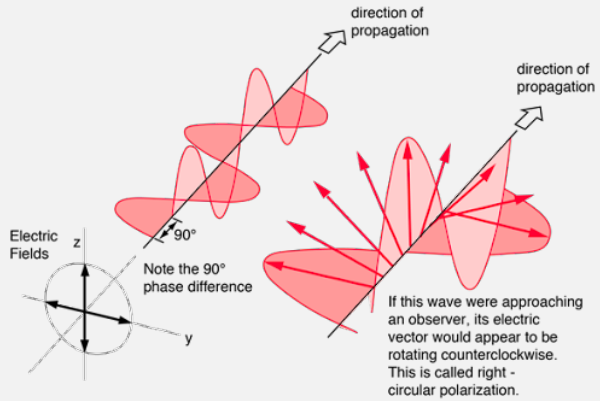
$n < 0$ θ DECREASES

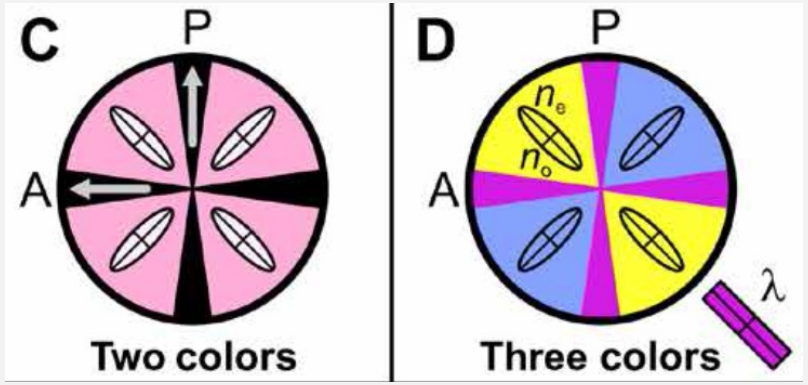
UNAMBIGUOUS RECONSTRUCTION OF THE DIRECTOR AND WINDING NUMBERS OF THE DEFECTS

- Wave plate use with POM
  - Incoming light beams split into ordinary rays and extraordinary rays associated
  - Inserting a wave plate at 45° with respect to the polarizers produces different interference colors along the slow and fast axes associated with *n*_e and *n*_o
- In general: plate operates by introducing a relative phase shift between the orthogonal wavefronts passing through the material
  - A phase shift between the ordinary and extraordinary components converts the incident linear polarized light vibrations into either elliptical or circularly polarized light.





