Light Microscopy Basics

Background, Concepts, and Applications

Michael O'Relley
Carl Zeiss Microscopy, LLC.
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Agenda

1	Overview
2	Major R&D areas of materials microscopy
3	Types of Light Microscopes
4	Contrast and Imaging Modes in Microscopy

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1. Overview
2. Major R&D areas in microscopy
3. Types of Light Microscopes
4. Contrast and Imaging Modes in Microscopy

Zeiss Beginnings

Early microscope,
18th century France

Stativ 1, 1857

ZEISS workshop in Jena, Germany

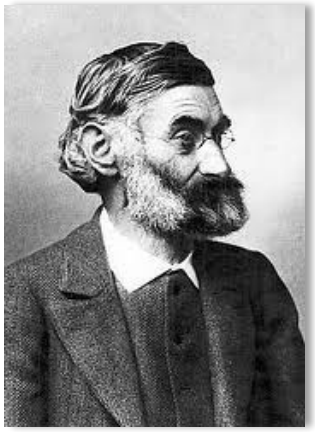
Abbe's equation for lateral resolution

$$d = \frac{\lambda}{2n\sin\alpha}$$

Zeiss Beginnings

Carl Zeiss

Began producing microscopes in 1846



Ernst Abbe

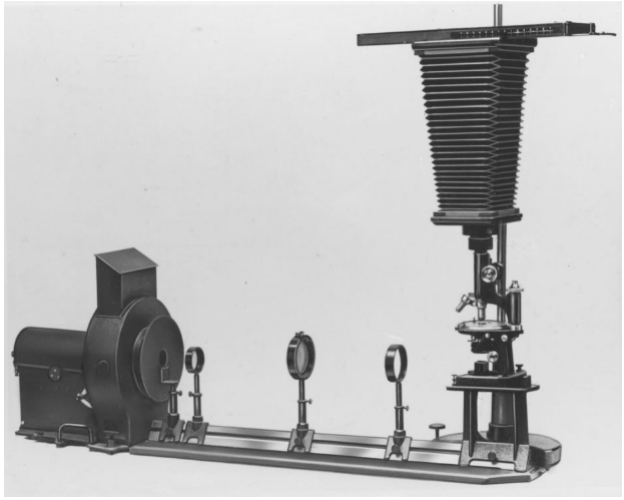
Physicist - developed mathematical foundations upon which microscopes are still designed today

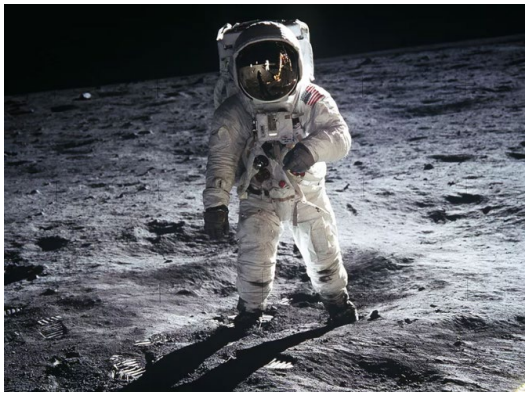
Otto Schott

Chemist - pioneered high-quality glass and glassmaking techniques

Zeiss Beginnings

Many microscopy innovations (first infinity optics, first aligning light path, first computer control, etc.) have been pioneered by Zeiss

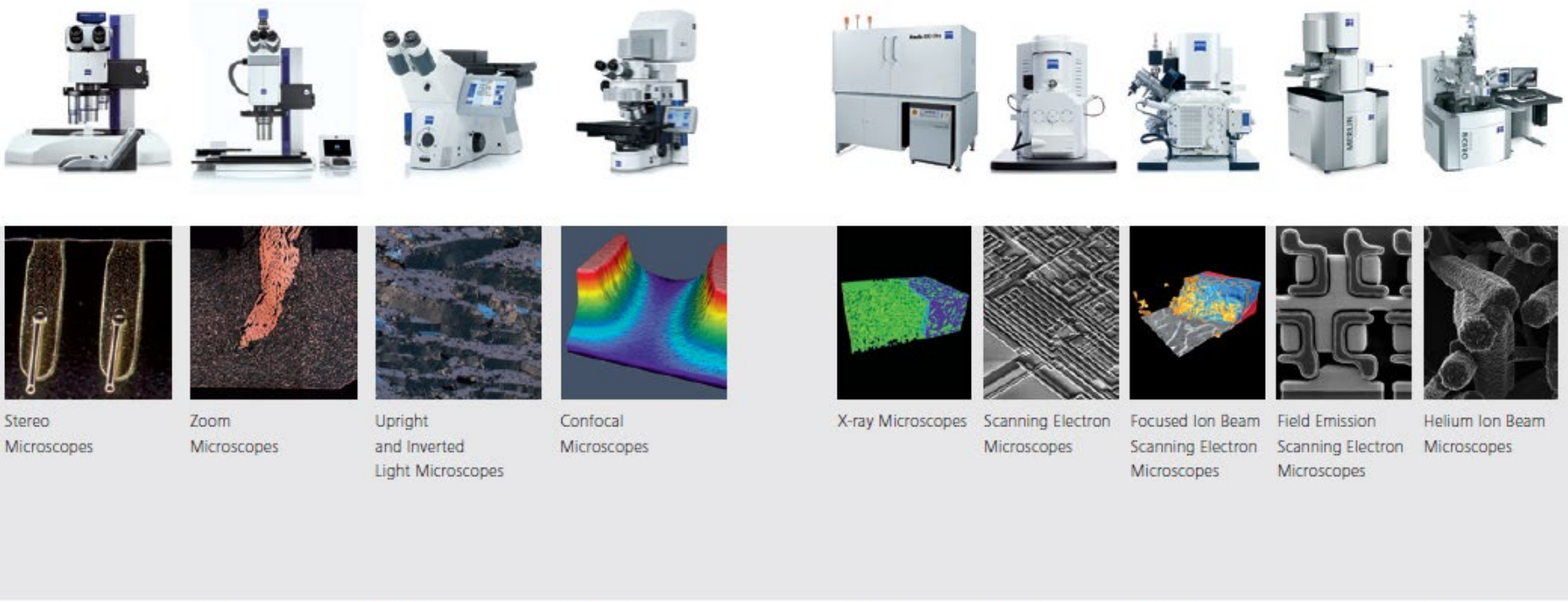




Zeiss Organization Today

Eyeglasses, sports optics, microscopes, SEMs, surgical instruments, coordinate measurement machines, semiconductor manufacturing systems

Zeiss Microscopes for Materials: Light, SEM, X-ray



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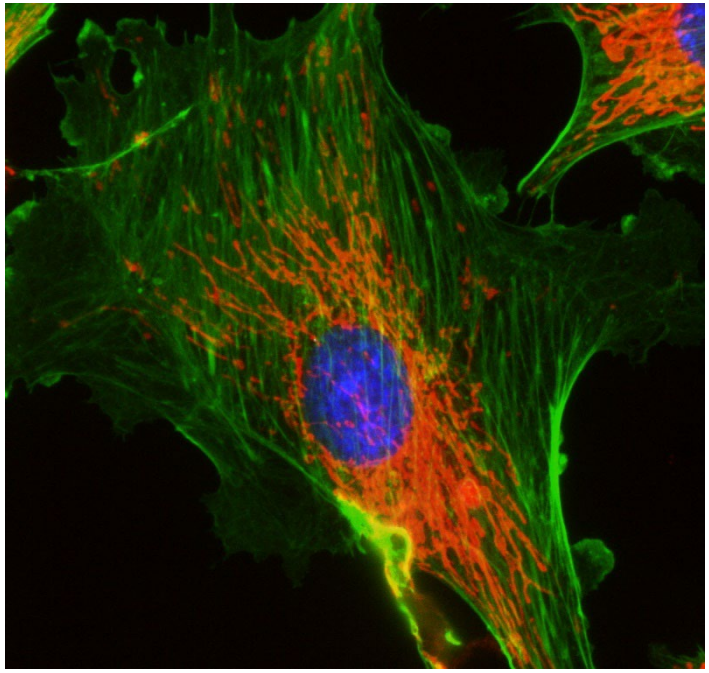
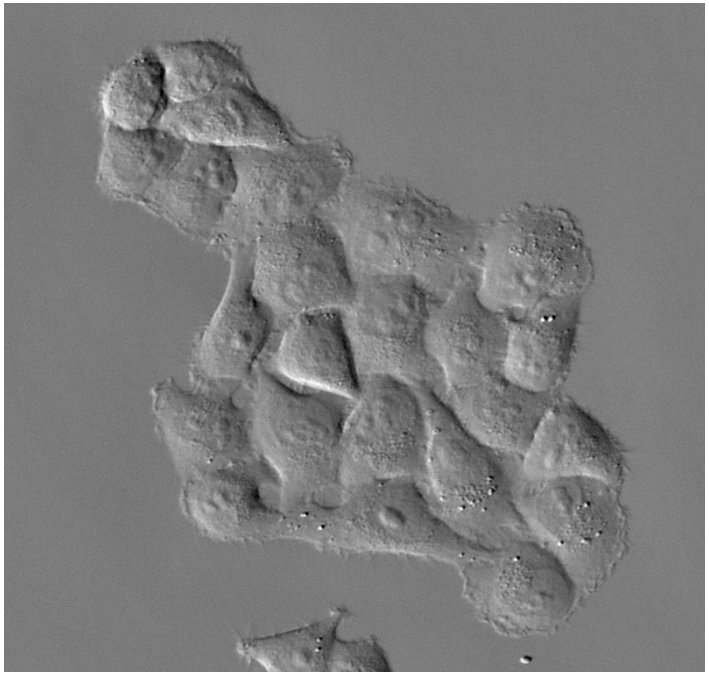
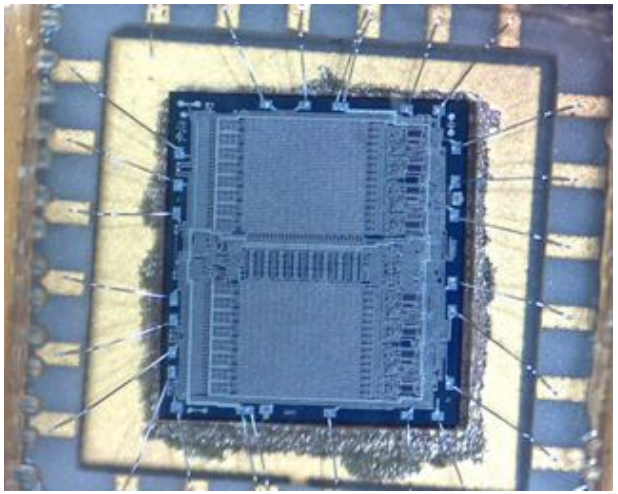
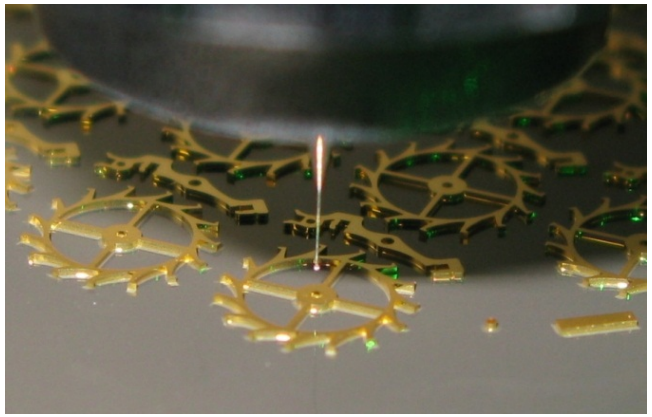
Biology, sure…

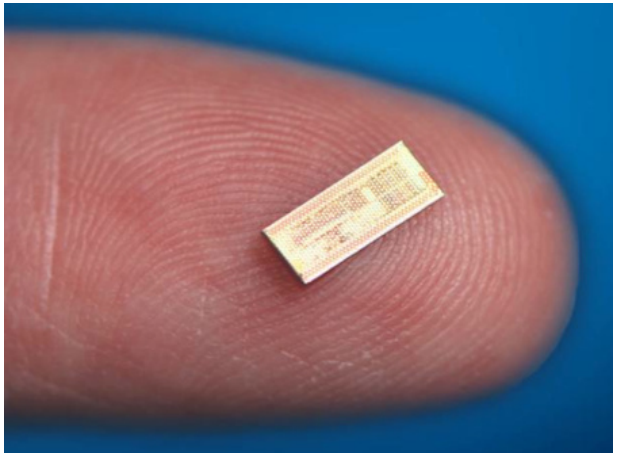




Semiconductors/Electronics/MEMS

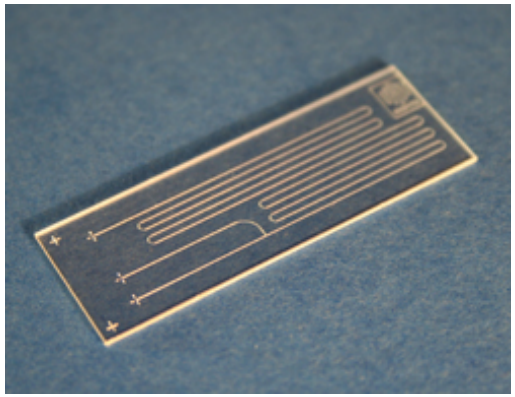




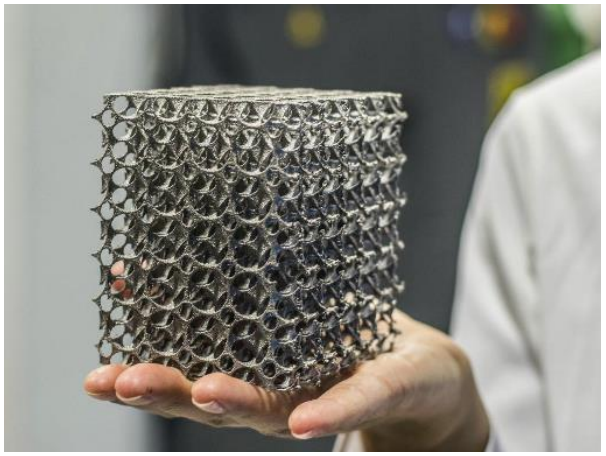


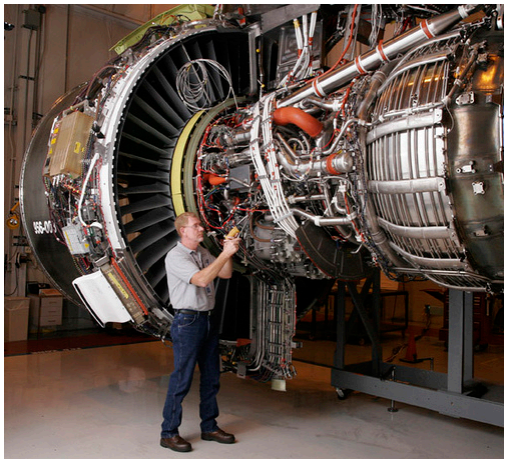
Composites

Polymers / Coatings



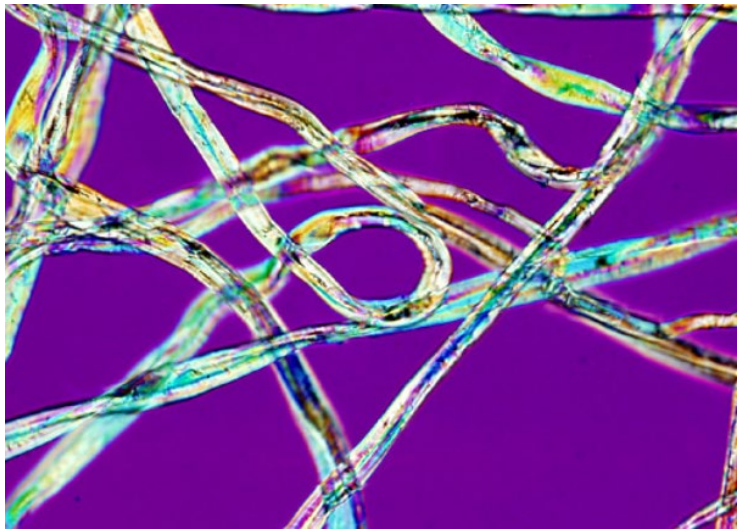
Metals





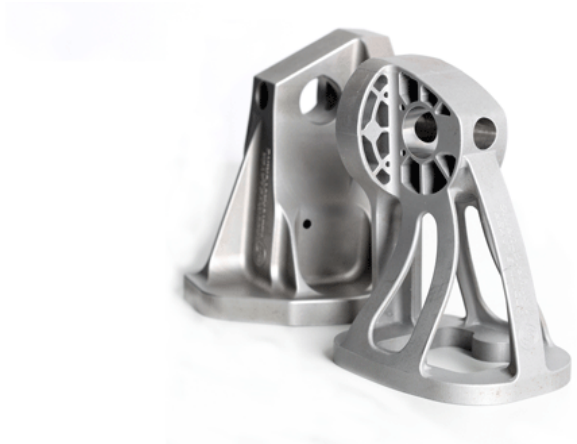
Geology, Oil, Gas, & Mining

Forensics



Energy and Batteries

Advanced Materials



Failure Analysis

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3. Types of Light Microscopes
4. Contrast and Imaging Modes in Microscopy

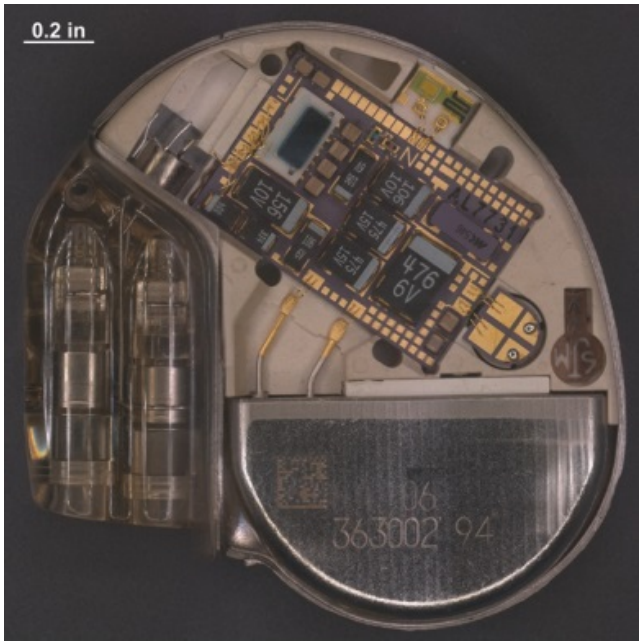
Stereo/Zoom Microscopes

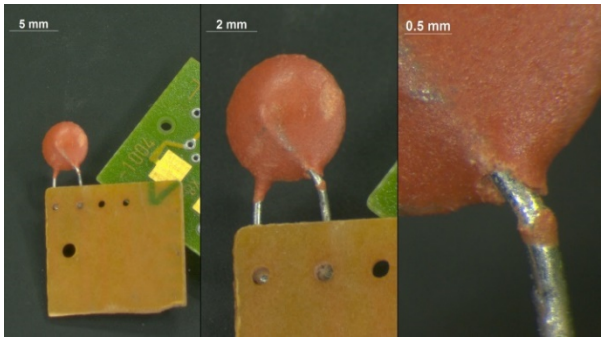
Stereo/Zoom Microscopes

Benefits:
- Large samples, large field of view, large depth of field
- Rarely have to manipulate sample for viewing
- Manipulate with hands / perform sample prep
- Stereoscopic image in eyepieces

