Microscopy for Biology Education

Practical Experiments for Education in Biology

ZEISS

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Part I

Basics of Microscopy

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Basics of Microscopy

This manual is intended for all trainers of bio-medical applications in individual instruction and at schools and universities.
It will provide you with basic information on microscopy, sample preparation, and practical instructions for biological experiments.

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Equipment

A microscope should be available for each participant; no more than two participants should share an instrument.
In larger classes, use a master instrument for the instructor equipped with an HD camera or connect the microscopes in your teaching space to create a digital classroom.

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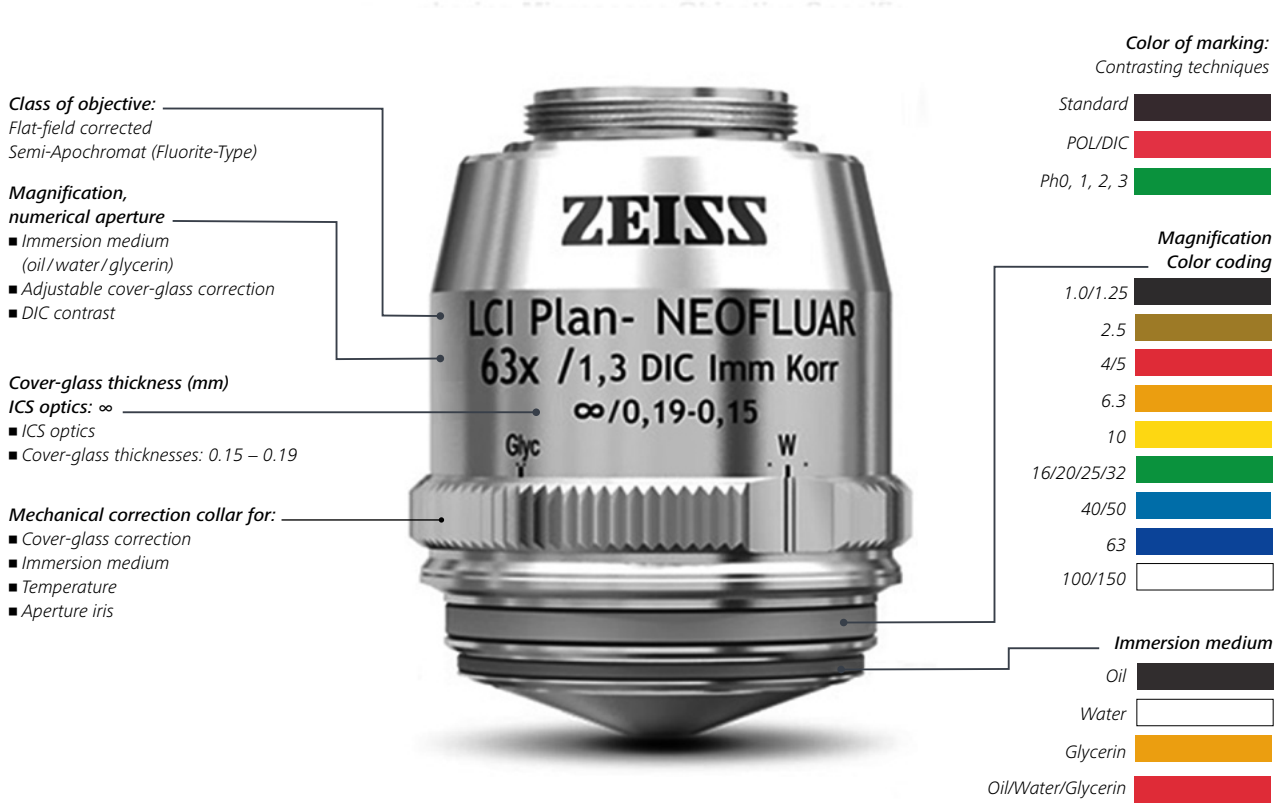
All microscopes should be equipped with a Koehler beampath and various contrasting methods (e.g. brightfield, darkfield, phase contrast) to demonstrate the various contrasting methods and their proper alignment to the students.

1. The Microscope and How It Works

The task of a microscope is to enlarge small details of a specimen, thus making them visible to the human eye.

The enlarged image is visually observed through the eyepieces.
The image formation within the compound microsope takes place in two major steps:
First, the objective forms the slightly magnified, real intermediate image. Second, this image is further enlarged by the eyepiece, which acts like a simple magnifying loupe. This virtual image is viewed with the human eye lens apparatus and projected onto the retina.
The eyepiece is typically of 10× magnification and ideally has a visual field number of 20 to 23.
The field of view number is the diameter of the intermediate image given in mm.