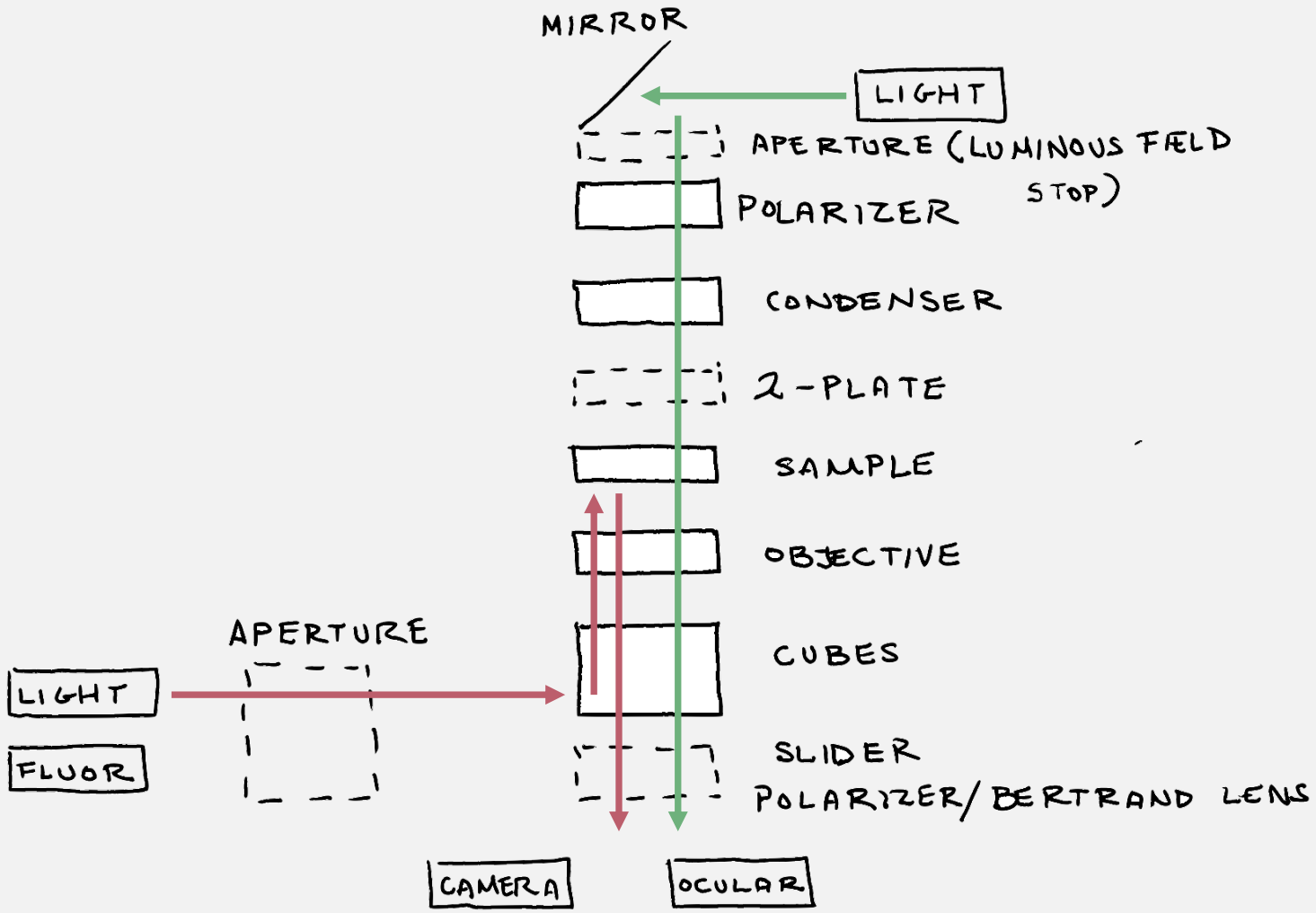




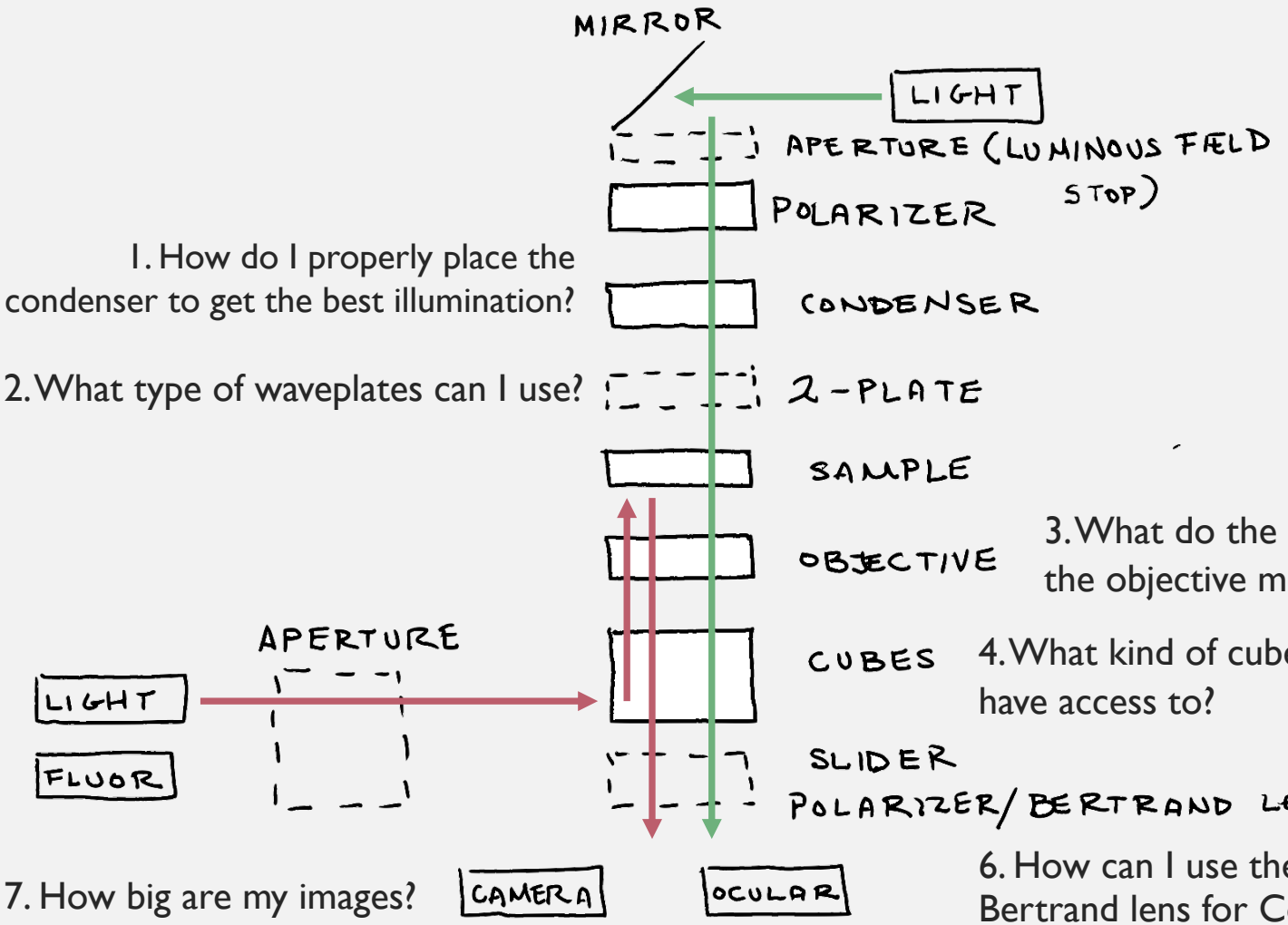
OTHER METHODS FOR STUDYING CONTRAST

- Differential interference contrast microscopy (DIC)
  - DIC works on the principle of interferometry to gain information about the optical path length of the sample, to see otherwise invisible features.
  - DIC works by separating a polarized light source into two orthogonally polarized mutually coherent parts which are spatially displaced at the sample and recombined before observation
  - The interference of the two parts at recombination is sensitive to their optical path difference
- Hoffman modulation contrast microscopy
  - Optical microscopy technique for enhancing the contrast
  - Like DIC microscopy, contrast is increased by using components in the light path which convert phase gradients in the specimen into differences in light intensity that are rendered in an image that appears three-dimensional
  - Consists of a condenser with a slit aperture, an objective with a slit aperture, and a polarizer which is fitted between the condenser and the illumination source and is used to control the degree of contrast.