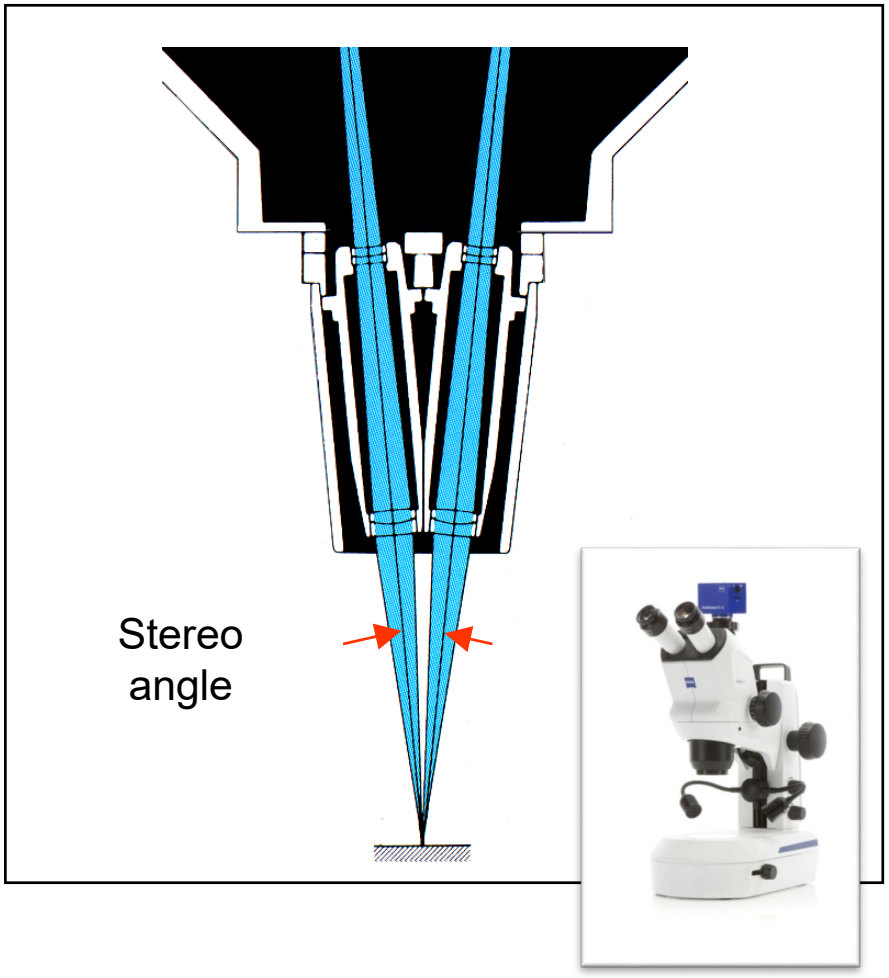
Constraints:
- Magnification- and resolution-limited

Applications:
- Viewing entire animals
- Routine inspection
- Dimensional measurement
- Fracture surfaces, mechanical testing
- First step in FA workflow





Stereo/Zoom Microscopes: Greenough Design



- Two separate microscope systems are inclined towards one another by a stereo angle of between 11 and 16 degrees
- Can add additional lenses to vary the mag ranges and working distances

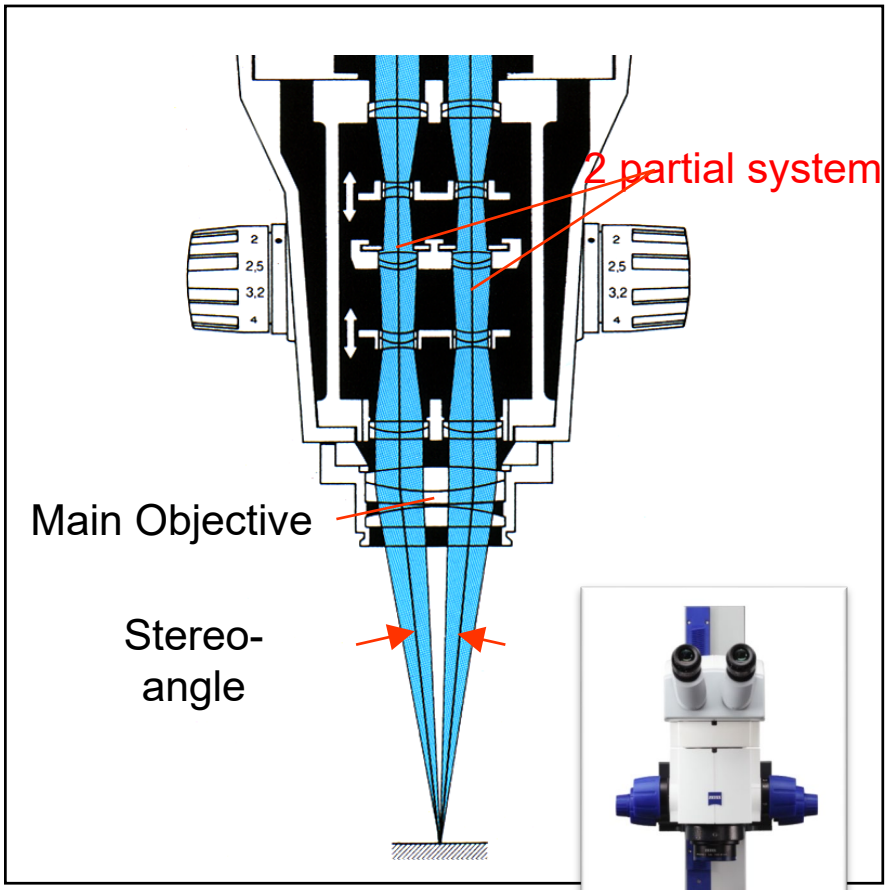
Benefits:

- High optical image quality: optical components in the center for best correction
- Compact design and moderate prices

Drawbacks:

- The axes of the optical systems are not perpendicular to the object field
- Reduced modularity due to the compact design

Stereo/Zoom Microscopes: CMO Design



- Two partial microscope systems run parallel to each other and share the same main objective
- The extra-axial beam pairs produce the stereoscopic angle

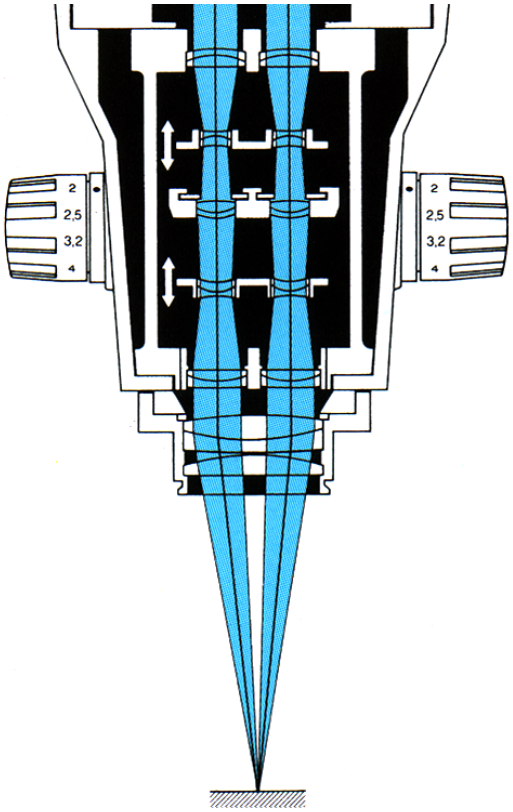
Benefits:

- Modularity: parallel beam paths above and below the magnification system permit the incorporation of additional equipment
- The axis of the objective is perpendicular to the object field: imaging easier with slider

Drawbacks:

- Main objective is used in the outside margin, maintaining image quality more challenging,
- More optical corrections → Higher price

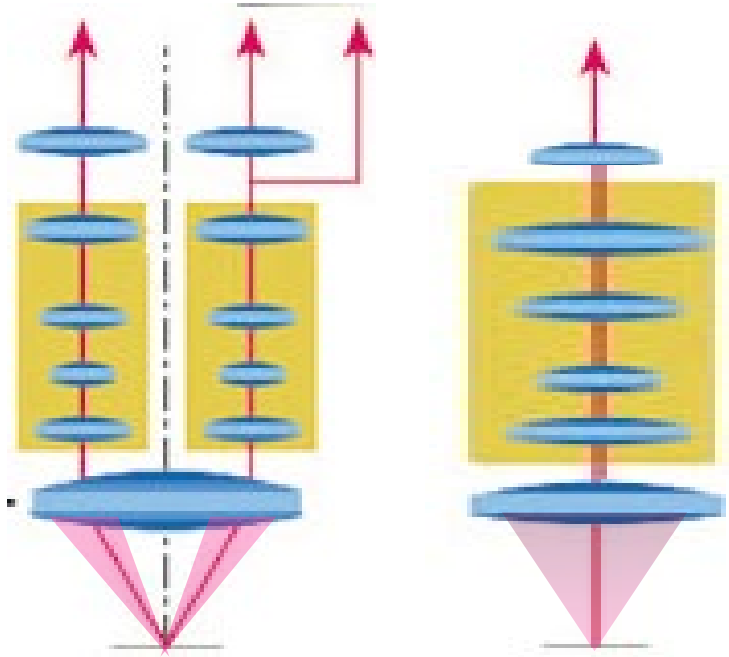
Stereo/Zoom Microscopes: CMO Design



Another drawback:

- Two adjacent light paths limit the numerical aperture of the optical system

Stereo/Zoom Microscopes: High-NA Zoom



Another drawback:

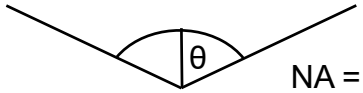
- Two adjacent light paths limit the numerical aperture of the optical system and therefore its resolution

If you have a single light path...

... you can make it much larger!

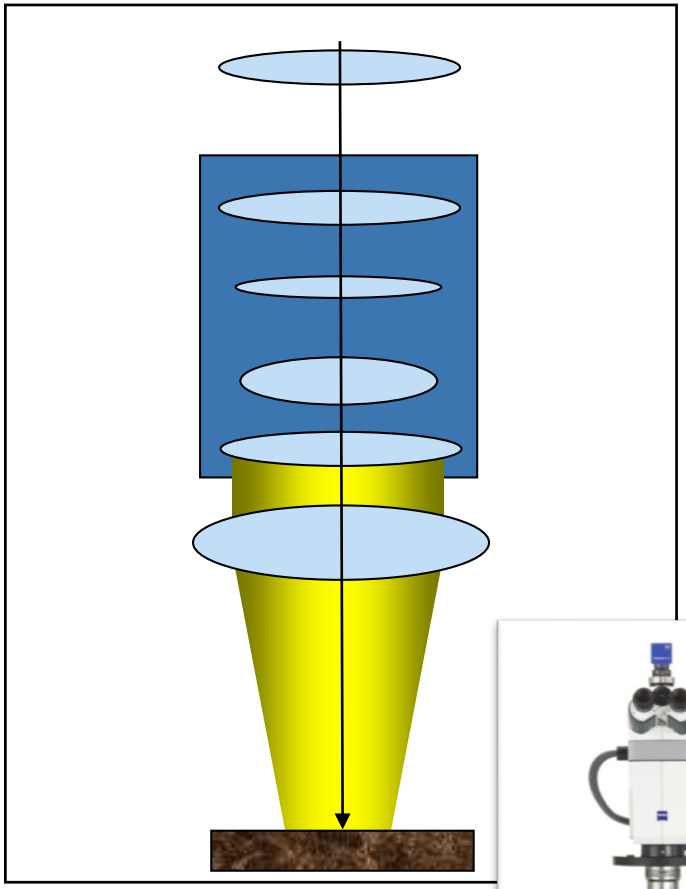
High-NA zoom microscopes have a single, super-wide light path, therefore a larger light-gathering angle and superior resolution

Resolution comes from:



NA = *n* sin θ

Stereo/Zoom Microscopes: High-NA Zoom

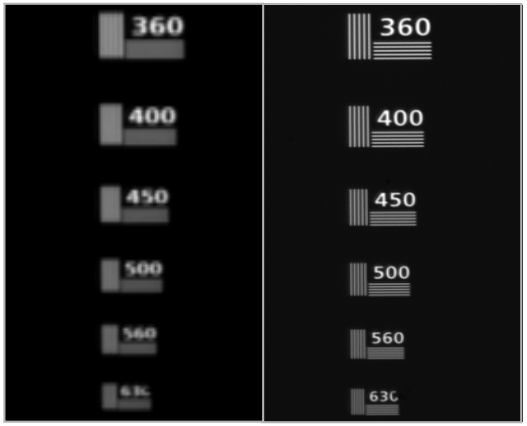


Benefits:

- Resolution: about double that of a traditional stereomicroscope, even at low zoom
- Can pack more information into a high-megapixel digital image
- Topography: more accurate due to smaller depth of field
- Brighter fluorescence imaging

Drawbacks:

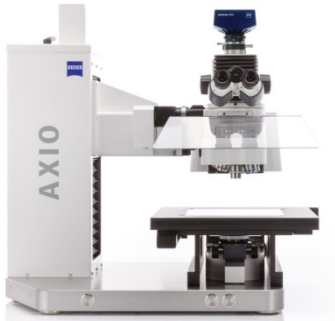
- Don't have a true stereo view
- Smaller depth of field
- Higher price



SteREO Discovery.V12
PlanApo S 1.0x
Beta: 70x

AxioZoom.V16
PlanApo Z 1.0x
Beta: 70x

Compound Upright Microscopes

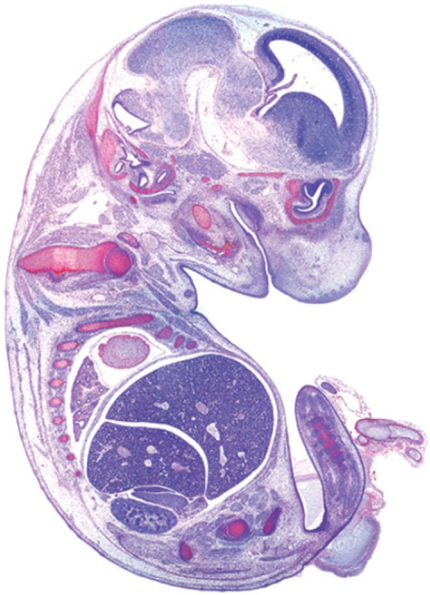


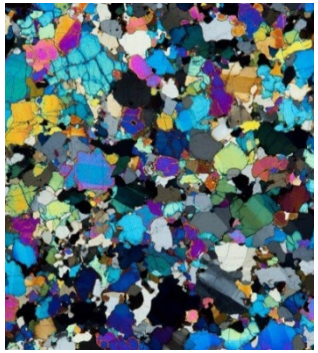
Compound Upright Microscopes

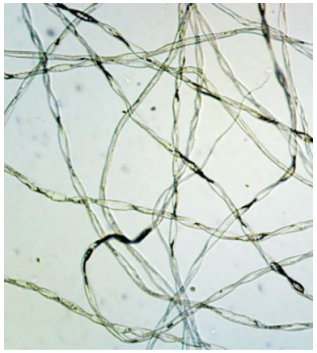
Benefits:
- High magnification and resolution
- Flexible sample type: slides, powders, mounts
- Large number of contrast modes possible

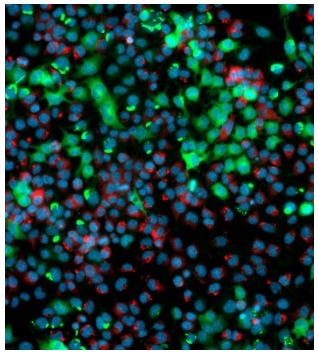
Constraints:
- Small working distance and depth of field
- Mounted specimens require leveling
- Samples usually require extra preparation

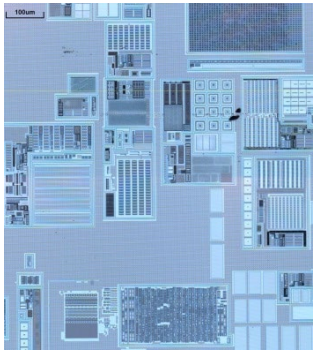
Applications:
- Materials microstructure, image analysis, characterization
- Heating/cooling: polymers, composites, materials science
- Samples on slides (cells, tissue sections, biomaterials, polymers)
- Petrography: geology, mineralogy, oil/gas exploration