Markings on the Objective



Demo 1

Classes of Objectives

Achro (Greek: colorless)
Achromatic objectives are corrected for two colors and do not produce strong bands of color (chromatic aberration) around the details of the image. Sometimes the prefix achro is replaced by the prefix "A," such as in "A-Plan."

Apochromat (Greek: free of color)
Objectives that have no visually detectable traces of color fringes. Apochromatic objectives are corrected for three colors.

D
Objectives are designed for use with or without an immersion liquid for establishing contact between the front lens and the sample surface (usually the cover glass surface). Most commonly used is the standard 0.17 mm cover glass; such objectives therefore frequently bear the marking D = 0.17. Sometimes a cover glass is not permitted (D = 0).

Epi (Greek: from above)
Indicates objectives suitable for reflected light work (Greek: "from above") such as the ZEISS Epiplan. Objectives for transmitted light lack this designation, for example the ZEISS Plan.

Korr objective
Objectives with a correction collar can be used with cover glasses of varying thickness.

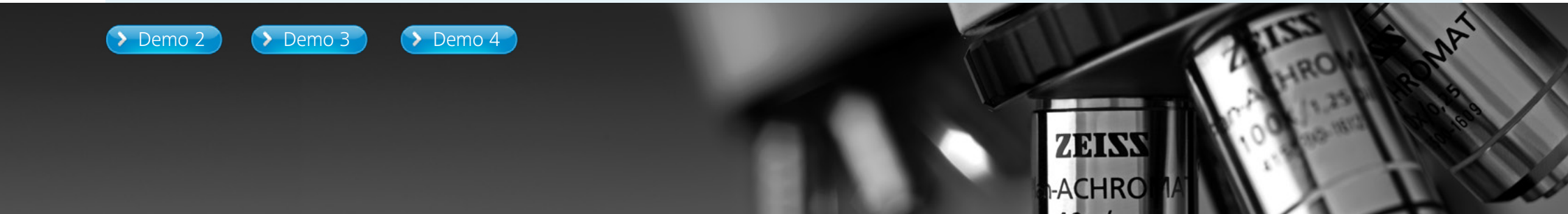
LD (long distance)
LD objectives provide an extended working distance usually with lower N.A. values, corresponding to a slightly lower resolution.

Oil, W, Glyc, LCI
As demonstrated in 1847 by the Italian physicist Giovanni Battista Amici (1786–1863), when direct contact is established between the front lens of the objective and the sample by means of a liquid such as water, image sharpness and brightness increase dramatically. These media are referred to as immersion liquids. Today synthetic immersion oils, glycerin, water, or silicone oil are used as immersion fluids. Immersion objectives can bear the marking Oil, W, Glyc, or LCI depending on their design.

Plan (Greek: flat)
Plan objectives are calculated to eliminate the field curvature providing a flat image, sharp to the edges. As flatness if image is of particular importance for reflected light applications, all Epi objectives are flat-field corrected all the way to the edge. This is not necessarily the case for transmitted light objectives which are used mainly in biology and medicine. In these cases, the simpler A-Plan types have a lower flat-field correction as compared with the higher class of objective, the N-ACHROPLAN.

Dry objectives
Objectives designed for use without an immersion fluid are referred to as dry objectives.
Dry objectives of low magnification are not sensitive for the use with or without a cover glass. For this reason they are marked "-" (insensitive).

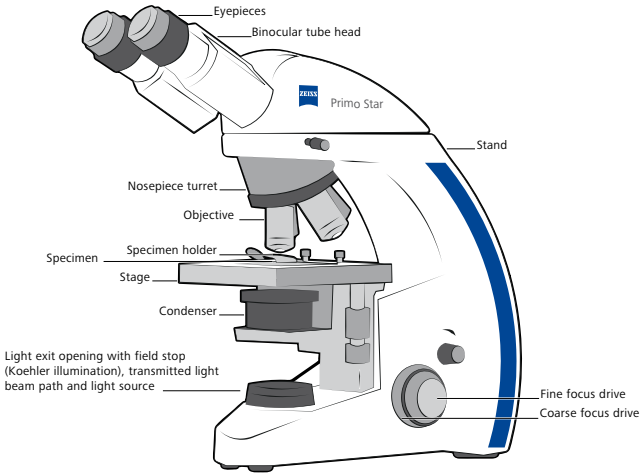
Demo 2 Demo 3 Demo 4



Depending on their characteristics, such as image flattening, color correction, and transmission, objectives are assigned to different classes such as achromat, semi- apochromat and apochromat.
If objectives are used under incorrect optical conditions, they produce images with considerable blurring. Typical mistakes are to use the wrong cover-glass thickness or medium.