OUR POLARIZED OPTICAL MICROSCOPE



FREQUENTLY ASKED QUESTIONS



1. How do I properly place the condenser to get the best illumination?

2. What type of waveplates can I use?

3. What do the symbols on the objective mean?

4. What kind of cubes do I have access to?

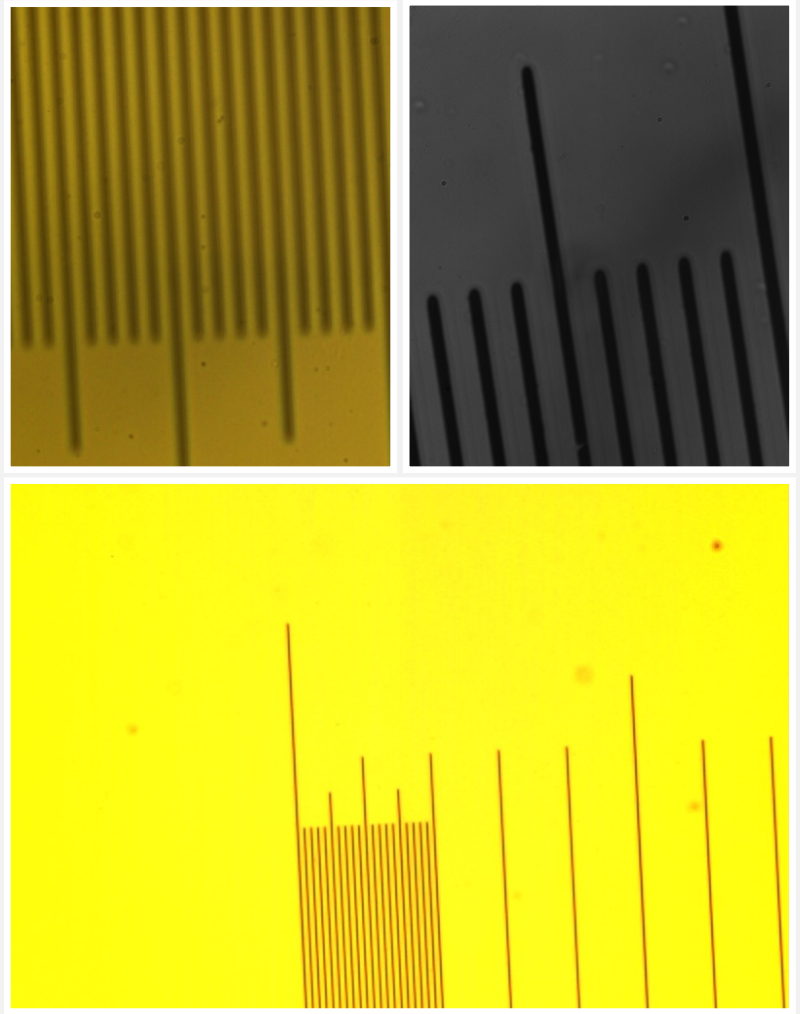
5. How can I use Fluorescent Microscopy?

6. How can I use the Bertrand lens for Conoscopic microscopy?

7. How big are my images?

DETERMINING IMAGE SIZE

- Calibration measurement with small ruler can be used to determine size of sample at different objective magnifications
- Each small line is 10 micrometers
- Imaged here: 60x, 20x, 5x
- We can formally take this measurement and calibrate for pixels in the future

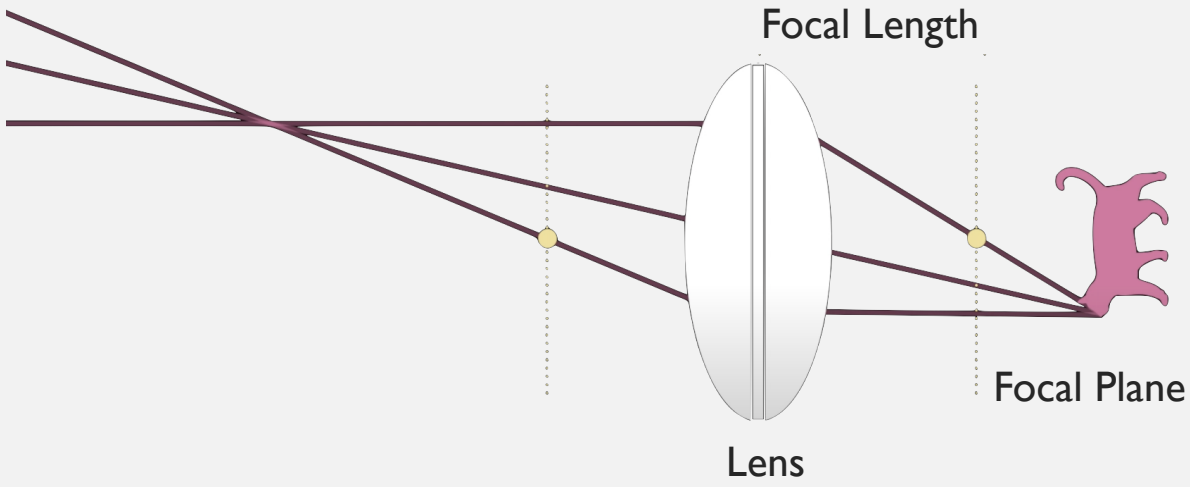


KOHLER ILLUMINATION

- Köhler illumination acts to generate an even illumination of the sample and ensures that an image of the illumination source (for example a halogen lamp filament) is not visible in the resulting image.
- Also, this illumination maximizes contrast and minimizes artifacts
- Achieving Kohler illumination depends on aligning optics with respect to the sample and each other
  - When you focus on your sample, you are aligning objective to the sample.
  - For best illumination, optics need to be aligned too!
  - Much needed for Conoscopic imaging

KOHLER ILLUMINATION

- Optics consist of lenses and diaphragms
  - Diaphragms can be open and closed to let more or less light through them
  - Lenses either focus light or defocus light (reversable) and follow simple rules for all light coming off objects