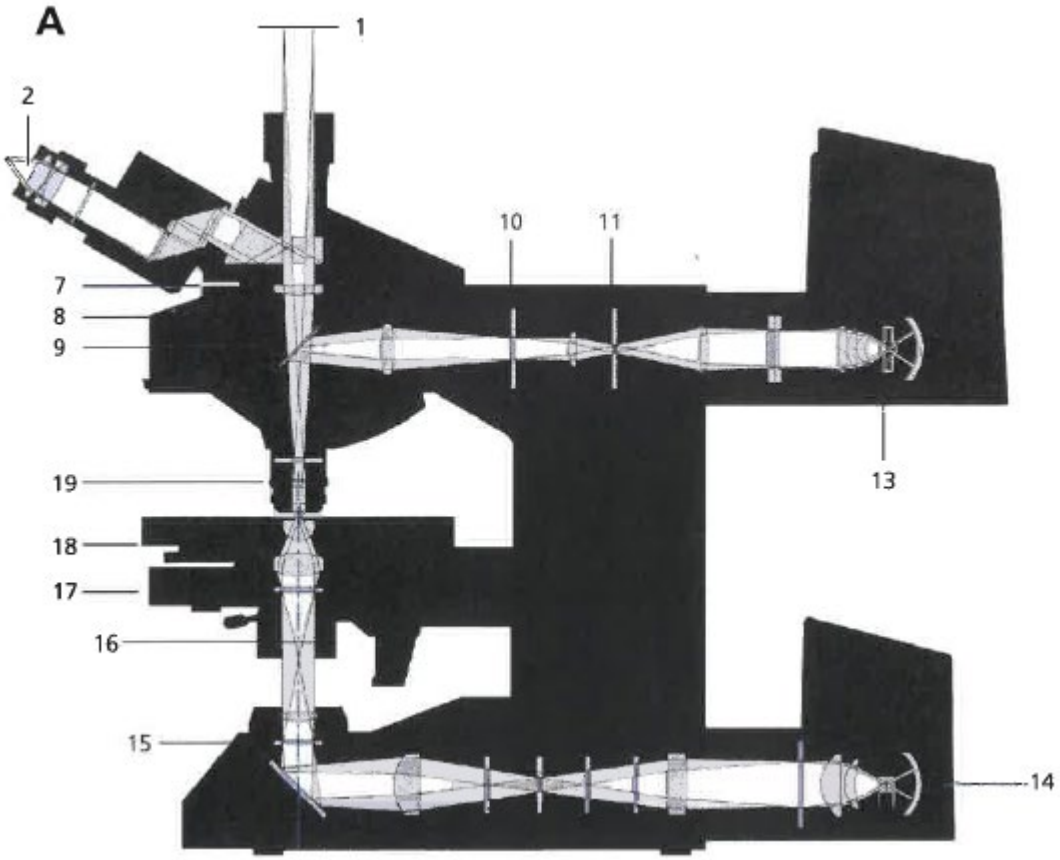








Compound Upright Microscopes



1 intermediate image plane phototube
2 eyepiece
3 intermediate image plane frontport
4 intermediate image plane baseport
5 beam path switching between baseport/ frontport/vis
6 sideport prisms
7 tube lens
8 analyzer
9 reflector module
10 luminous field diaphragm
11 aperture diaphragm
12 filter slider
13 HBO lamp
14 HAL lamp
15 luminous field diaphragm
16 polarizer
17 aperture diaphragm
18 condenser
19 objective

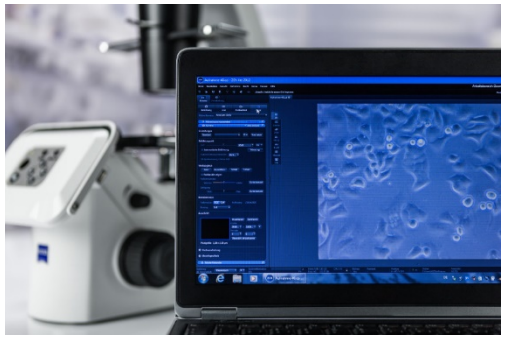
Compound Inverted Microscopes

Compound Inverted Microscopes

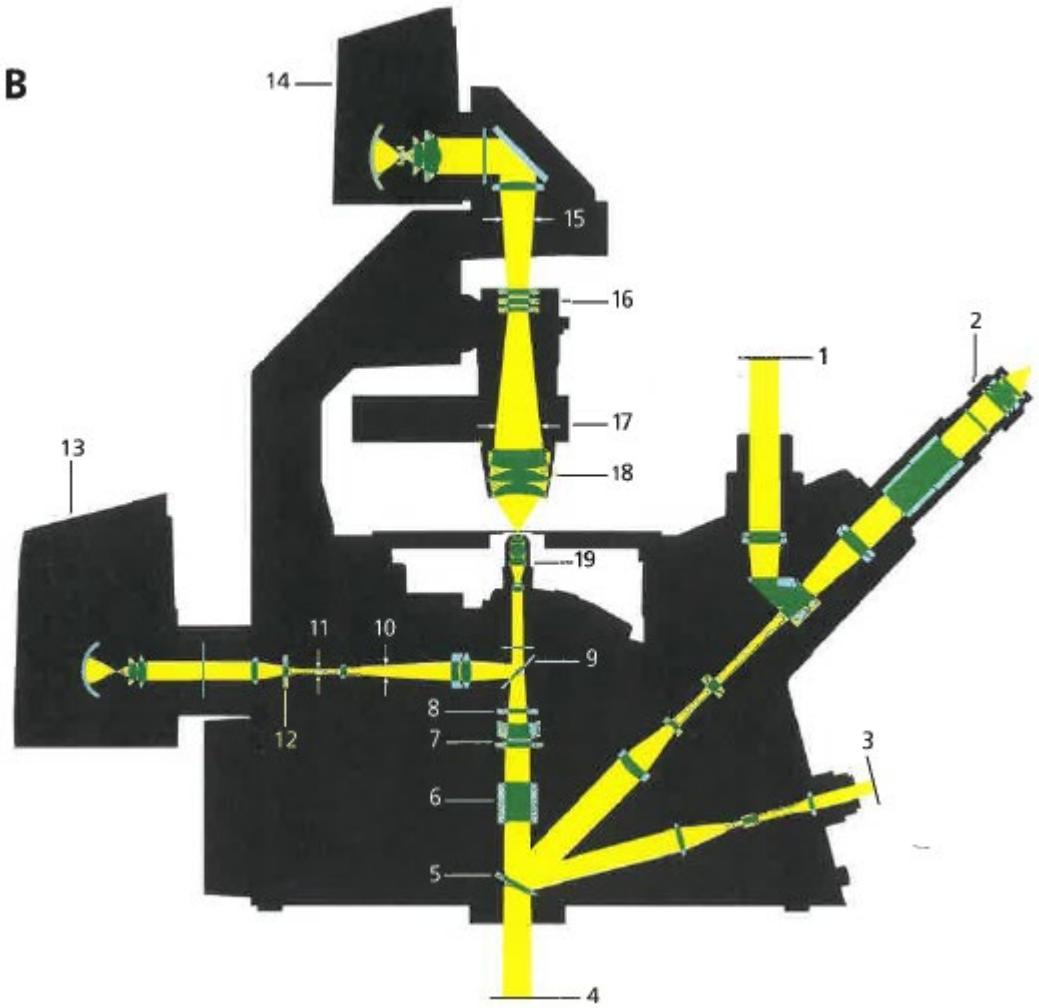
Benefits:
- High magnification and resolution
- Mounted samples require no leveling (upside down)
- Sample can have tall height above focus plane (dishes)

Constraints:
- Small working distance and depth of field
- Less flexible to varied materials sample sizes (mounts, small coupons, and slides only)

Applications:
- R&D: microstructure, image analysis, characterization
- Metallography: steel, copper, cast iron, titanium
- Any sample which can be cut, mounted, and polished
- Biology: cell culture, live cell imaging, glass-bottom dishes, wells



Compound Inverted Microscopes



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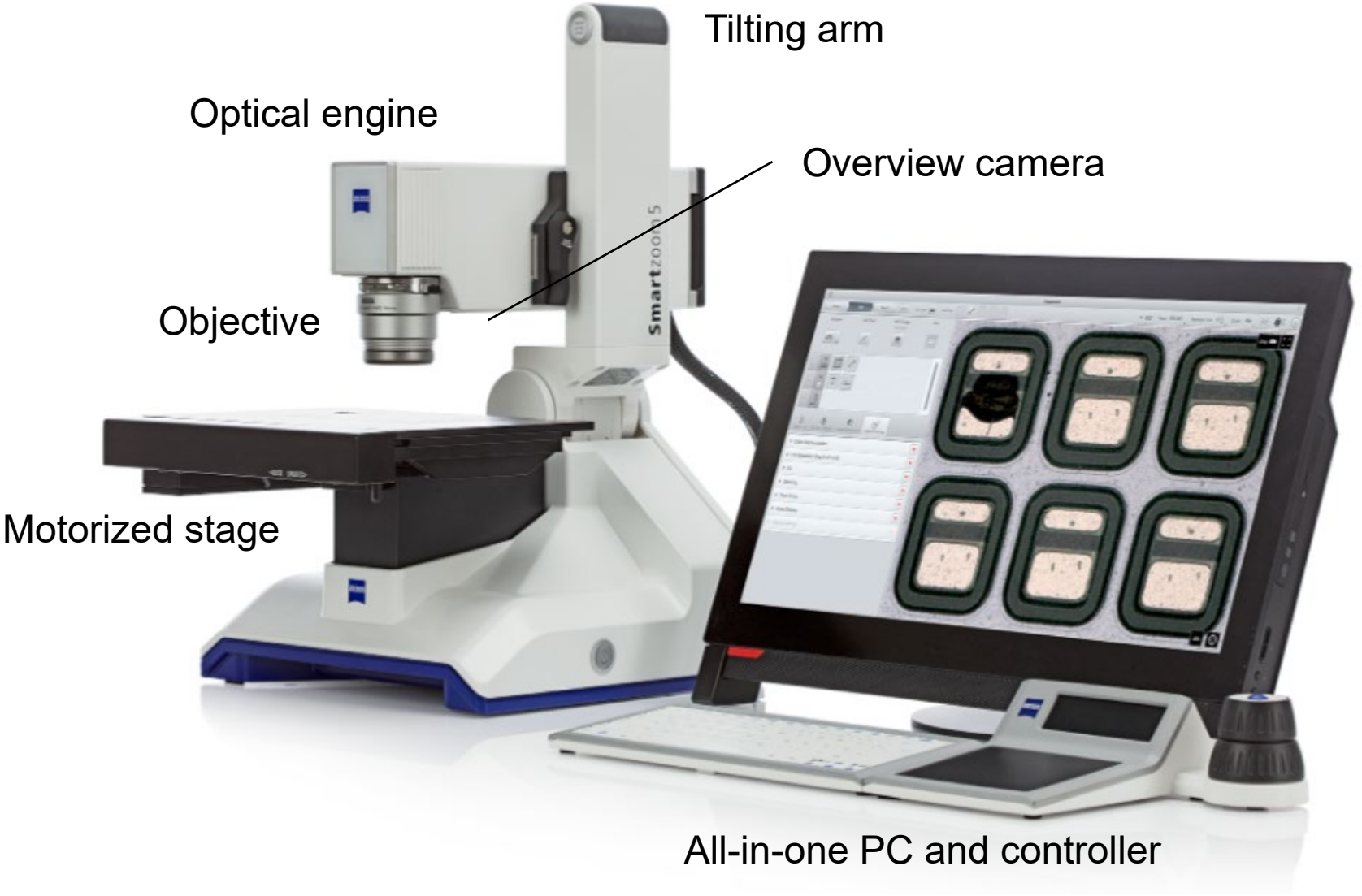
Digital Microscopes

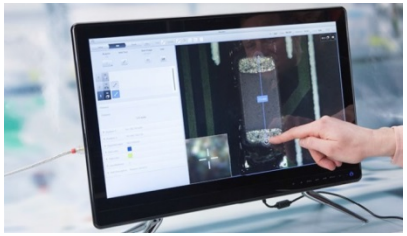
Digital Microscopes



Digital Microscopes

Tilting imaging head

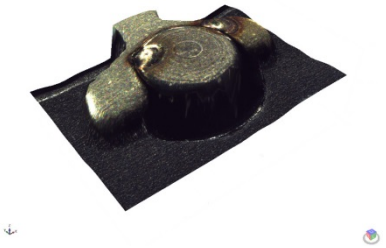
Sample protection



Integrated workflow

Integrated illumination

Hot-swappable objectives



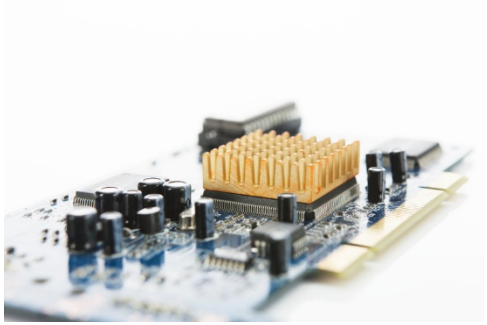
3D imaging

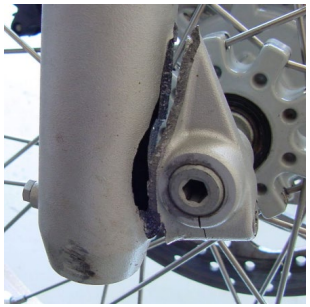
Digital Microscopes

Benefits:
- Small footprint
- Fast learning curve and easy operation
- Wide variety of samples
- Fast topographic images
- Tilted imaging

Constraints:
- No eyepieces
- Limited modularity/expandability
- Limited optical resolution

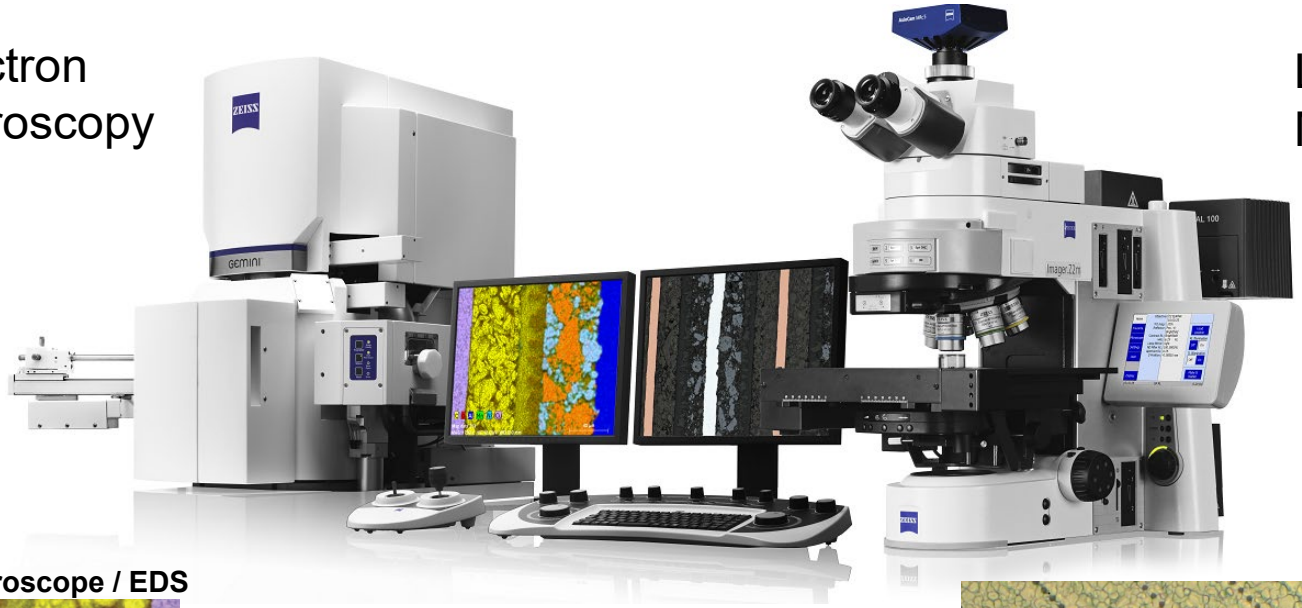
Applications:
- Part inspection, measurement, quality control
- Incoming failure analysis
- Macro complement to higher-magnification tools





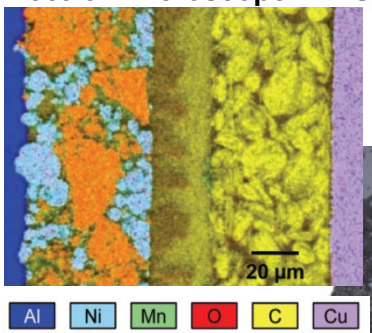
Correlative Microscopy

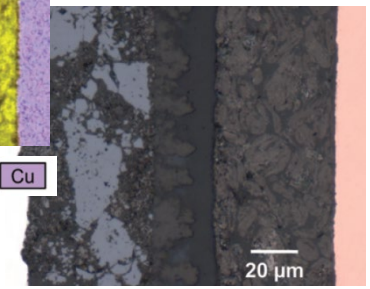
Electron Microscopy

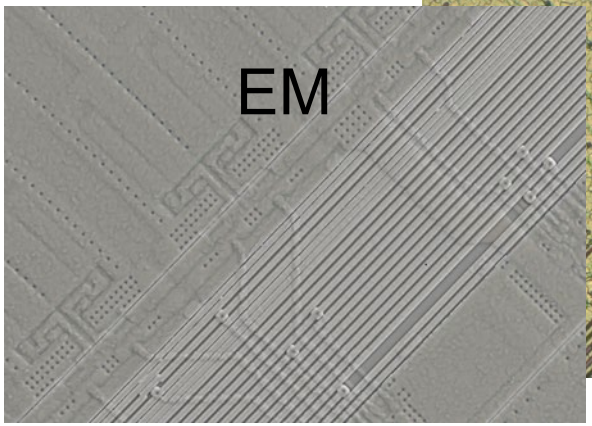


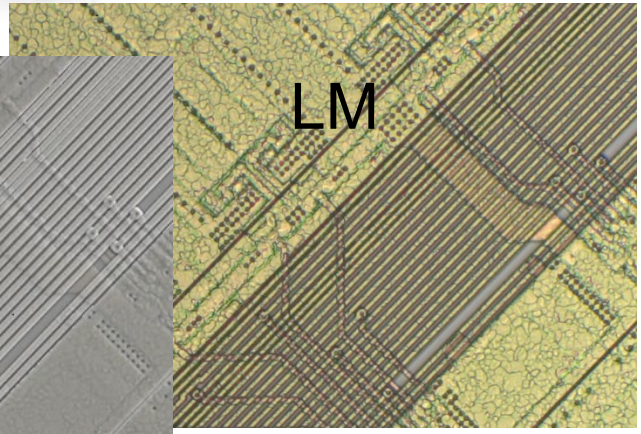
Light Microscopy

Electron Microscope / EDS









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Transmitted Light - Brightfield

The condenser containing the aperture diaphragm (2) and the luminous-field diaphragm (A) normally contained in the stand base are the aids which make all this possible. A closer look reveals that the luminous-field diaphragm is imaged on the specimen by means of the condenser. The luminous-field diaphragm determines which part of the specimen is illuminated. The aperture diaphragm, however, is imaged on the "pupil" of the objective (3) and regulates the illumination of this pupil. The entire optics are computed in such a way that aperture angles of the light cones are correctly set together with the aperture diaphragm.