Adjusting the eyepieces ensures that both the human eye and the camera are focused on the same, coincident focal plane. If you need eyeglasses or contact lenses, wear them. First, adjust all focusable eyepieces to the zero mark (if there are crosshairs, this is the red dot; without crosshairs, the white dot is used). Look into the fixed or focusable eyepiece already adjusted to the zero mark. With this eyepiece, focus on a small, distinct structure (the right eye is always used to look through the right eyepiece) using the fine focus knob on the microscope. The image for the other eye is adjusted by bringing the image of this structure into focus using only the eye lens focussing ring on the eyepiece.
In the unlikely event that the camera focus is not coincident with the adjusted eyepieces, the height of the camera adapter must be altered.

Built-up of the Compound Microscope



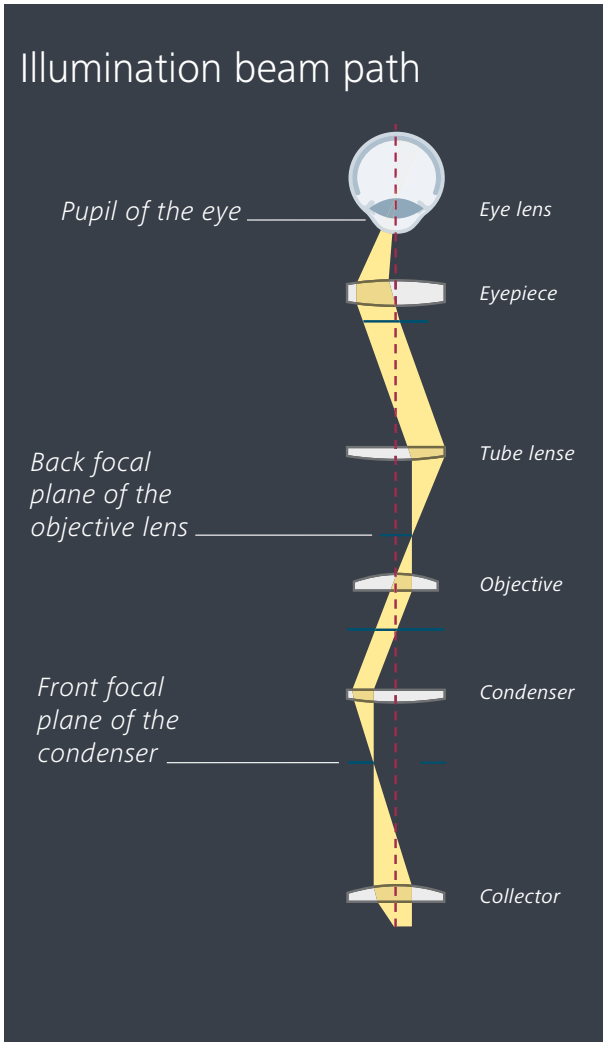
The microscope stand houses all components for illumination ind image capturing.

The stand carries the binocular tube head, which accomodates the eyepieces. Tubes for different field of view numbers are available. Typically, simple tubes offer a fixed viewing angle (mostly 30°). Ergotubes have adjustable viewing angles and/or heights. The binocular tube head often is outfitted with a camera port (trinocular tube or phototube). Numerous adapters for different camera types and sensor sizes are available.