  - Off of an object you can form a collimated beam of light (at focal plane) or an image of the object

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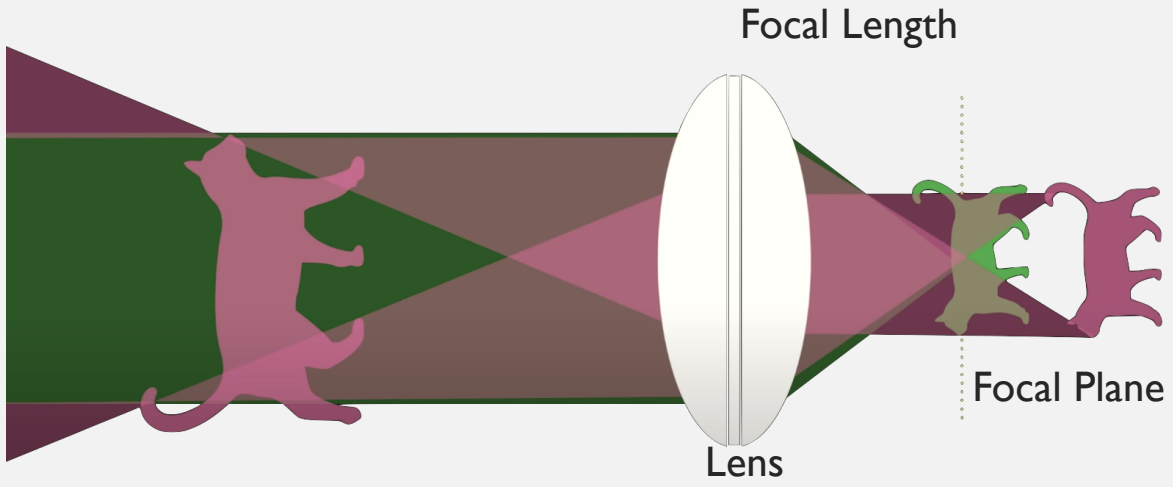
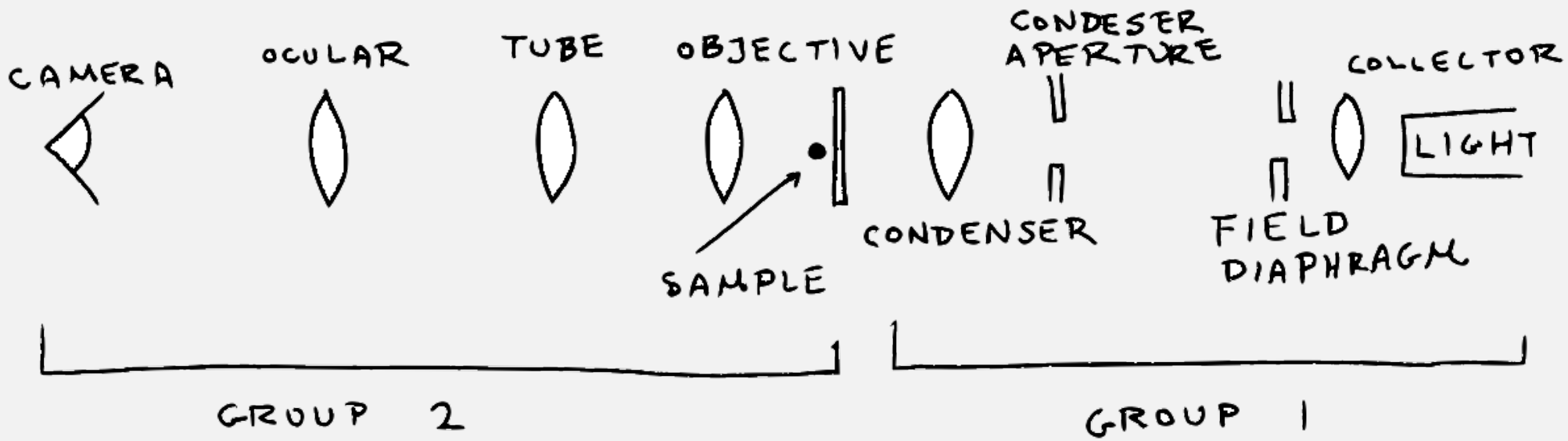


  - Off of an object you can form a collimated beam of light (at focal plane) or an image of the object