Therefore, the microscope contains two different groups of optical planes which belong together.
The first group consists of:

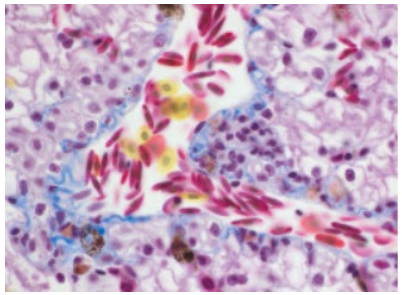
1 = lamp filament
2 = aperture diaphragm
3 = objective pupil
4 = pupil of the observer's eye

This group defines the beam path of the pupils and determines the resolution of the microscope.

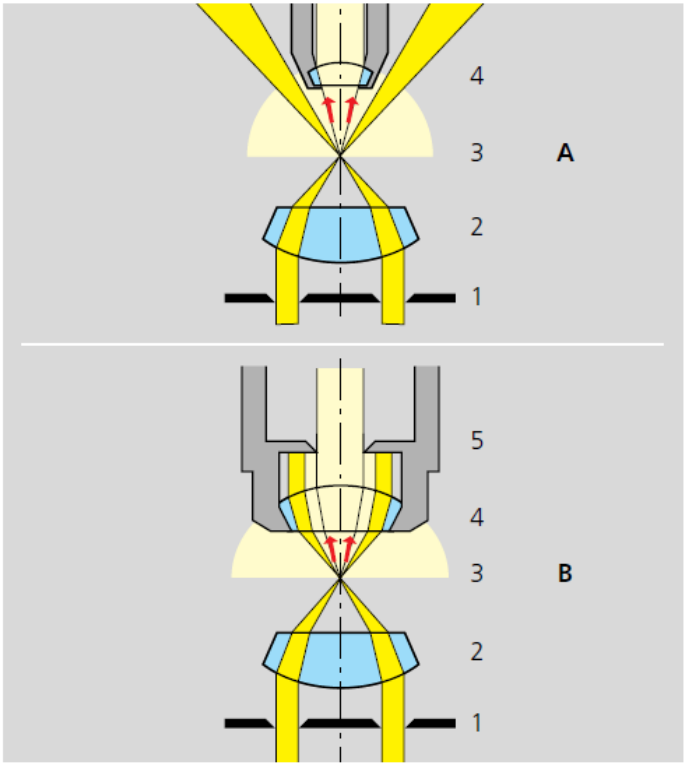
The other group contains:

A = luminous-field diaphragm
B = specimen plane
C = intermediate image in the eyepiece
D = retina of the observer's eye

A to D are the important optical planes in the image-forming beam path. The image becomes visible here and the image limits are set.



Transmitted Light - Darkfield

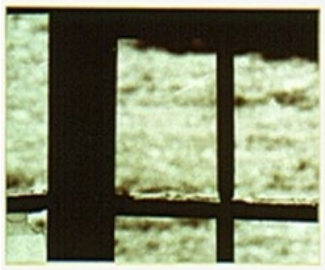


18.2

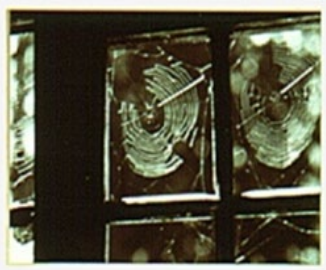
An artificial dark background is created in the microscope using a annular stop (1) in the condenser. Although the condenser optics (2) then illuminates the sample (3), it does so with a hollow cone of light. The light does not hit the objective (4), but passes it by on the outside. If there is no sample, the image seen in the eyepieces remains completely dark. However, if objects, e.g. small particles of bacteria, are in the object plane, light is laterally diffracted away from the straight path. Provided that this light hits the aperture cone of the objective, it is gathered by the objective and fused to form an image. The object becomes brightly visible in front of a dark background.

For this, it is necessary for the objective aperture to be smaller than the inner aperture of the illuminating light cone (case A). However, objectives with an integrated variable iris diaphragm (5) are also available to shutter out the indirect light even if it falls into the aperture cone of the objective (case B). This permits the use of very high apertures for darkfield.

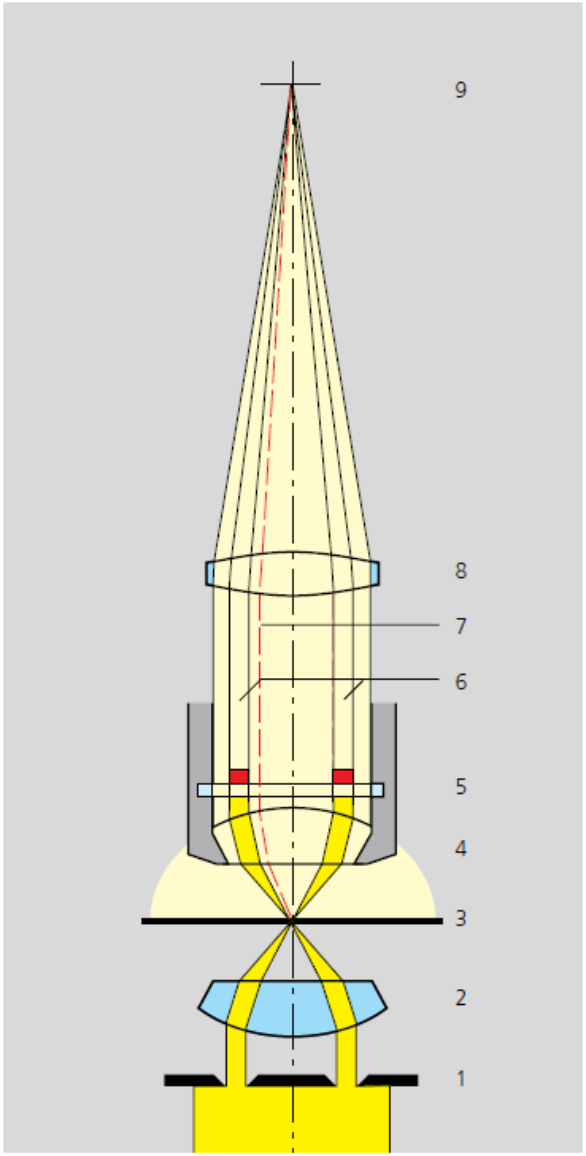
Spider web
Brightfield



Spider web
Darkfield



Transmitted Light – Phase Contrast

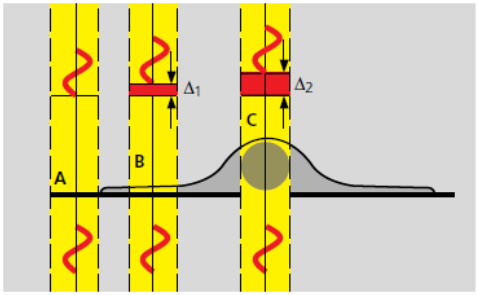


19.2

Phase contrast makes these tiny differences visible by the use of optical devices – i.e. it translates them into differences in intensity. The optical effect used consists of a shift of phase in the light ray. During their journey through cell nuclei, cytoplasm or water, the light waves are shifted by small degrees, since these media have slightly different refractive indices. The higher the refractive index of a medium, the smaller the speed or velocity of light in the medium. As a result, a light wave which has passed through a cell nucleus, lags behind the light waves which only had to pass through water. The amount of "lag" is called phase shift. Before their entry into the sample, the waves are still "in phase", but this is no longer the case when they have passed through the various materials. The amount of the phase shift behind the sample depends on what media (refractive indices) the waves had to pass through on their paths and how long the paths were in these media.

The human eye cannot see these phase shifts in the microscope image. It can only distinguish between different intensities and colors. Therefore, the phase contrast technique uses optical tricks to translate phase shifts into "grey values".

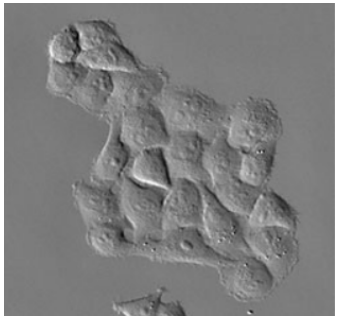
Much like darkfield, the aperture diaphragm is replaced by a phase stop (1) which illuminates the sample (3) via the condenser optics (2). However, here the entire light bundle enters the objective (4) and an image of the phase stop (1) is created in the objective pupil (5). A "phase ring" is attached to the objective pupil (5) which does two things: firstly, it attenuates – like a grey filter – the pronounced bright light coming from the phase stop of the condenser, and secondly, it adds a constant phase shift to this light. If the specimen contains objects such as cells and their nuclei, they guide the light from the



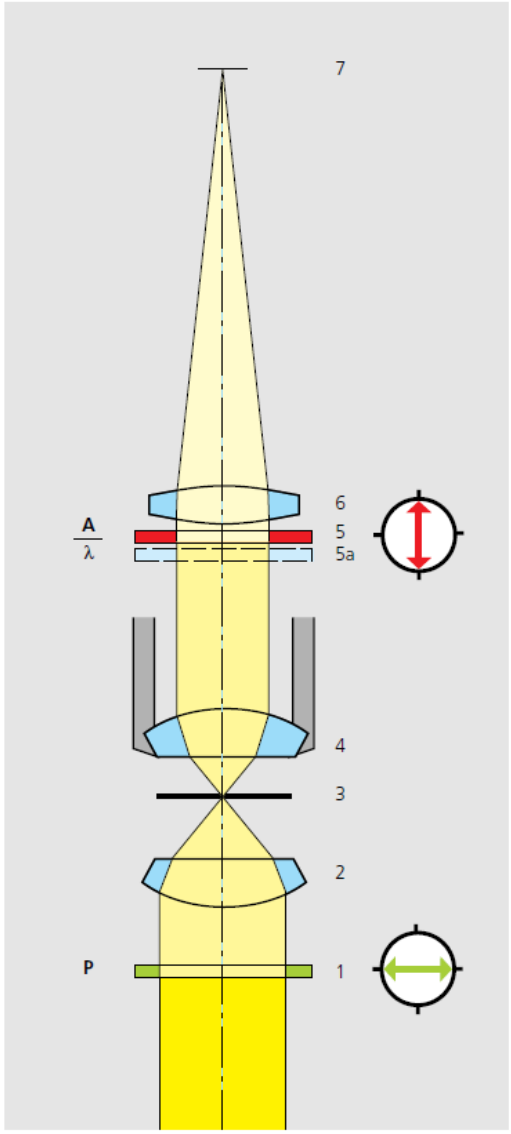
19.1

direct ray to new paths (7). This light will not pass through the phase ring in the objective, i.e. it will neither be attenuated nor will it be "retarded". All the partial rays are fused to form the intermediate image (9) by the tube lens (8).

The partial rays which have all been "retarded" to varying degrees are superimposed in the intermediate image, where they amplify or attenuate each other, depending on the phase position. Since the direct ray was strongly attenuated by the phase ring in the objective, the much weaker, diffracted light can become effective. The result of these interference processes in the intermediate image are bright and dark spots without which the cell to be examined would not be visible to the eye. Optimum contrast is created by selecting the right retardation and attenuation for the light waves in the phase ring of the objective.



Transmitted Light – Polarized



22.2

In this method, polarized light is used; it consists of light waves which all feature the same direction of vibration, i.e. which are linearly polarized. This very "ordered" light is generated by polarizers which filter out a privileged plane from the statistical confusion of vibration directions prevailing in natural light.

It is an important fact that two filters of this type do not let any light pass when they are arranged one behind the other in the beam path at an angle of 90° to each other. The first filter sorts out the vibration directions in such a way that the second filter cannot let pass this very selection. The second filter is called "analyzer", since it allows the privileged direction of the first filter – called "polarizer" – to be checked.

The appropriate arrangement is relatively easy to implement in the microscope. The polarizer (1) on the condenser – near the aperture diaphragm – ensures that the specimen (3) is illuminated with linearly polarized light via the condenser. The analyzer (5), arranged at an angle of 90° to the polarizer (1), is located behind the objective. The tube lens (6) forms the intermediate image (7).
If no specimen is on the microscope stage – or only an empty, clean microscope slide – the image will remain completely dark. When illuminated, many specimens turn the vibration direction of the polarized light out of the plane produced by the polarizer. Such specimens are mainly birefringent materials, in which the refractive index depends on the vibration direction of the incident light. This is mainly the case with crystals, such as starch or minerals, but also with polymers. If such materials are viewed under the polarization microscope between the crossed polarizer and the analyzer, bright areas can be seen in the image because light is partially transmitted by the analyzer.

The drawing of the beam path also includes a so-called *auxiliary object* (5a), also termed *lambda plate*. In polarized light, this lambda plate converts contrast to colors. As in phase contrast, path differences are used for this purpose, although this time with polarized light and birefringent material in the auxiliary object. The path differences generated lead to an extinction of certain wavelengths in the light, i.e. only certain colors remain from the white light and create beautiful, colored pictures.

Mechanical stress in the glass results in so-called stress-induced birefringence, which – in turn – influences the polarized light. Therefore, Pol examinations in the microscope require condensers and objectives which are free of such internal stress. Such objectives can be recognized by the "Pol" marking inscribed in red.

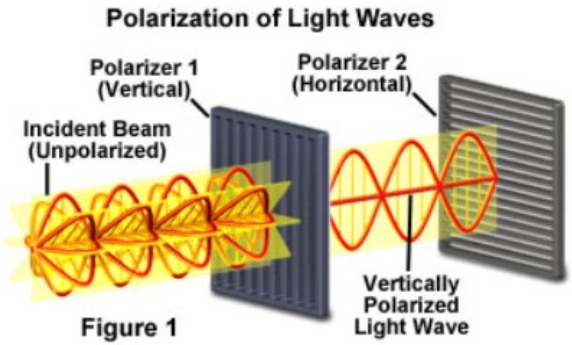
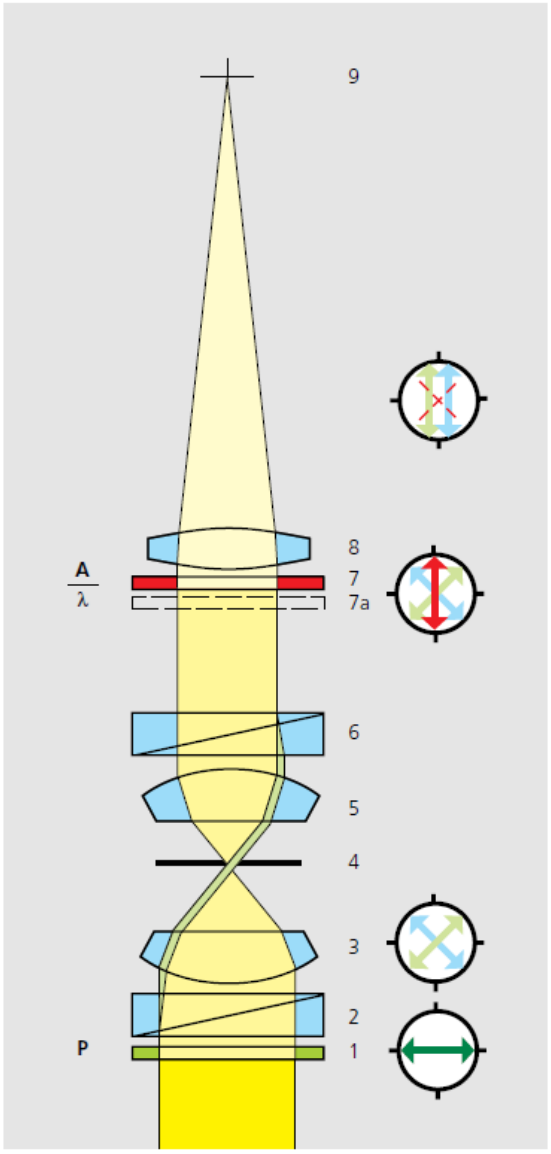


Figure 1

Transmitted Light – Differential Interference Contrast



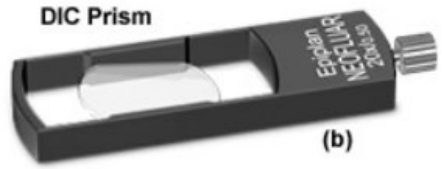
23.1

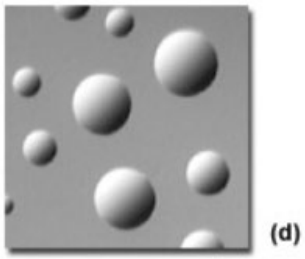
This highly efficient contrasting technique is based on the Pol contrast technique (page 22) as far as the components used are concerned. In its function, it is indeed related to DIC in reflected light (page 32). However, DIC in transmitted light is slightly more complicated than in reflected light because, firstly, two birefringent prisms are used, and, secondly, the path difference in the object is created in a different way.

Fig. 23.1 shows the beam path which, initially, is identical to that of polarized transmitted light. Additionally, the two birefringent prisms (2) are inserted in the condenser and near the objective pupil (6). The condenser prism (2) performs a vectorial decomposition of the previously linearly polarized light into two vibration directions which are perpendicular to each other, and laterally shifts these partial beams in such a way that a lateral displacement of $\Delta x = k \cdot \lambda$ occurs in the specimen. λ is the wavelength of the light used and k is a number which normally is smaller than 1.

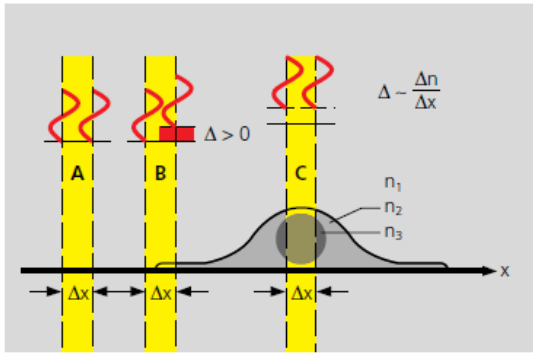
If the two partial beams now pass through exactly the same structures, no further path difference will occur in the specimen (cases A and C in Fig. 23.2). However, if the two partial beams "see" slightly different conditions, each of them will "experience" its own path difference which accompanies it on the remaining path to the intermediate image (case B in Fig. 23.2). The second prism (6) cancels the splitting process again behind the objective, and analyzer (7) selects those components from the now phase-shifted wave trains which lie in its vibration direction.

It is only now – with a *common vibration plane* – that the two partial beams can interfere with each other and therefore convert path differences to intensity differences which can be seen by the eye. A λ-plate (7a,λ) permits additional color contrast to be produced. The resulting images look like reliefs because this method displays only "lateral" changes. DIC is therefore also ideal for the optical sectioning of unstained, thick objects.