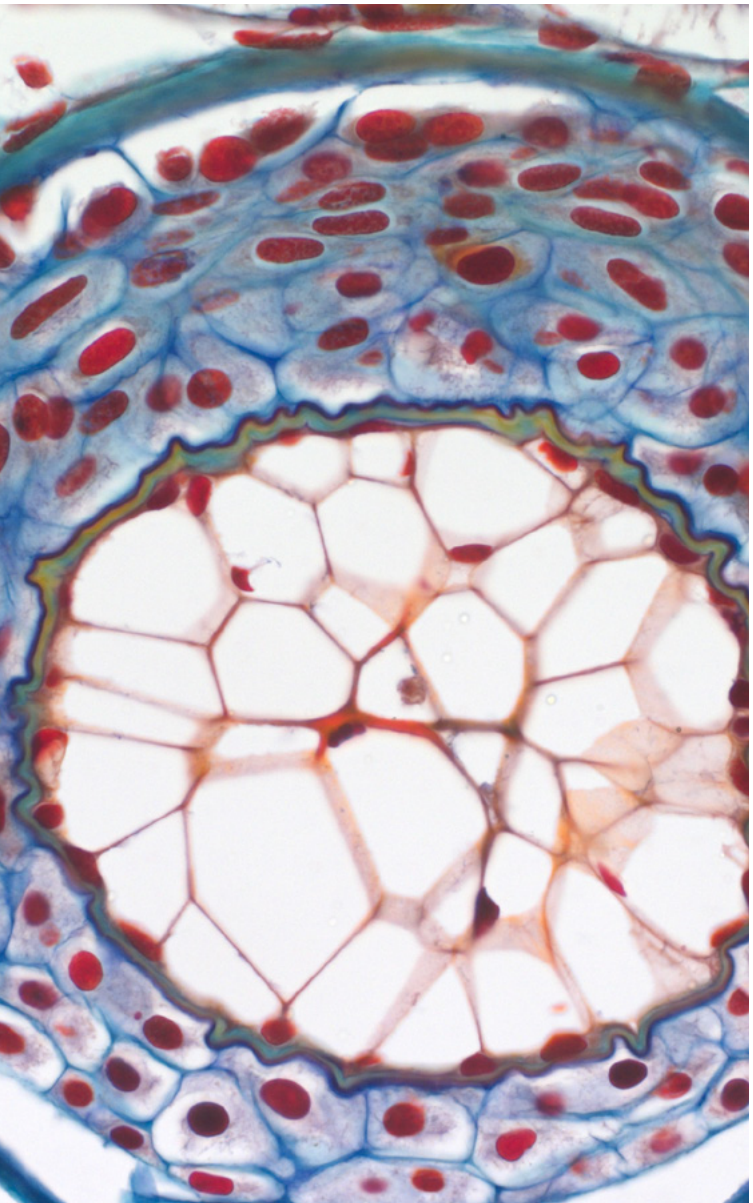
The different objectives are mounted within threaded openings of the objective nosepiece. The thread dimensions are specific for the microscope manufacturer. Different object guides are available for different applications (such as polarized light, reflected light, immersion slides). The condenser carrier below the stage will house different types of condensers. For Koehler illumination, the stage carrier is height adjustable and centerable. Both the condenser and the reflected light illumination beam path utilize an aperture diaphragm. The aperture diaphragm is also referred to as a contrast diaphragm or condenser diaphragm. The aperture diaphragm is used to control resolution and image contrast in Koehler illumination. The lamp house, often with an adjustable collector lens system (for optimal illumination homogeneity) contains the light source (halogen or LED). The field diaphragm controls illumination homogeneity and reduces stray light. The alignment method is referred to as the Koehler illumination method.

Transparent samples are usually observed in transmitted light. Opaque samples are looket at in reflected light.

For fluorescence, microscopy samples are illuminated/excited in reflected light.



Basics of Microscopy



To detect the fine structural details within a specimen two major preconditions-resolution and contrast-have to be fulfilled.

Resolution

A detail becomes visible only when it can be distinguished from those around it. This distinction of the individual details of a specimen is referred to as resolution. Resolution increases the smaller the distance between details which can still be distinguished from one another. In a conventional light microscope, the smallest revolvable distance between neighbored structures is approximately 0.3 – 0.2 µm. An important indicator of the resolving power of an objective is its numerical aperture (N.A.). Resolution is dependent on the N.A. of the objective, the light wavelength of the illuminating light utilized, and the refraction index of the medium between the front lens of the objective and the sample surface.

Contrast

There must be a difference in brightness between the structure or a specimen and its surroundings, otherwise it is invisible to the human eye even under adequate resolution and magnification. This difference in brightness to the surroundings is referred to as contrast. Contrast is affected by sample preparation, the distance between the sample and the cover glass, and the thickness of the cover glass in transmitted light. In reflected light, the reflexiveness and diffraction behavior of the samples affect image contrast.

Magnification

Details must be dispalyed with a sufficient size. In other words, they need to be magnified, in order to become detectable by the human eye or a camera. Magnification ranges from low to medium to high.

Low
(objective magnification 1.0× to 5×):
Large overview images for sample sizes up to 25 mm are used most often in screening applications. Challenge: it is difficult to homogeneously illuminate large object fields. The large depth of field makes dust visible.

Medium
(objective magnification 10× to 40×):
Medium magnification is suitable for most applications. Challenge: color reproduction, spherical aberration due to incorrect sample conditions.

High
(objective magnification 63× to 100× (150×)):
Small samples, fine structures. Challenge: stray light which reduces the contrast in finely structured details, insufficient resolution, color reproduction, spherical aberration, illumination intensity (image brightness).