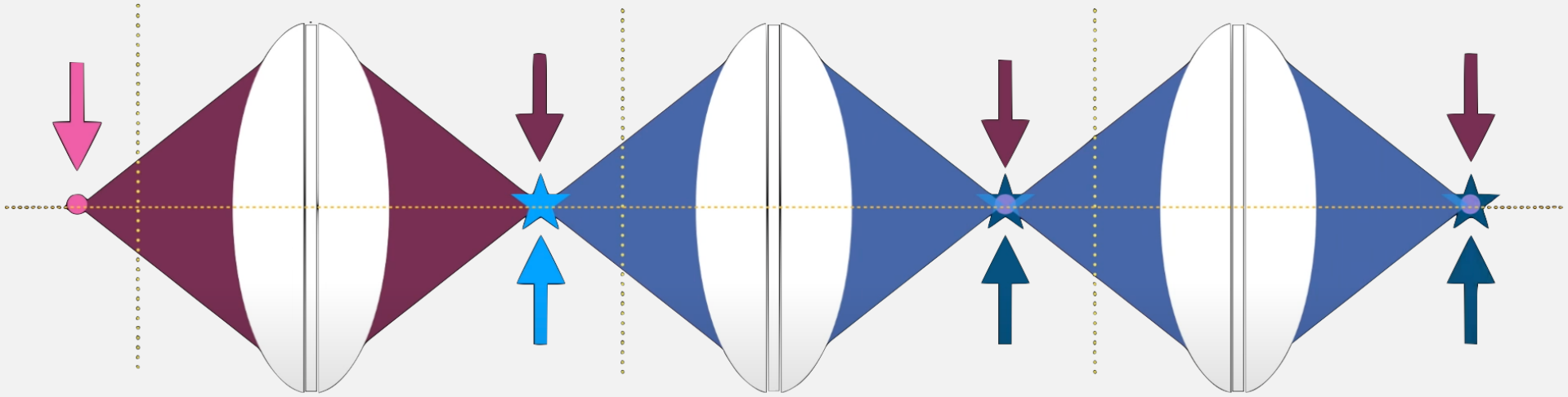
KOHLER ILLUMINATION

- Microscope is a series of lenses and diaphragms
- Optics can be placed in two groups
  - Group 1: Illumination
  - Group 2: Image forming



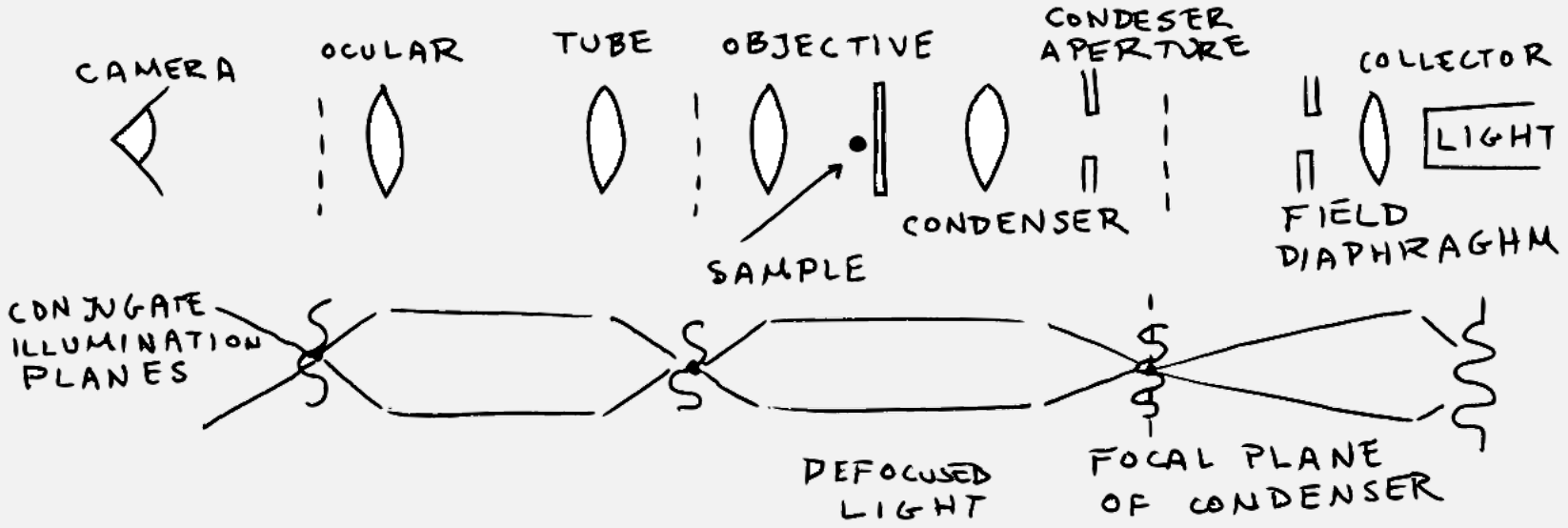
KOHLER ILLUMINATION

- For Kohler illumination we focus on the camera, the objective, the sample, the condenser, and the field diaphragm
- Goal is to evenly illuminate sample and not have an image of bulb on sample or camera
  - We can think of conjugate focal planes