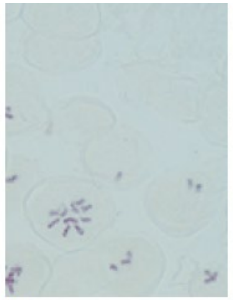




Pseudo 3D Relief



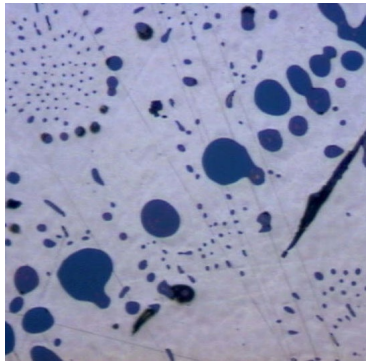
23.2


23.3a

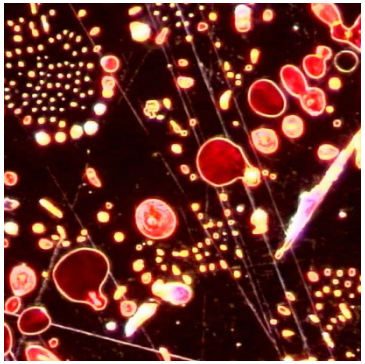

23.3b

Reflected Light Techniques

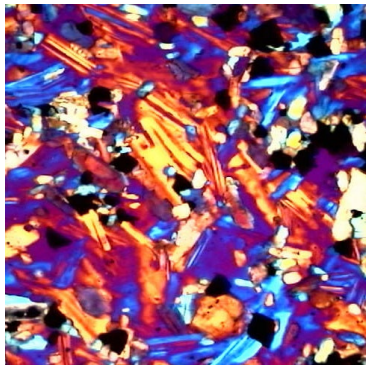
BF Reflected



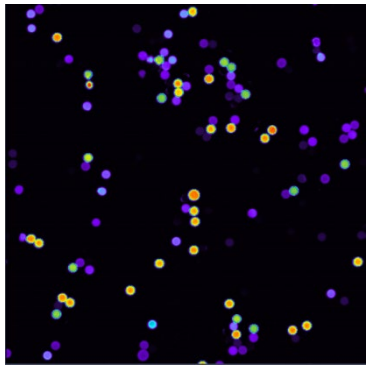
DF Reflected



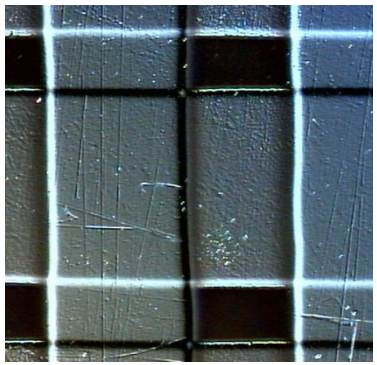
Pol Reflected



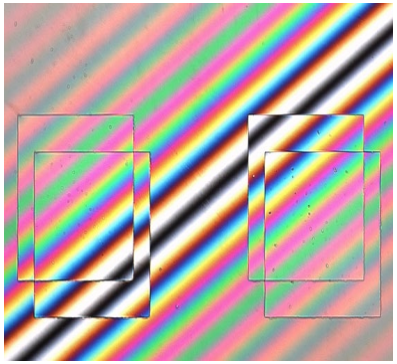
Fluorescence

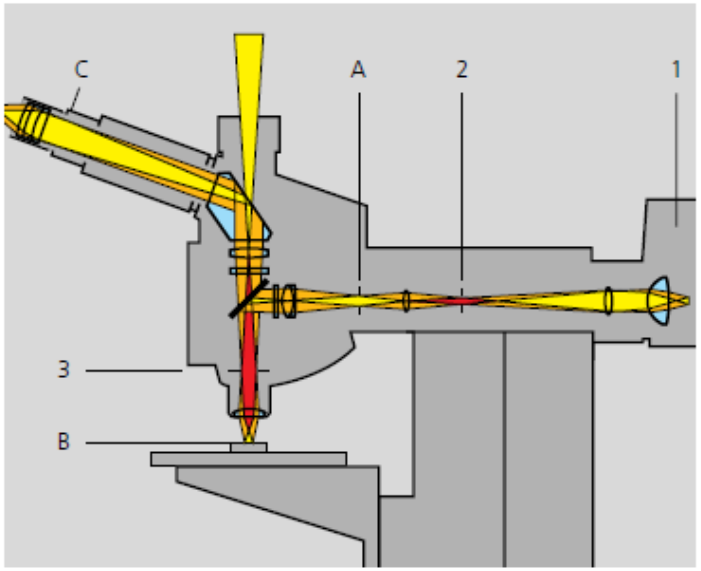


"C"-DIC & DIC Refl.



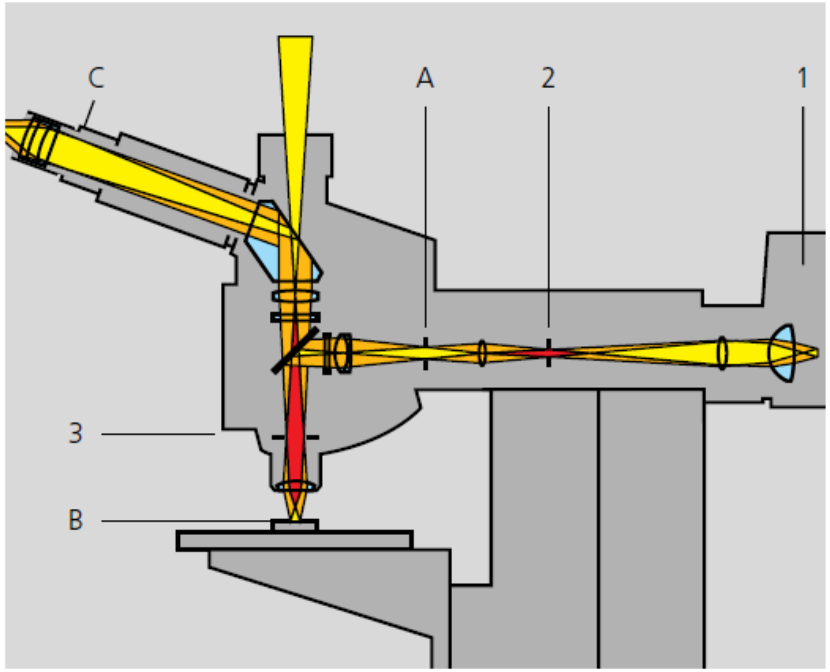
TIC Reflected





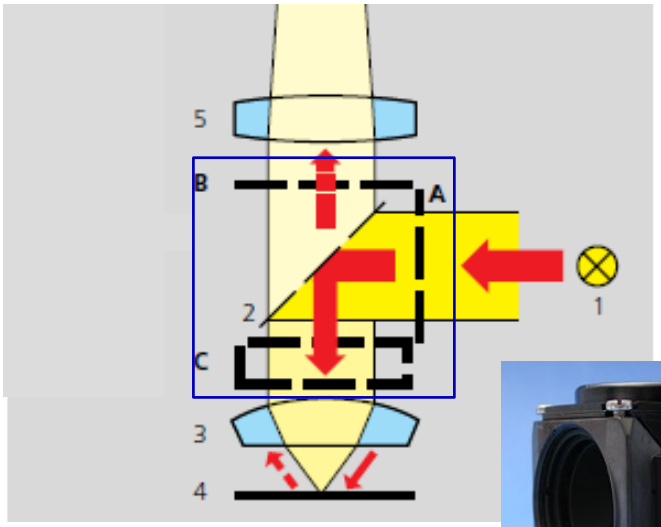
11.2

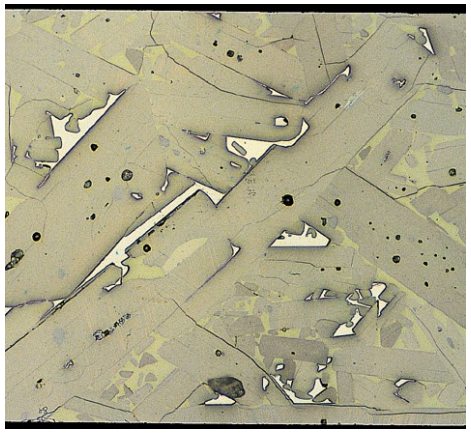
Reflected Light - Brightfield



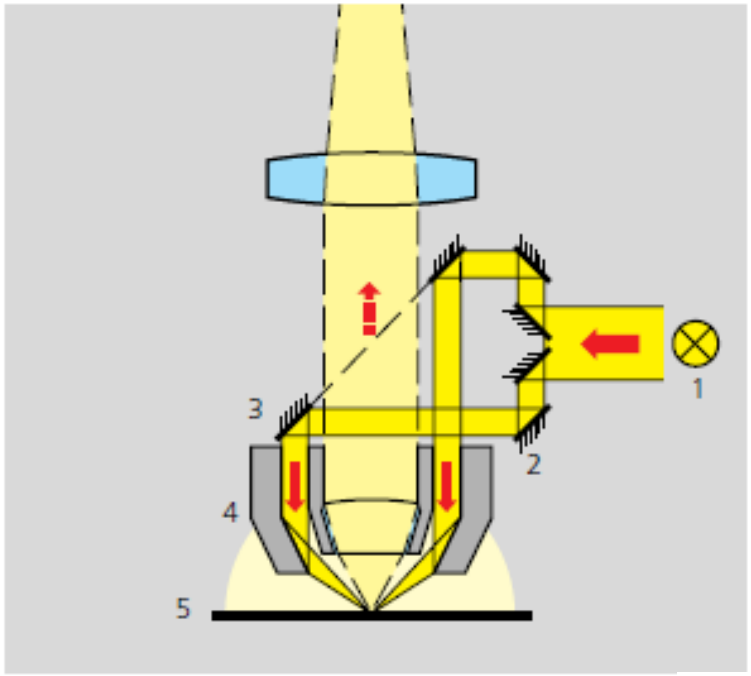
11.2

The beam path for reflected light shows that the light from light source (1) travels via aperture diaphragm (2) and luminous-field diaphragm (A) to the beam splitter. Here, about half the light is is reflected towards the objective pupil (3) and bundled onto the sample surface (B) by the objective. The surface reflects or scatters the light back into the objective. On the return journey, the beam splitter, in turn, lets about half the light pass to the tube lens which produces the intermediate image (C), this then being additionally magnified and observed using the eyepiece.





Reflected Light – Darkfield

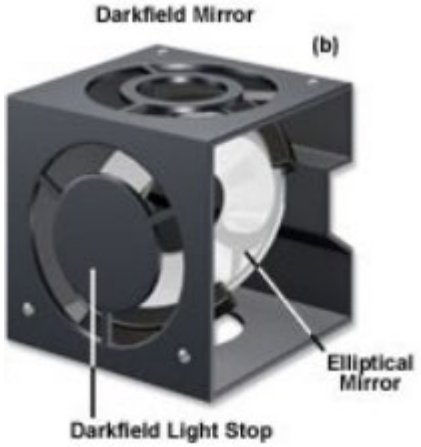


Contrasting methods in reflected light:

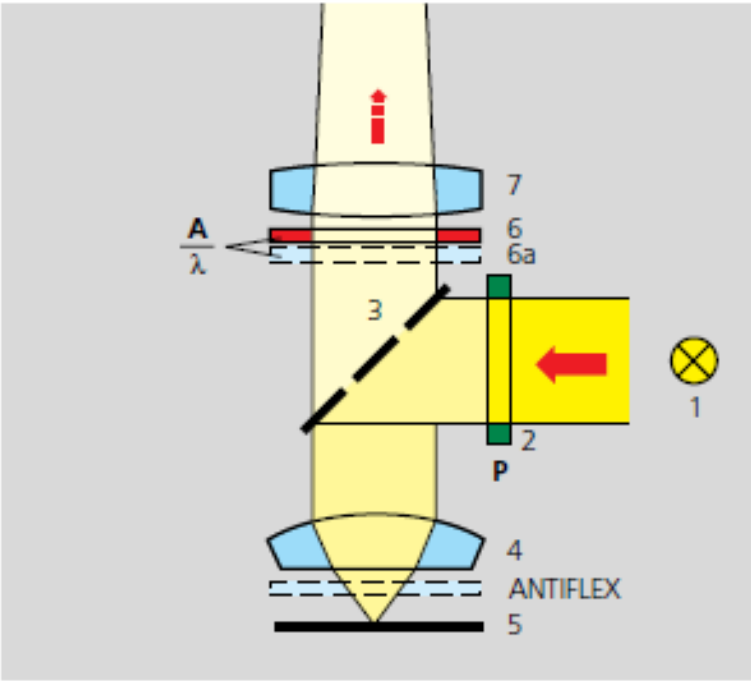
Darkfield in reflected light (DF)

This method is ideal for the inspection of surfaces. The light coming from the reflected-light illuminator is directed downwards towards the objective (4) via a mirror step assembly (2) and a mirror with an oval hole (3). Having passed the outer sleeve of the objective, it hits a ring-shaped concave mirror which directs the light rays for grazing incidence on the sample surface (5). If the object were a perfect mirror, no light would be reflected into the objective and the image would remain dark. Existing structures, however, direct light towards the objective and become brightly visible against a dark background.

Important: open the luminous-field and aperture diaphragms so that the light beam will illuminate the mirror assembly (2)!



Reflected Light - Polarization



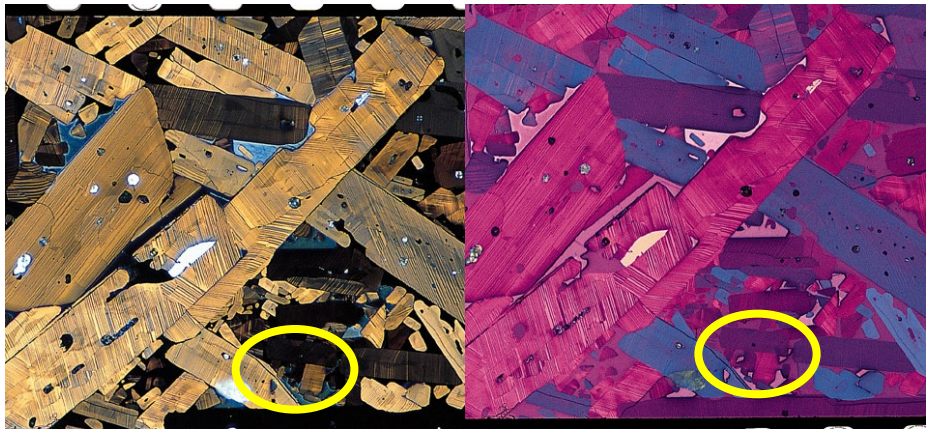
Polarization contrast in reflected light (POL)

Suitable for surfaces with structures which change the state of polarization during reflection, e.g. structure grains in samples of ore. The illuminating light hits a polarizer (2,P) first and is imaged, linearly polarized, on the sample surface (5). Behind the beam splitter, it hits the analyzer (6,A), which allows only the depolarized portion of the light to reach the tube lens (7). As in transmitted light, an optional lambda plate (6a,λ) enables the changeover from grey contrast to color contrast.

When objectives of a very low magnification are used, a so-called Antiflex cap (a rotatable λ/4-Platte in front of the objective) permits otherwise unavoidable reflection to be also eliminated from "dark" sample surfaces.

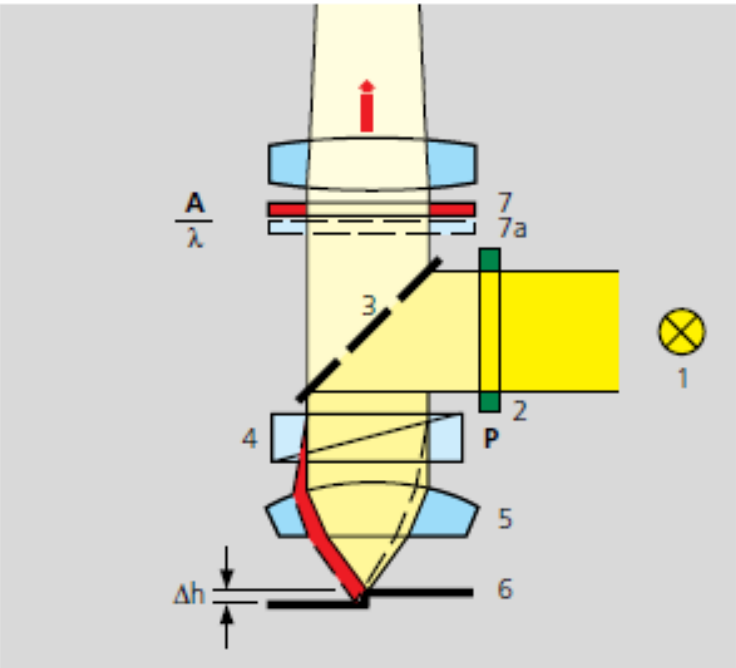
Crossed Pol

Pol with lambda plate



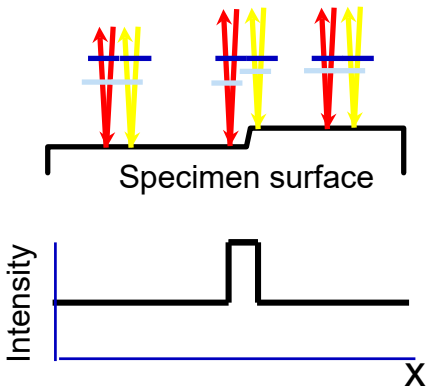
Notice detail visible with color contrast - POL with lambda plate

Reflected Light - DIC

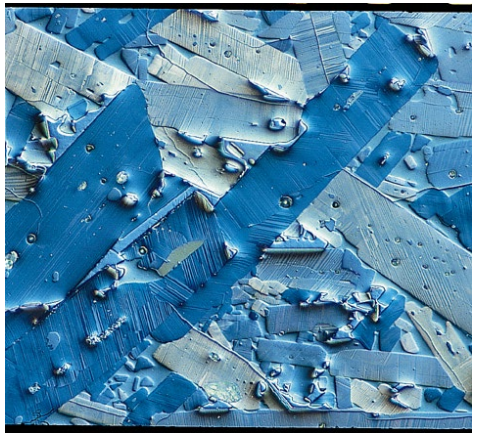


Differential Interference Contrast in reflected light (DIC)

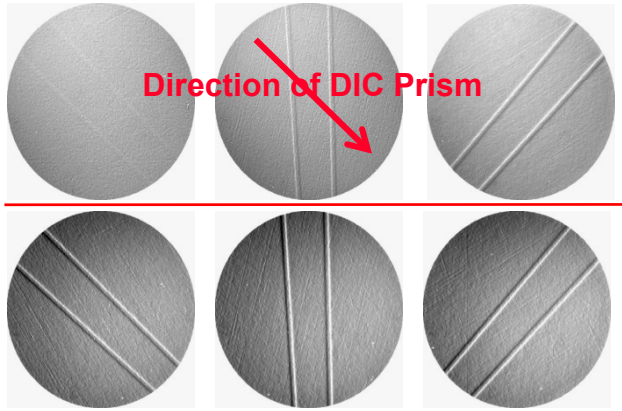
As an extension of polarization contrast, this method also allows the visualization of minute elevation differences in surfaces. A birefringent prism (4) is used, which splits the polarized light beam into two partial beams on its way to the sample. These partial beams hit the sample (6) with lateral displacement from each other. If the surface is completely flat, nothing will happen. However, if there is a small step between the two partial beams, one of the two beams must travel a path which is 2Δh longer and is assigned this path difference. Once the partial beams have returned via the DIC prism (4) and the analyzer (7), they feature the same vibration direction again – due to the analyzer – and can interfere with each other in the intermediate image. The path difference assigned on the surface then changes into grey values which can be seen by the eye: steps become visible as a relief. As an auxiliary object, the lambda plate (7a) changes the grey values into colors again.