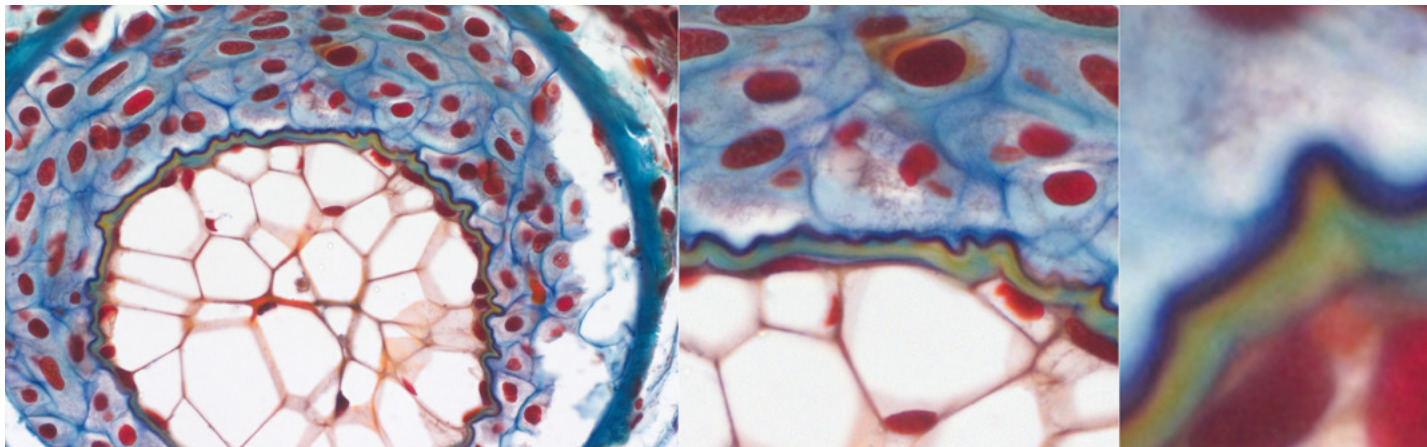
Sharpness

This is not a scientific term but nevertheless continues to be used. Sharpness is best defined as the line contrast of resolved structures. Without sufficient contrast, resolution cannot be detected.

Depth of Field

This is the "thickness of the optical section" of an object through which a sharp image is observed through the entire stack. Images with high resolution exhibit a very thin object section which appears sharp through the entire stack: depth of field and image resolution are mutually dependent. A 20×/0.5 objective typically has a larger depth of field (2.53 µm) than a 20×/0.8 objective (1.32 µm).For the image sided focal depth (e.g. in the camera sensor plane) the term depth of focus is used. It is reciprocal to the depth of field.

Demo 5



Microscope Types

Upright and Inverted Microscopes

A distinction is made between microscopes for upright observation (the sample is located below the objective) and those for inverted observation (the sample is located above the objective). Inverted microscopes are the instrument of choice for work with cell cultures (as in petri dishes) and are also suitable for advanced cell biology applications (such as 3D fluorescence imaging). Inverted and upright microscopes are suitable for use with transmitted and/or reflected light. Inverted and upright microscopes use only a single beam path for image creation and so are unable to provide stereoscopic images. The images produced with these are called monoscopic or non-stereoscopic images.

Stereo Microscopes and Zoom Microscopes

Microscopes with two beam paths can produce stereoscopic images and are referred to as stereo microscopes. They have a limited resolution (N.A. < 0.144). In addition to providing stereoscopic images, they also offer large working distances.

Demo 6

Koehler Illumination in Transmitted Light

August Köhler (1866–1948) invented this illumination method for transmitted and reflected light in 1892. Koehler illumination results in a uniformly illuminated microscopic image with maximum illumination homogeneity. It is achieved by projecting an image with a small iris diaphragm, called the field diaphragm, into the plane of a sample already in focus. In this way, the image from the light source cannot appear in focus together with the object plane. And, so the light source in the focal plane is imaged on the rear surface of the objective.
In Koehler illumination, only that object field diameter is illuminated which is imaged by the objective. The result is an image free of stray light. This gives the operator control over the relative strengths of high resolution and strong contrast.

Koehler illumination is aligned for transmitted and reflected light. The method for each is of a slightly different complexity and both involve several alignment steps. When the microscope objective is changed, the alignment process must be repeated.

Koehler illumination is used for objective magnifications of 10× and higher.

Contrary to Koehler illumination, the light source image unintentionally or accidentially can be imaged into the specimen plane. This often happens in stereo microscopy, where a Koehler illumination is not possible for optical reasons. This undesirable situation is called critical illumination opposing the Koehler illumination.