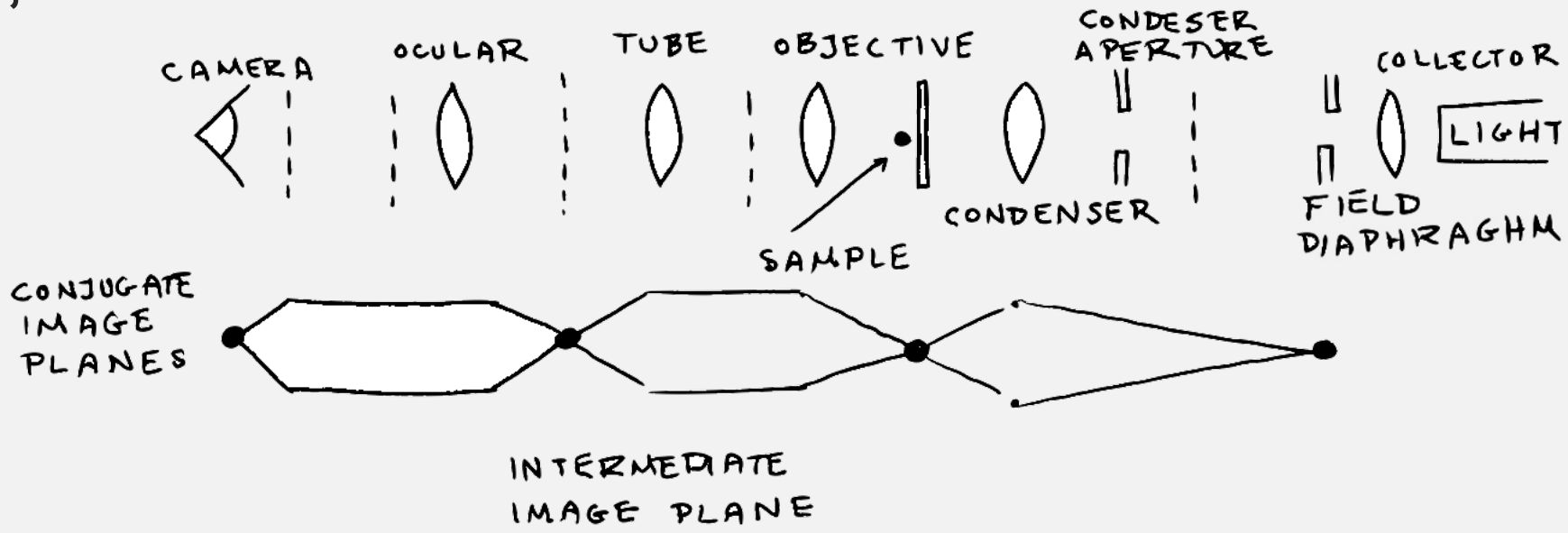
KOHLER ILLUMINATION

- There are two types on conjugate focal planes in a microscope
  - First, conjugate Illumination planes (Image)
  - Key: making sure that the image of the light source is in the focal plane of the condenser
  - To get this we need to understand the second conjugate focal planes



KOHLER ILLUMINATION

- There are two types on conjugate focal planes in a microscope
  - Second, conjugate image planes (Image)
  - Key: an in-focus image of field diaphragm should be superimposed on the image of the sample on the camera
  - Aligned condenser forms image of FD while illuminating sample with defocused light
  - We can adjust the condenser to make sure this is the case

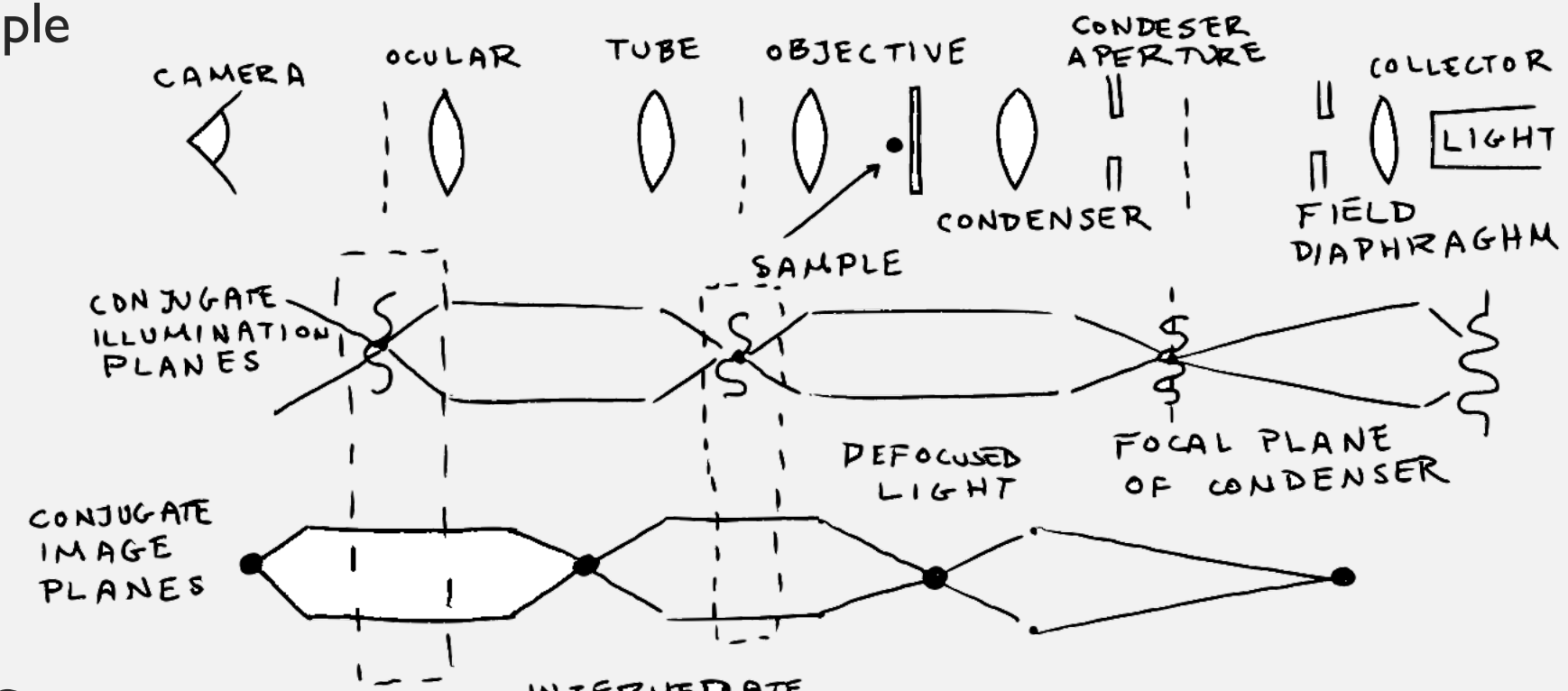


KOHLER ILLUMINATION

- How to align a microscope Kohler illumination
  - Place objective correctly by focusing on sample
  - Close the field diaphragm to get it in field of view
  - Move condenser axially to focus field diaphragm (move condenser so that the FD is centered)
  - Sample will be illuminated with defocused light!
  - Illumination must be aligned relative to the image formation
  - This should be done every time you change the objective

CONOSCOPIC IMAGING

- Conoscopic imaging uses polarized convergent light to view birefringent samples
- We look to see the interference pattern in the back focal plane of the objective behind the sample