3D relief image with nanometer (in Z) surface features visible.



Reflected Light – C-DIC [*Circular* DIC]



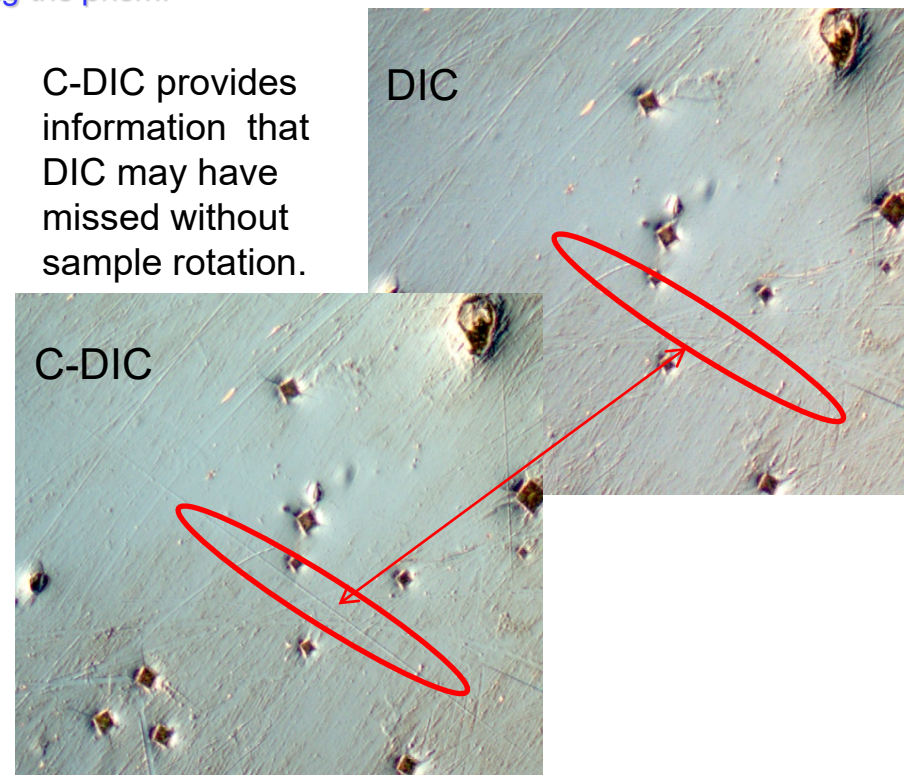
In conventional DIC, the stage has to be rotated to maximize the contrast .

Using C-DIC, maximum contrast can be adjusted in any structure direction by rotating the prism!

CDIC is similar to DIC but uses ¼ lambda plates with both the analyzer and polarizer to produce circular polarized light along with a rotatable prism.

Rotatable CDIC Prism(s) with CDIC reflector cube

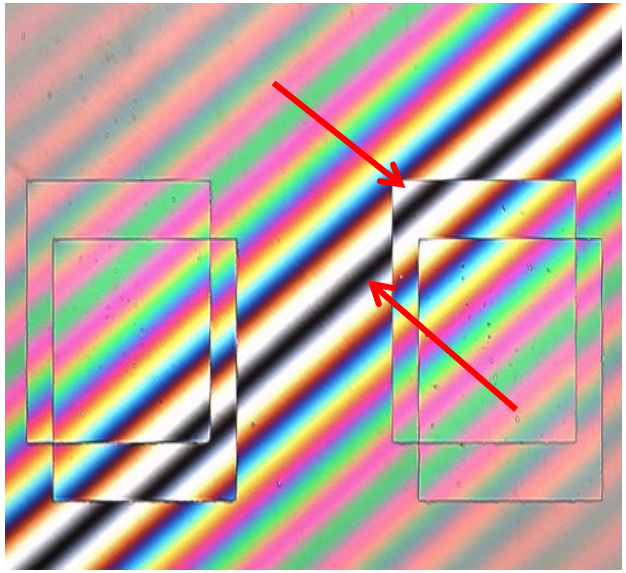
C-DIC provides information that DIC may have missed without sample rotation.



Reflected Light – C-TIC [*Circular* TIC]

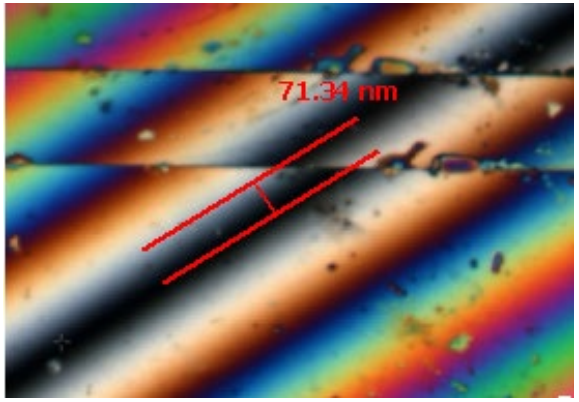
Quantitative interferometric technique for step height measurement

Total **I**nterference **C**ontrast – An optical interferometer for simple step height distances on the sample surface.



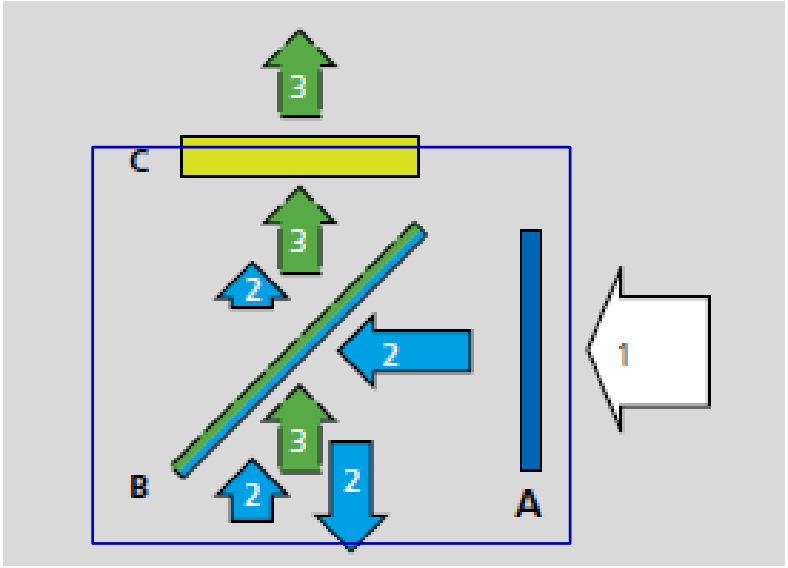
C-TIC is similar to CDIC but the TIC prism has a larger shear distance. This creates a clear double image. The shift of the interference bands created then can be measured height distances from 10nm to several microns depending on the sample.

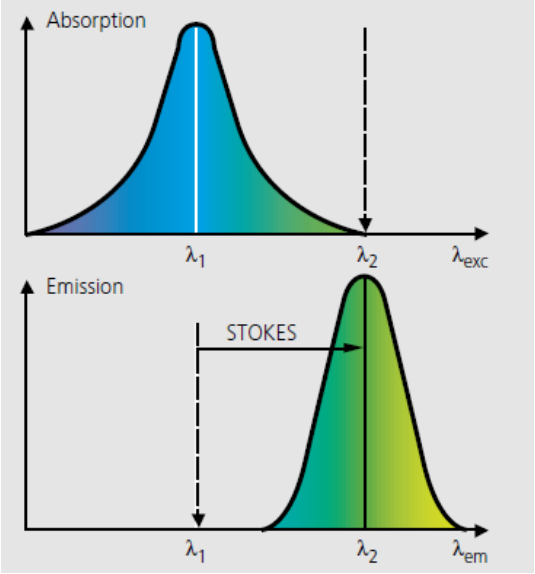
Consists of CDIC Reflector module and a TIC Prism (much like CDIC)

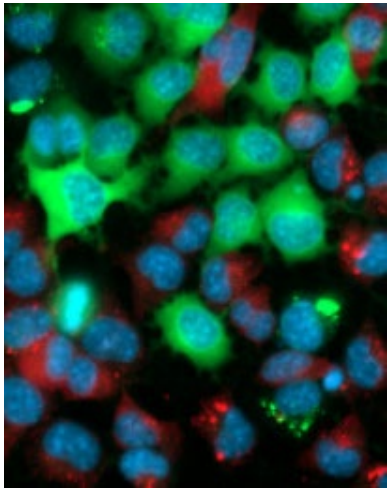


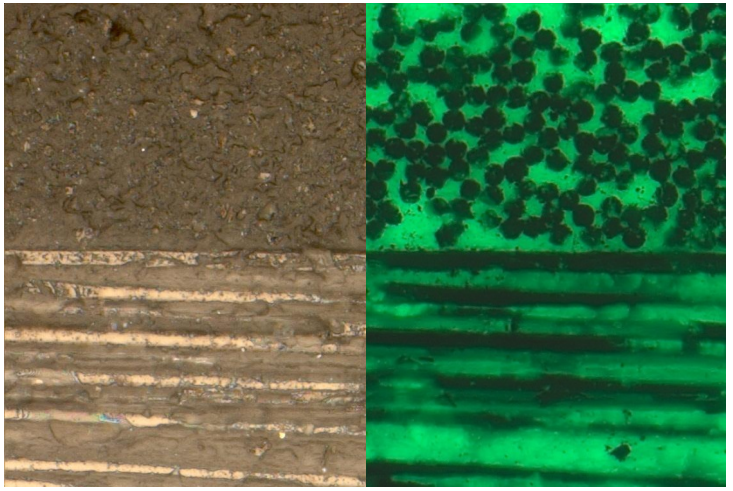
Reflected Light – Fluorescence

The exciter filter (A) (Fig. 27.2) filters almost monochromatic light (2) out of the light source radiation (1). The properties of the dichroic beam splitter (B) are fascinating: it reflects the short-wave excitation light to the objective almost without any loss, but allows the fluorescence light (3) returning from the specimen via the objective to pass through almost completely. At the same time, most of the excitation light is reflected again and can therefore no longer affect the formation of the intermediate image. Above the beam splitter, the emission light and the remainder of the excitation light hit the barrier filter (C). Only the fluorescence light can pass the filter almost unhindered because its wavelengths are longer than those of the excitation light.



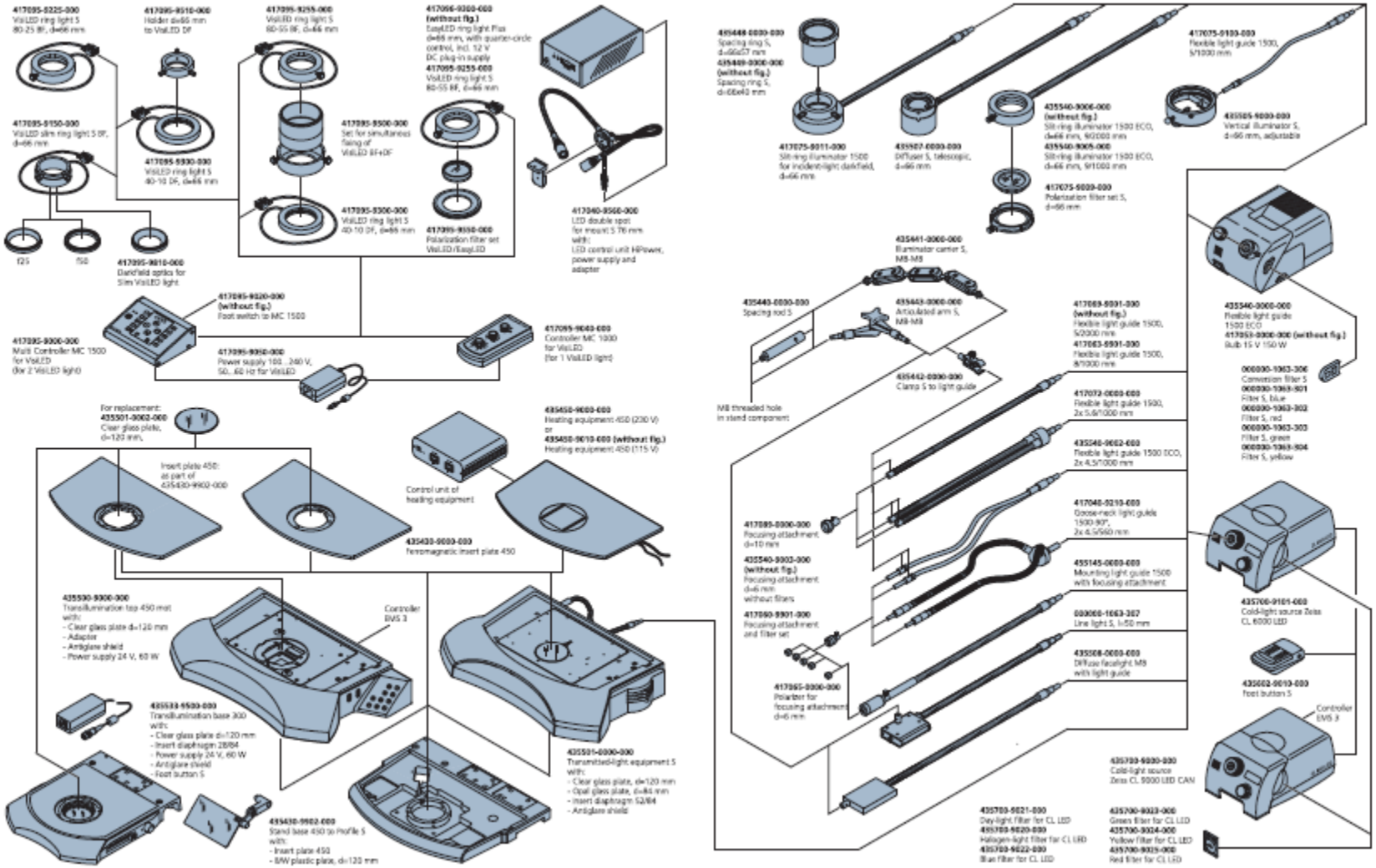




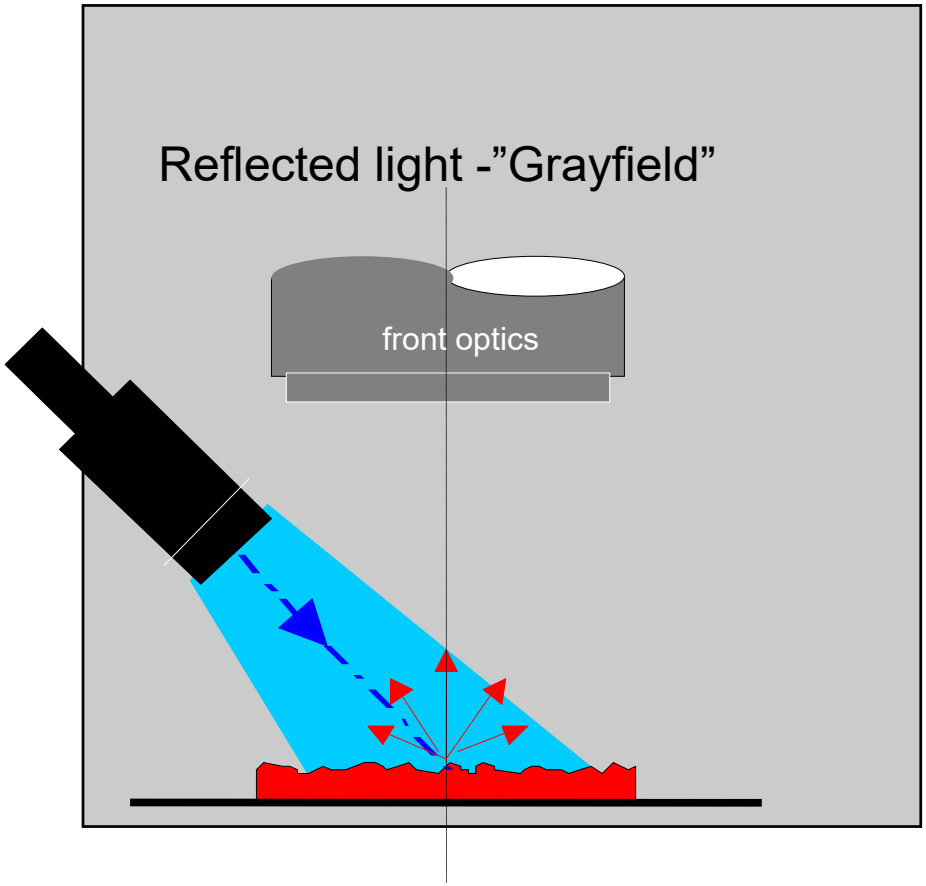


Brightfield Fluorescence

Stereomicroscopy illumination techniques



Oblique Illumination – Reflected Light Illumination



Application

Observation of richly textured specimen surfaces

In case of strong shadows to illuminate the specimen from two sides

Simple, flexible often low cost illumination

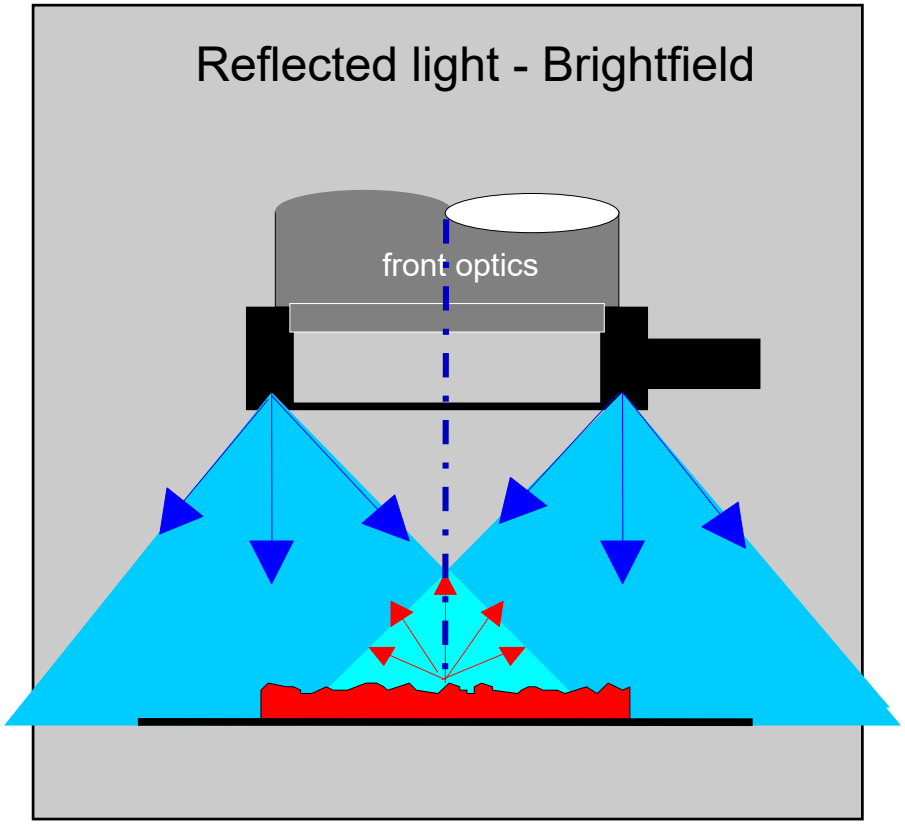
Oblique Illumination – Reflected Light Illumination

Manufacturing date indicator

1-arm spot light guide:
Emphasis on surface texture, dark shadows.