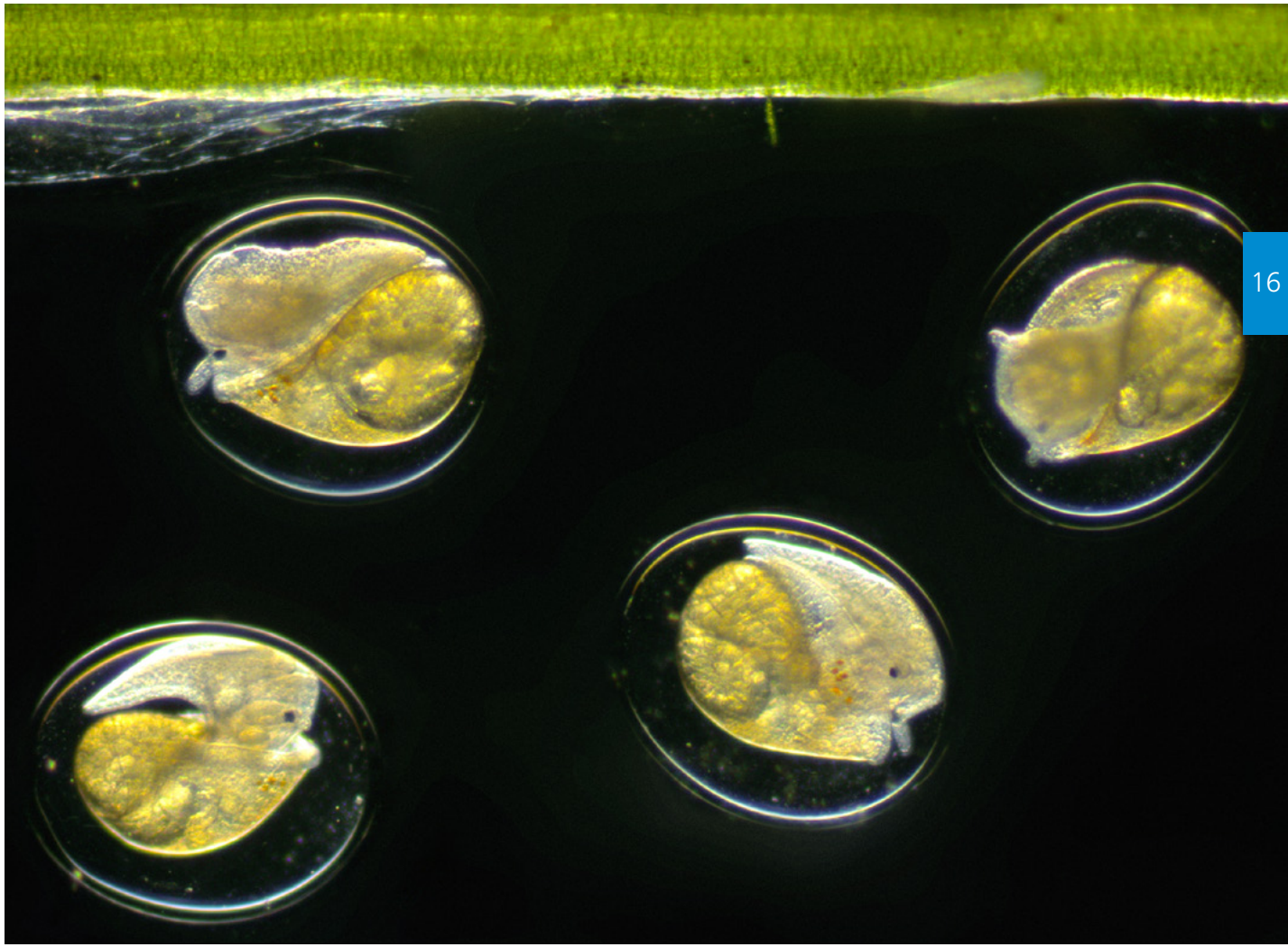
Demo 7

Dedicated Contrasting Techniques

Darkfield

The darkfield method utilizes light which did not pass through the objective lens, but rather bypasses it and strikes the surface of the sample obliquely. In contrast to brighfield, only the light diffracted by the specimen can enter the objective. The illuminating light is guided outside of the objective front lens. These appear very bright. Areas oriented perpendicular to the optical axis reflect the light past the objective lens and appear in the image as dark. This contrast method is especially useful to detect very minute and isolated structural details.

Demo 8

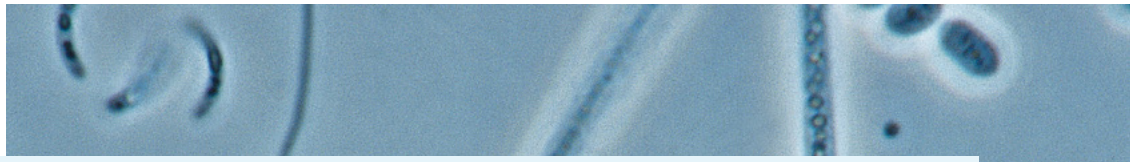


Phase Contrast

The Dutch physicist Frits Zernike (1888–1966) derived the phase-contrast technique mathematically. For this theory, he was awarded the Nobel Prize in 1953. Transparent samples (phase objects) are normally thin and therefore provide only weak contrast under brightfield illumination. Phase-contrast illumination transforms such faint images of translucent, thin structures into images with strong contrast.

Thin samples (e.g. single cell layer, protozoa etc.) are well suited for observation under phase-contrast illumination. Poor results are obtained with phase-contrast illumination on samples which are at a large distance from the cover glass or are too thick.