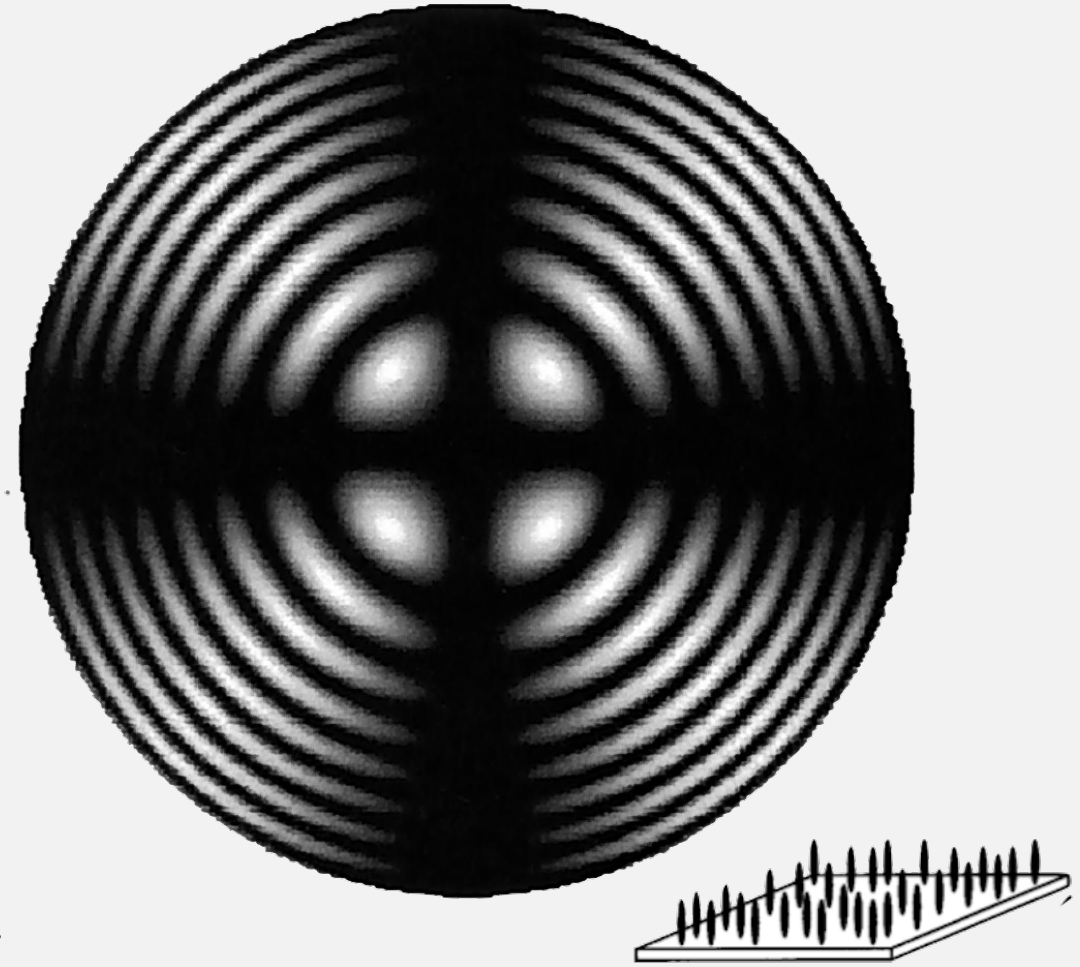
Conjugate conoscopic image planes

CONOSCOPIC IMAGING AND OBJECTIVE USE

- To view the pattern: insert Bertrand lens (upside down and on the left side) or remove the eyepiece to view the back focal plane of the objective
- Conoscopic observation is carried out at x40 or higher magnification
- It is important to make sure the objective is BF *ie. not phase contrasted*
  - When imaging a sample via brightfield illumination, the contrast between the sample and background can be minimal, as it only depends on absorption.
  - Phase contrast microscopy increases image contrast by converting phase changes into amplitude changes at the image plane.
  - All objectives are either BF and unlabeled, or labeled PhI, Ph2, etc.
  - There is a proper condenser that matches each objective, which can be changed on the microscope