2-arm spot light guide:
Emphasis on surface structure with homogeneously illuminated background and reduced shadows.

Reflected Light Brightfield – Brightfield Ring Lights, Diffusor

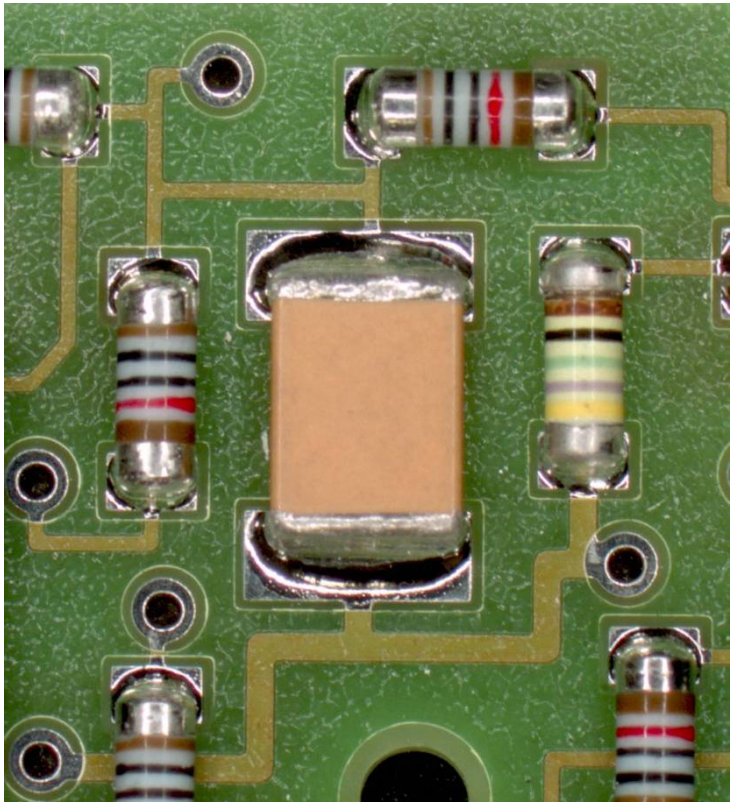


Application

Homogeneous, shadowless illumination of plan, more color-intensive specimens

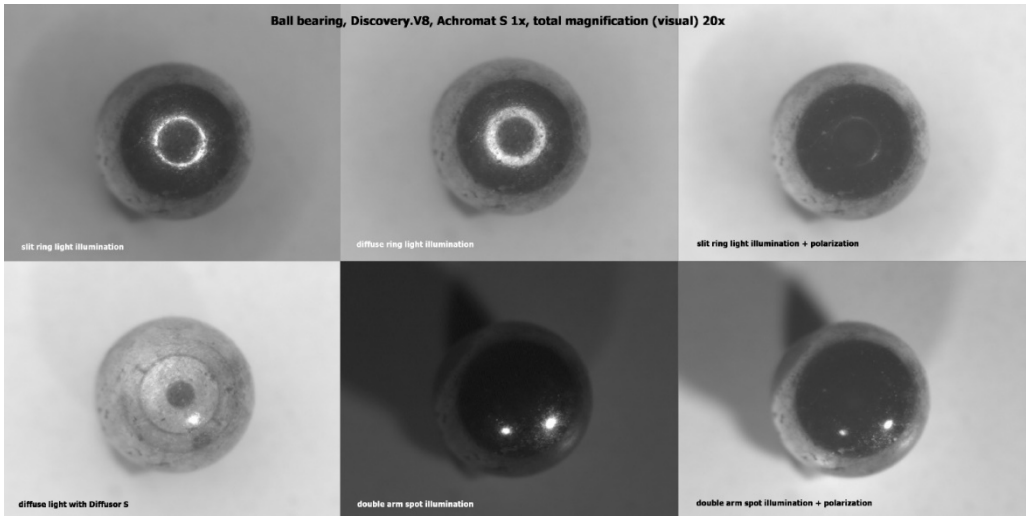
The diffuser is suitable to illuminate specular surfaces

Reflected Light Brightfield – Brightfield Ring Lights, Diffusor

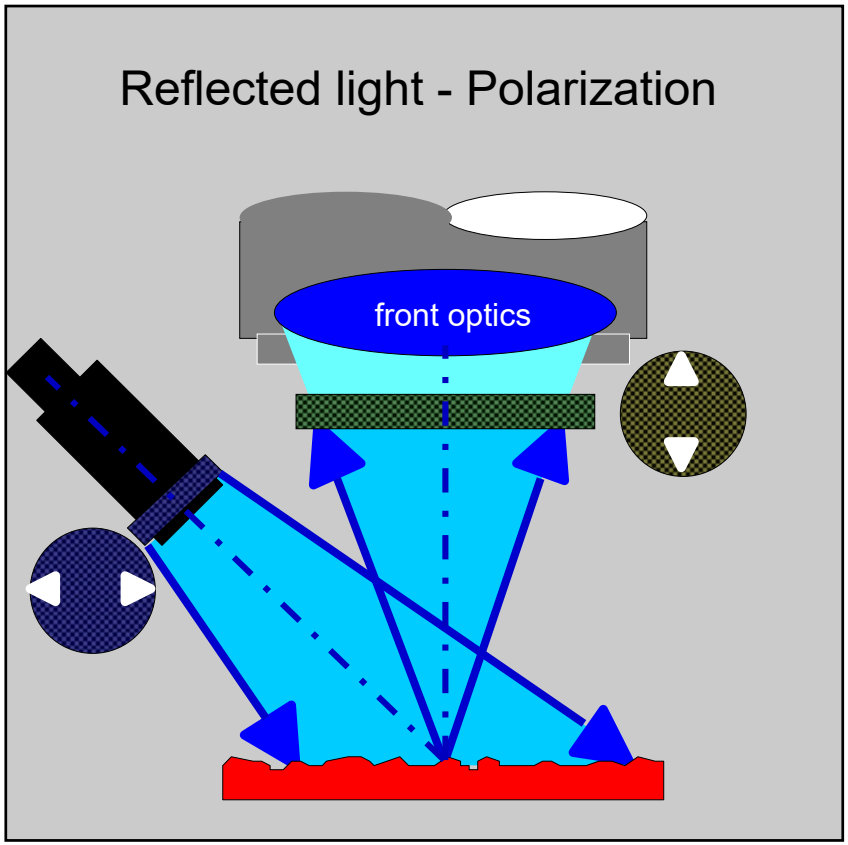


Printed circuit board
Shadowless illumination

Ball bearing
Avoid seeing your own illuminator



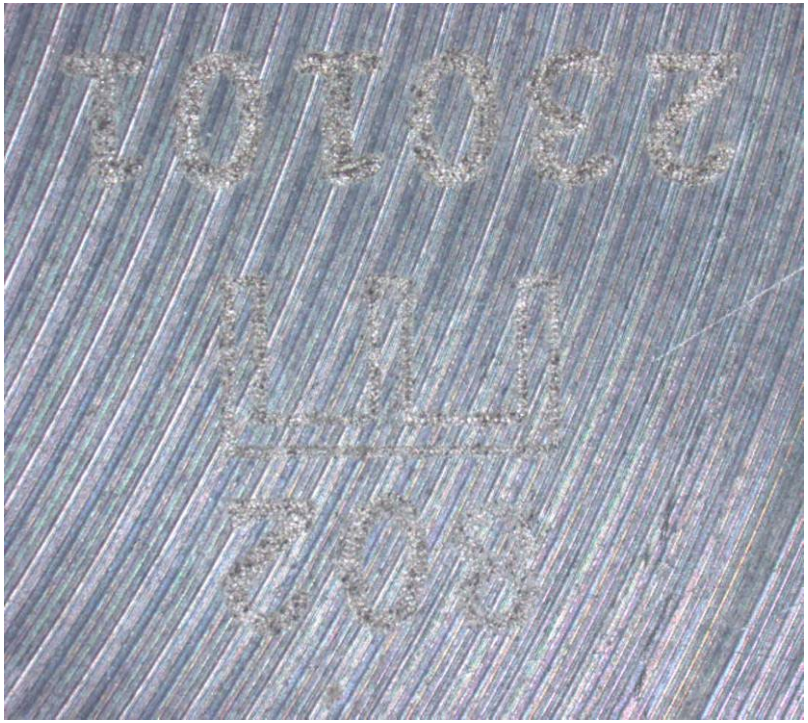
Oblique Illumination – Contrast Enhancement by Polarized Light



Application

Elimination of undesired reflexes on metallic or otherwise specular reflecting surfaces (glossy)

Oblique Illumination – Contrast Enhancement by Polarized Light

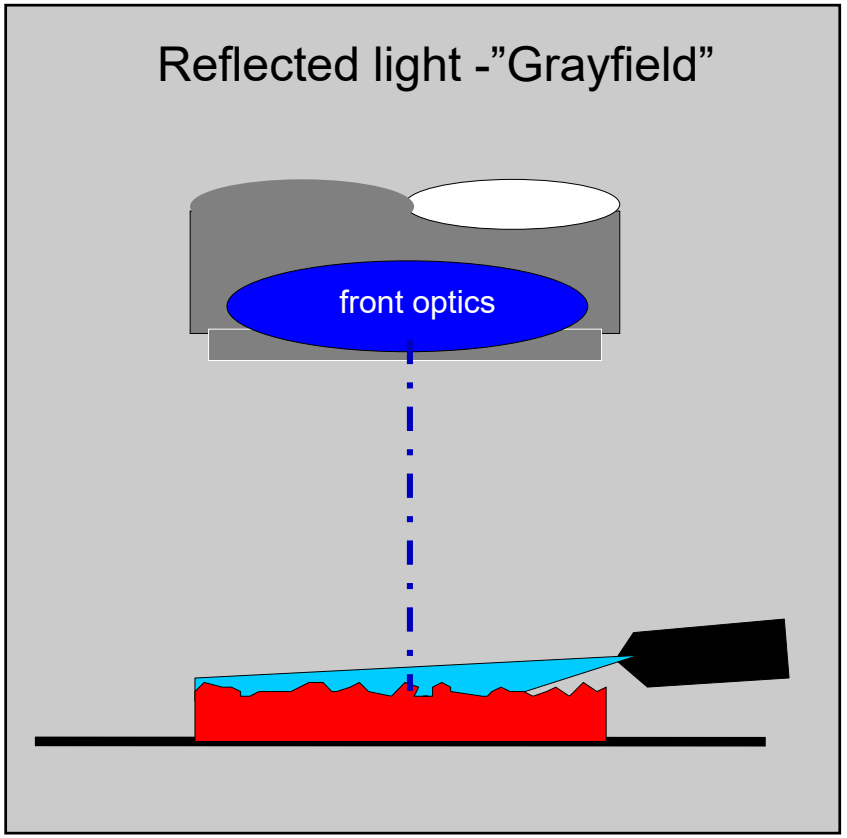


Engraved letters

Annular ring light:
The letters are almost invisible.

Annular ring light with polarizer and analyzer:
The letters are clearly readable.

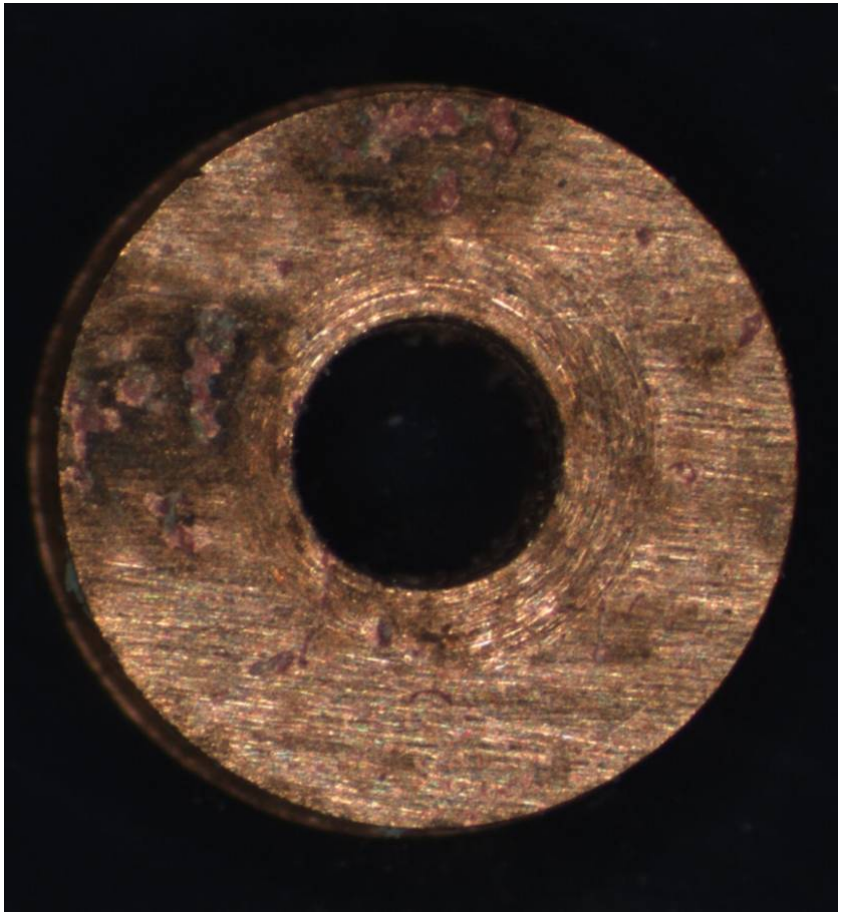
Oblique Illumination – Reflected Line Light



Application

Large area illumination at an extreme grazing angle, makes objects and structures of minimum height visible (forensics)

Oblique Illumination – Reflected Line Light

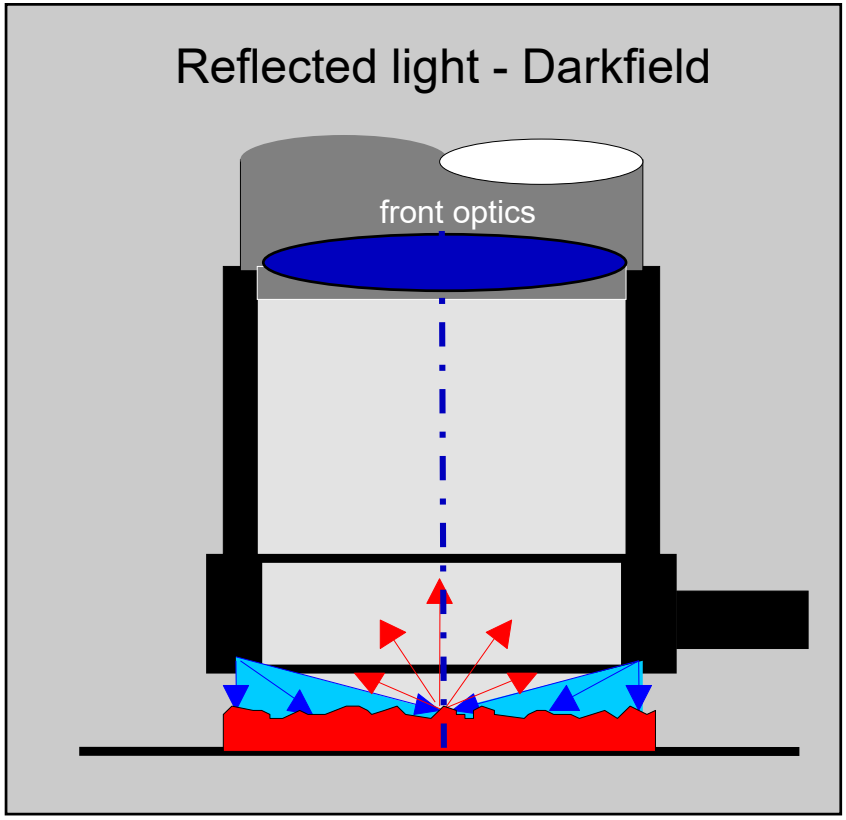


Washer

Ring light illumination:
The image of the washer shows little detail.

Line light illumination:
Glue residues and tool marks are clearly visible.