Demo 9



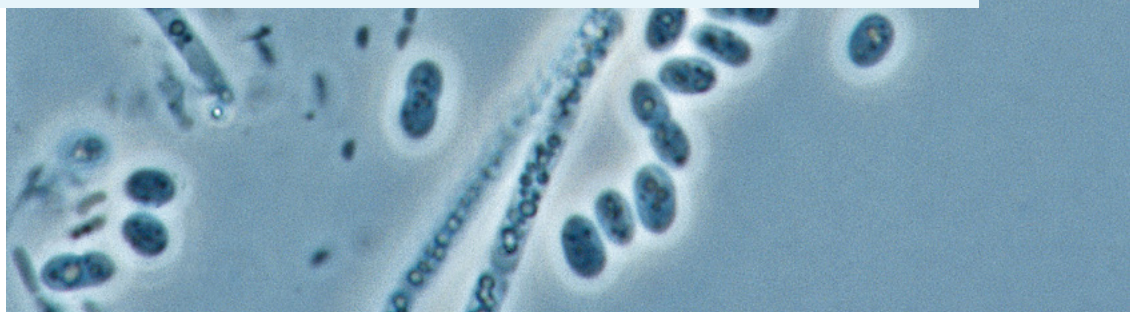
Phase-Contrast Objectives

For phase-contrast microscopy, dedicated objectives and matching ring stops inside the condenser are required.
The permanent phase ring structure inside the objective is mounted on a glass plate (phase plate for 10× objective) or on the surface of a lens (in most objectives). The diameters of the phase rings are classified in numbered groups which are based on ring diameter and objective aperture. Condenser-aperture size number Ph0 indicates 5×, Ph1 indicates 10×, Ph2 indicates suitability for most dry objectives, and Ph3 indicates suitability for all immersion objectives. The objective's back focal plane, containing the phase stop image as well as the phase ring, is observed using a Bertrand lens slider or an auxiliary microscope. The gray phase-ring structure inside the objective and the bright annular condenser aperture which is projected onto the back focal plane, are visible. By using the correct alignment tools, the annular condenser aperture can be centered in relation to the phase-plate image. Today, phase-contrast illumination is used only as a transmitted light method.

For best results in white-light phase-contrast microscopy, it is recommended to use LED light sources or HAL lamps with a suitable conversion filter. Otherwise, the phase-contrast image does not possess the optimum grayish background color.

Contrast intensity in phase-contrast microscopy is highest in green light as the annular structure of the phase objective is optimized for light with a wavelength of 550 nanometers. A strong green filter is used for this purpose.