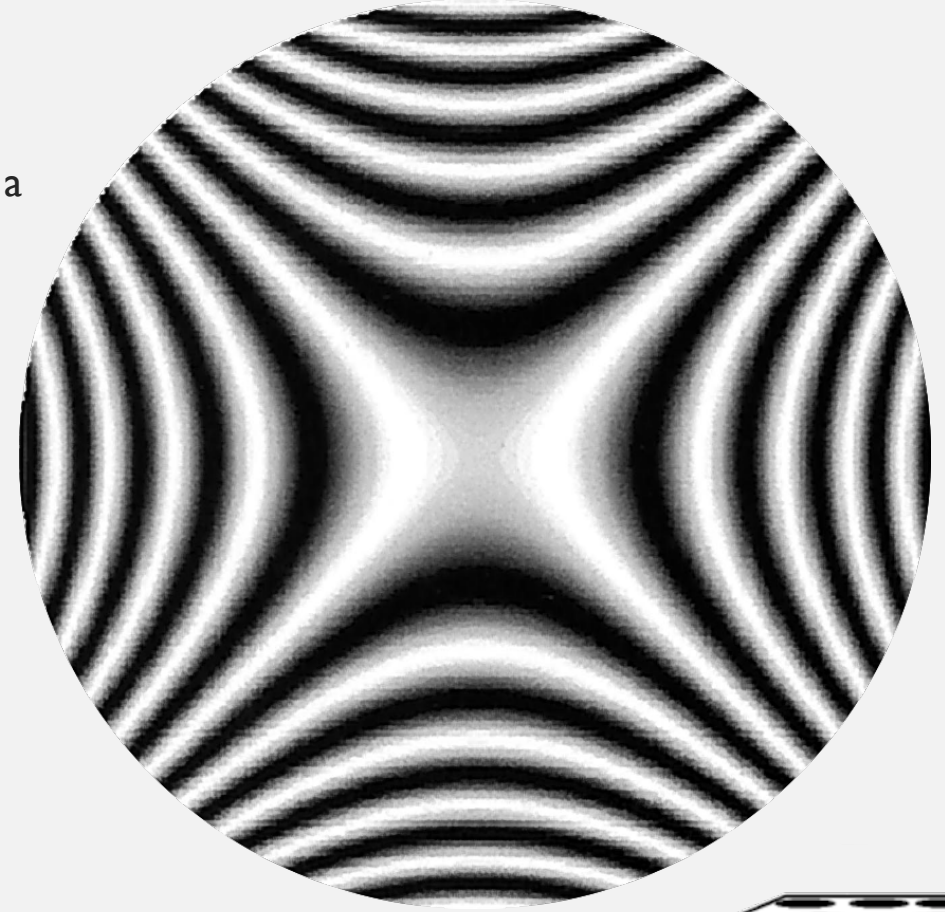
CONOSCOPIC IMAGING

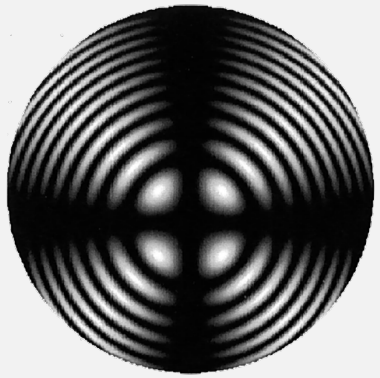
- Conoscopic figures consist of dark fringes and extinction brushes (isogyres)
- Homeotropic Anchoring
  - Exists when a uniaxial crystal's optic axis is aligned normal to the viewing surface
  - Interference figure will have concentric, circular fringes and an extinction cross
- The center of the interference figure corresponds "to zero-order retardation"
  - Light that propagated through the sample to this center point has propagated along the optic axis with no birefringence
  - Has traveled along the path of minimum phase difference.

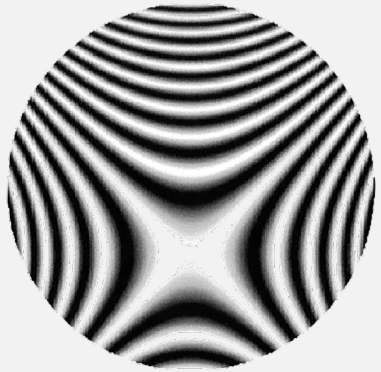


CONOSCOPIC IMAGING

- Planar Alignment
  - Exists when crystals aligned parallel to the surface
  - The interference figure will be a set of conjugate hyperbolas centered on a common locus
- Light propagating to the locus will have traveled along a path of relative maximum phase difference resulting from the high birefringence along this path
- Method for understanding birefringence tilt angles in the samples that produce these images can be found at
  - *Analysis of the conoscopic measurement for uniaxial liquid-crystal tilt angles (Winter)*





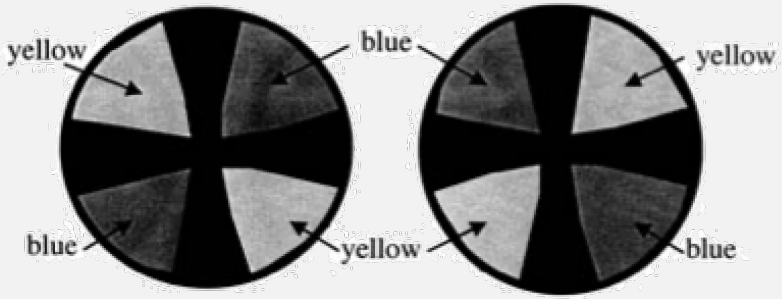


CONOSCOPIC IMAGING

- What are the uses of conoscopic imaging?
  - Discriminate between uniaxial and biaxial samples
    - Uniaxial: Maltese cross pattern, does not change when sample is rotated
    - Biaxial: "two sets of hyperbolic lines, with the apexes at the points where the optic axis pierces the plane perp to the direction of light propagation"???
  - Distinguish optically positive and negative materials
    - A waveplate can be added to determine which of the ordinary or extraordinary beam is the fastest

Uniaxial direction of light propagation II to optical axis

Biaxial



optically positive optically negative

FLUORESCENT IMAGING

- FL light source: X-Cite 120Q Iris FL Light Source
- This can be switched in and out with the halogen lamp used for reflection with appropriate tool
  - Warning! Do not look through microscope with no filter cube (UV = Ouch)
  - Please be careful with the halogen light source (The wires are finnicky)
- In each experiment a Zeiss made filter cube is used in reflection mode
- The cube first filters all light out except of a certain bandwidth to excite the sample
- As the sample emits light, it is passed through a second bandwidth filter
- Samples with appropriate emission and absorption spectra can be viewed with this imaging with or without POM depending on the sample
- A complete list of filters can be found here: https://www.micro-shop.zeiss.com/en/us/shop/filterAssistant/filtersets/
- All available filter cubes are also listed at osujilab → facilities → zeissaxio

FILTER CUBES, WAVEPLATES, SLIDERS

- Filter Cubes
  - The microscope is currently equipped with 8 possible filter cubes
  - These filter cubes consist of different combinations of bandwidth filters, polarizers, mirrors, and beam stops
- Sliders
  - The microscope is currently equipped with 6 possible sliders
  - These include, polarizers, unique lenses, and diaphragms
- Waveplates (Lens Sliders)
  - The microscope is currently equipped with 2 waveplates
- For a full list of available parts osujilab → facilities → zeissaxio

QUESTIONS OR COMMENTS?

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