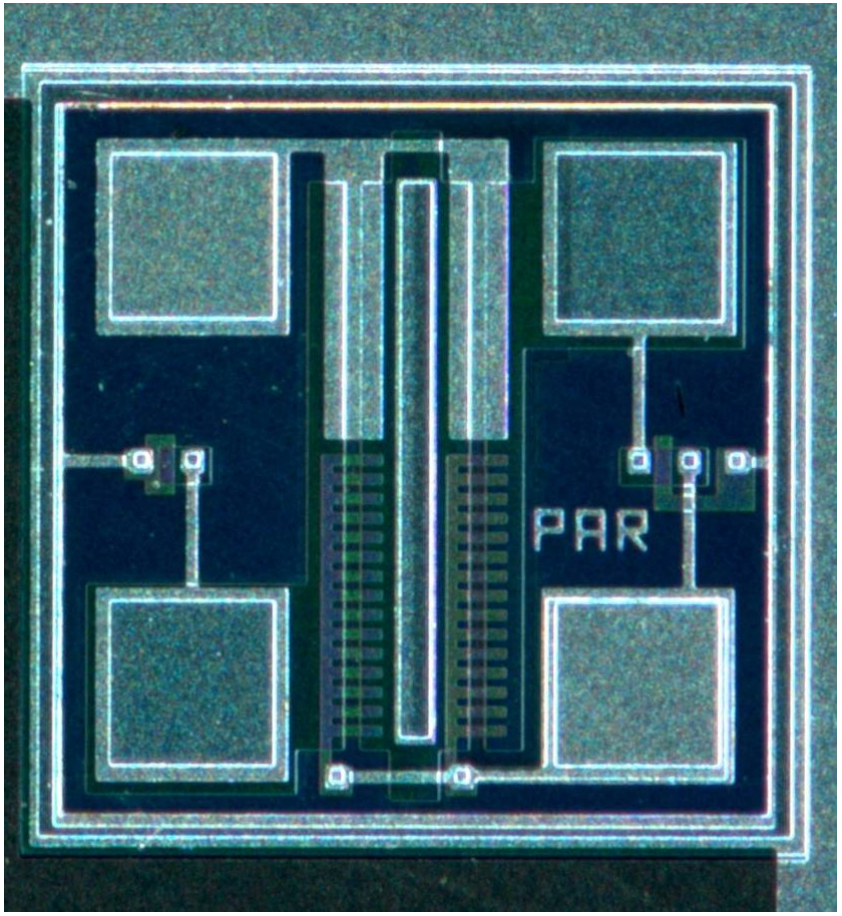
Reflected Light Darkfield
Darkfield Slit-Ring Light



Application

Fine and/or low contrast
specimen structures appear
bright against a dark background
Ideal for exacting demands
on resolution and contrast
A must for pigment analysis

Reflected Light Darkfield – Darkfield Slit-Ring Light

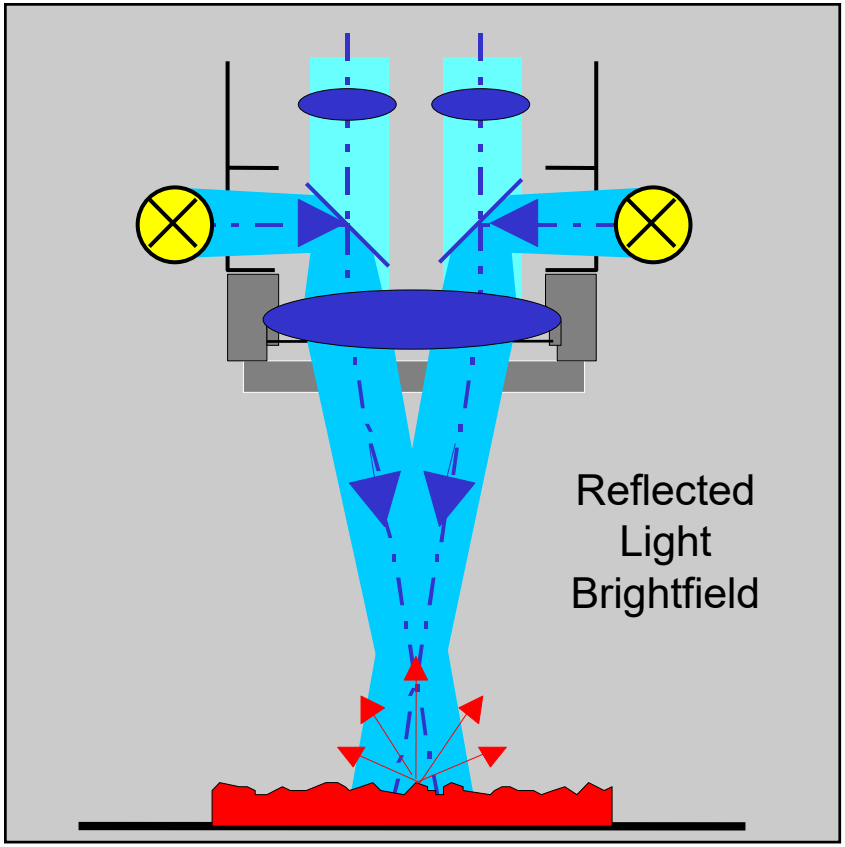


Wafer surface

Highly reflecting areas appear dark or black.

Light scattering areas of the wafer and dirt shine brightly.

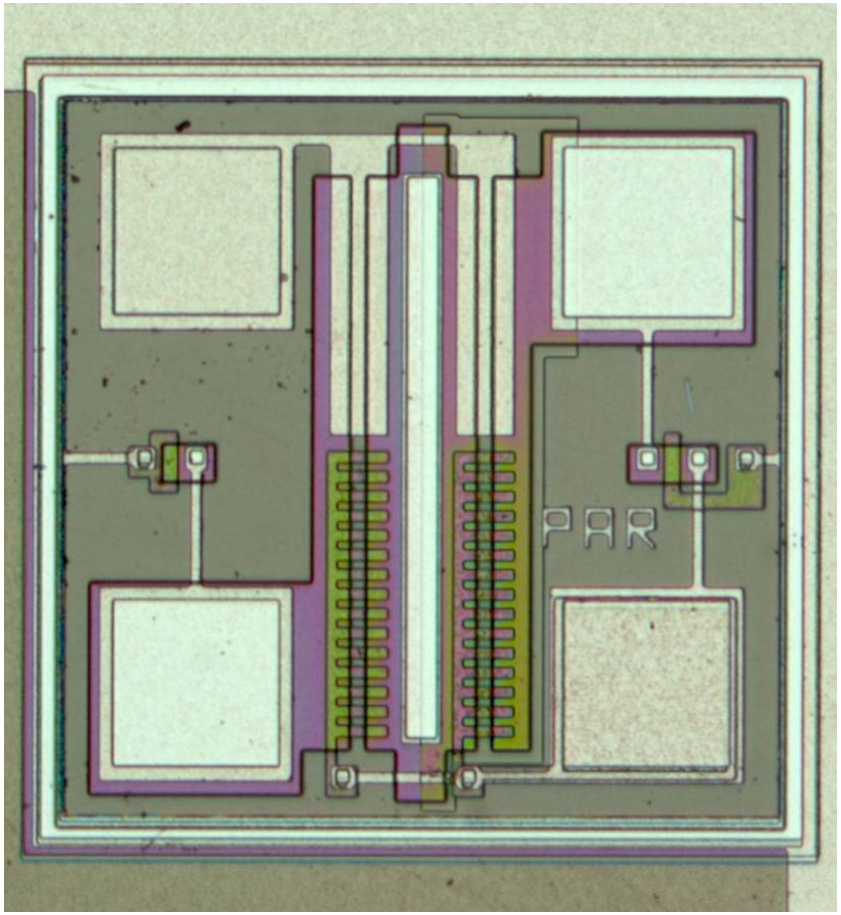
Reflected Light Brightfield – Brightfield Coaxial Illumination



Application

Makes flat, highly but irregularly reflecting surfaces visible
e.g. wafers, LCD's or
polished metal sections

Reflected Light Brightfield – Brightfield Coaxial Illumination

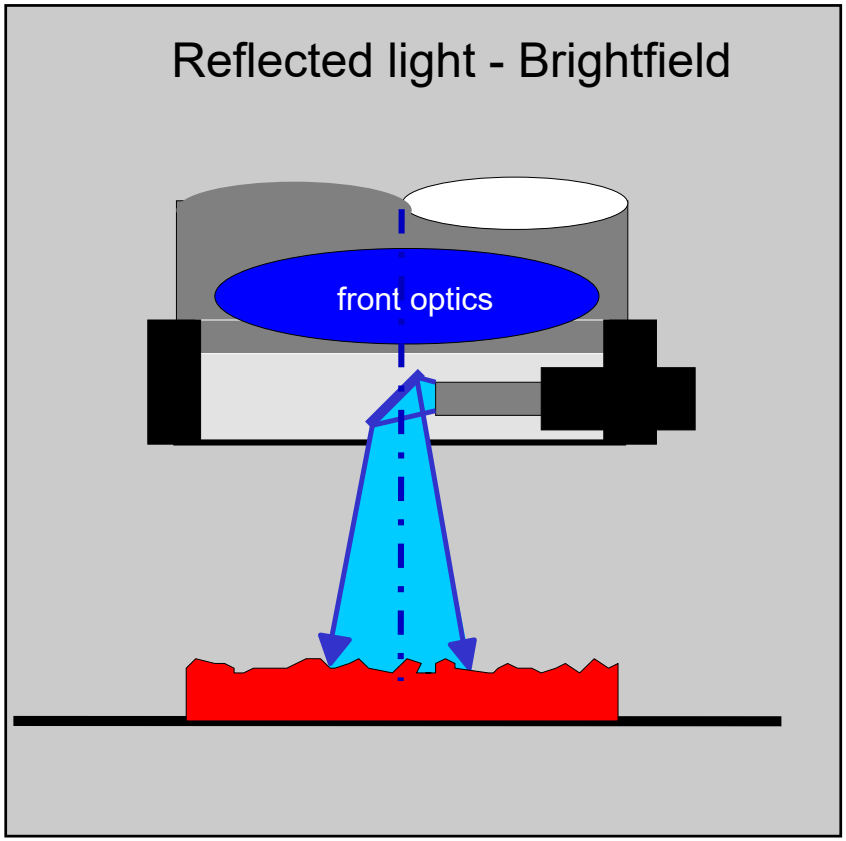


Wafer surface

Highly reflecting areas appear bright or in color.

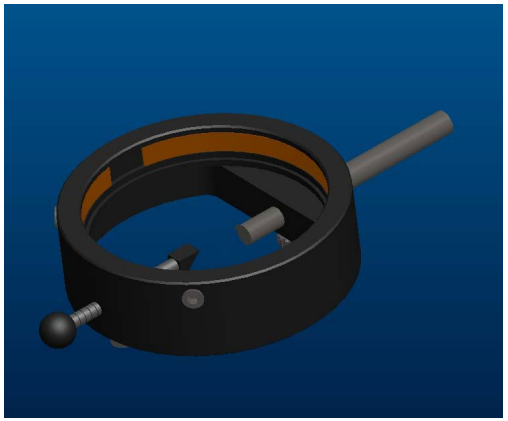
Light scattering areas of the wafer and dirt appear dark.

Reflected Light Brightfield – Brightfield Vertical Illumination

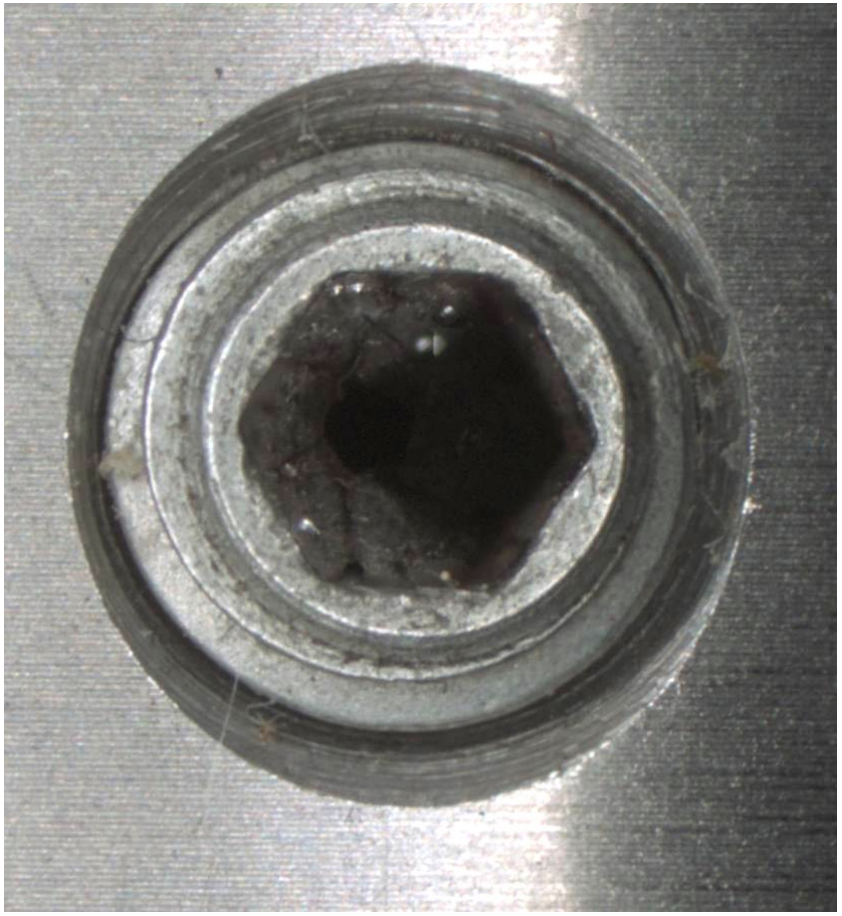


Application

Perpendicular light beam illuminates depressed specimen details
Shadowless observation of the insides of holes and cavities



Reflected Light Brightfield – Brightfield Vertical Illumination

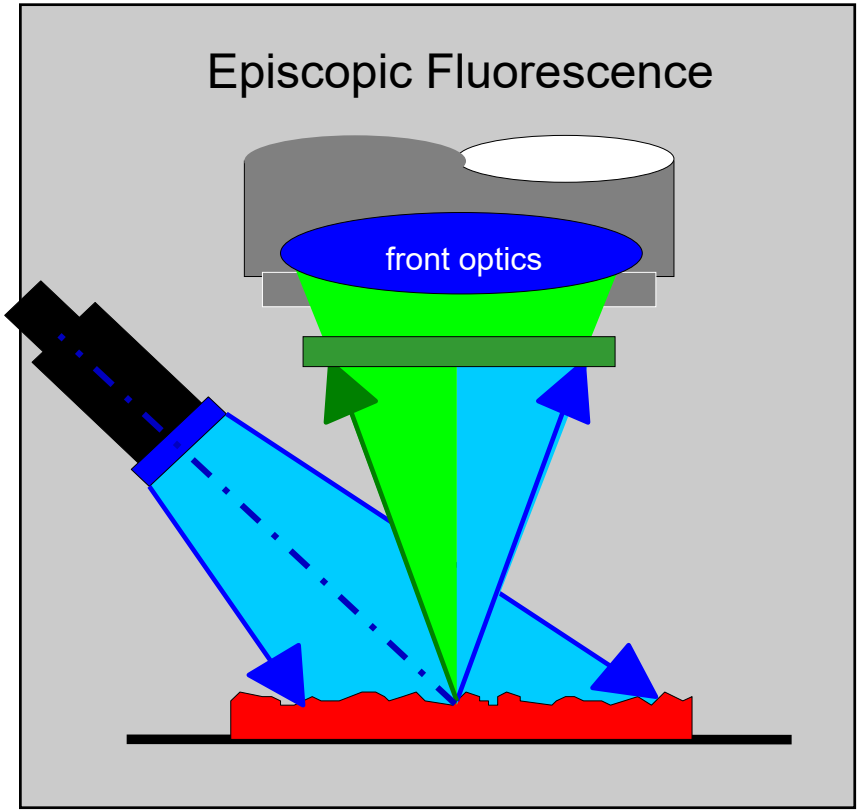


Threaded hole

Ring light illumination:
The hole in the center is shaded.

Vertical illumination:
The hole is fully illuminated.

Fluorescence Illumination – Contrast Enhancement by Fluorescence



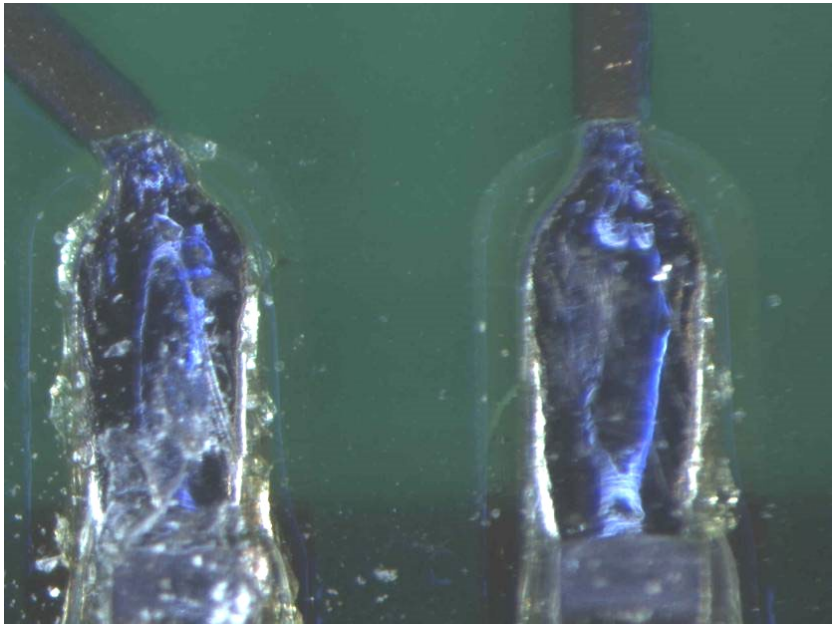
Application

Simplest (concrete) to most critical (microelectronics) fluorescence applications

Reflection-free fluorescence images

Strong 3D effect

Fluorescence Illumination – Contrast Enhancement by Fluorescence



Solder joints

Flexible light guide:
Solder joints appear to be intact.

Fluorescence illumination:
Cracks in the solder joints lights up in green.

Appendix

1 Overview

2 Major R&D areas of materials microscopy

3 Types of Light Microscopes

4 Contrast and Imaging Modes in Microscopy

We make it visible.