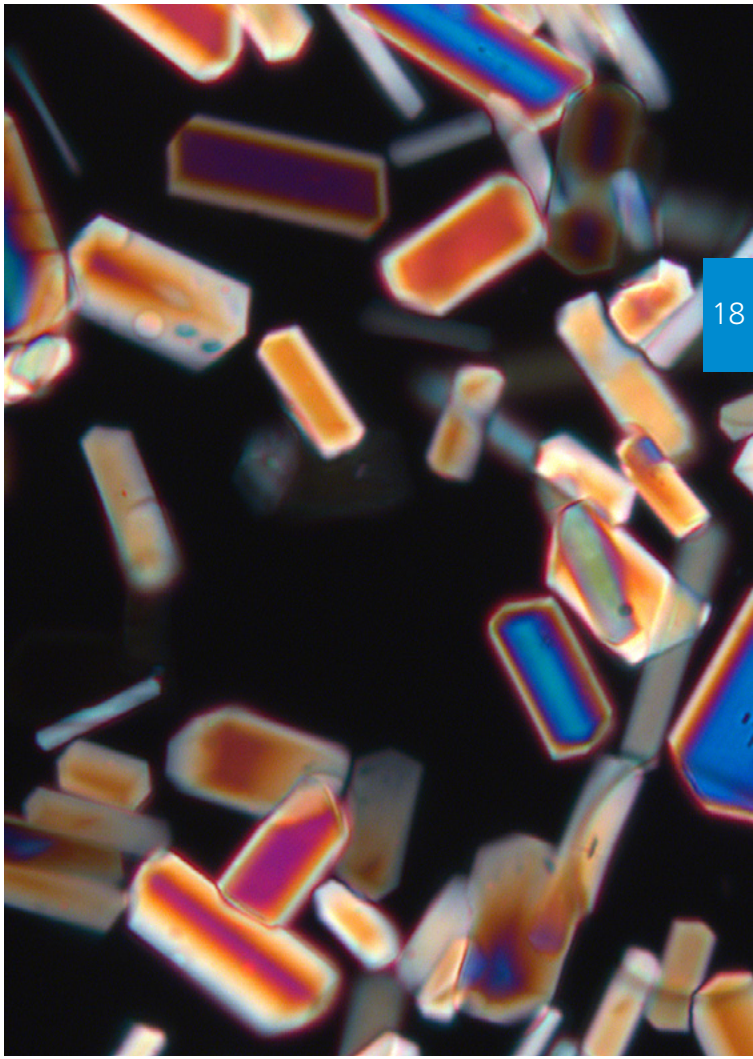
Demo 10



Oblique Illumination

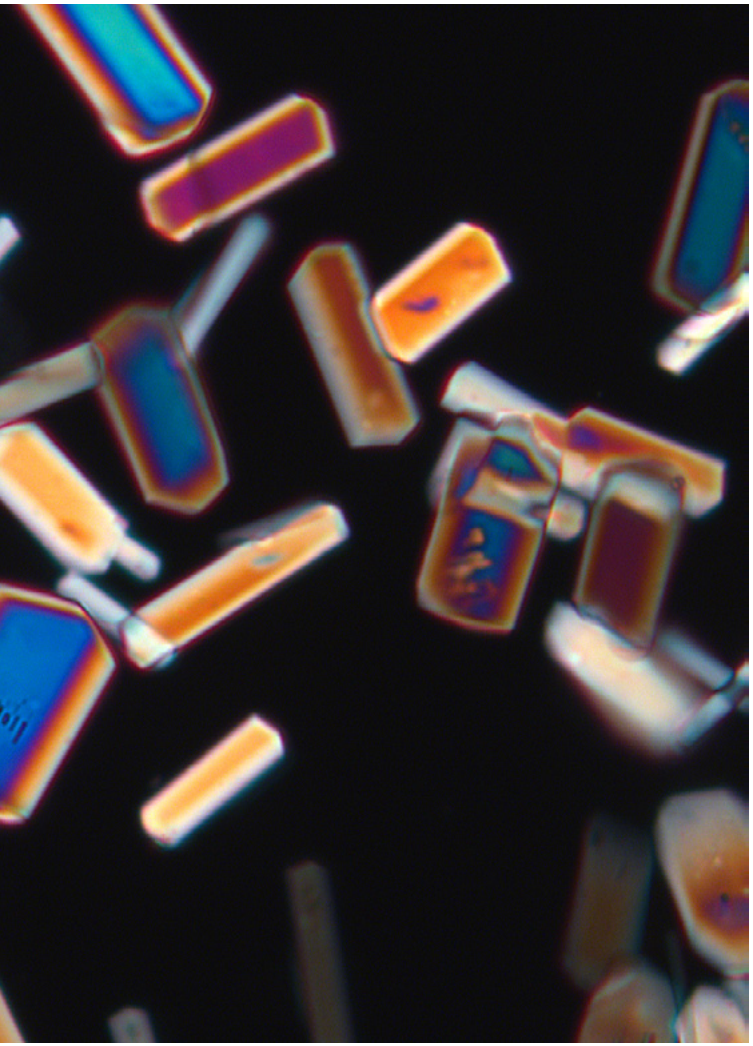
Oblique illumination is recommended for contrasting objects which are too thick for phase contrast. Oblique illumination produces a relief image which shows the finest structural details. The sample must be transparent. Oblique illumination is created by introducing the illuminating condenser beams at an angle.

Demo 11



Polarization Contrast

Many materials, such as most crystals – including some biological structures such as muscle cells are birefringent. This phenomenon fulfills an important diagnostic function in mineralogy, forensic microscopy, polymer research, or the quality control of textile fibers. In polarized light microscopy, transmitted illumination is typically used. But reflected light is also applied to visualize the contrasts in the grain structure of opaque metals such as aluminum, zirconium, etc. In stereo microscopy, polarized light is often used to block undesired reflections from shiny surfaces such as those on light bulbs and highly polished metals. For simple polarization contrast illumination, the microscope must be equipped with two crossed polarizers. In most cases, at least one polarizer and an analyzer are used for a polarization microscope. The two are oriented perpendicular to one another.



The illuminating polarizer is aligned so that it transmits in the W–E direction and the analyzer so that it transmits in the N–S direction. Polarizers and analyzers are typically referred to as polars. The phenomenon of double refraction depends on the alignment of the sample when observed between the crossed polars. For this reason polarization microscopes are equipped with a stage which can be rotated and preferably centered and which has an object guide.

Birefringence is detected between crossed polars by rotating the samples once through a complete rotation of 360 degrees. Birefringent materials oscillate four times with maximum brightness and darkness between the crossed polars.

Additionally, a lambda plate can be placed in the beam path. By definition, the direction of vibration of the slow light inside the lambda plate is from SW to NO and is referred to as N-gamma. Between crossed polars, the lambda plate generates a distinct violet background to the image. This color is also referred to as first-degree red or as sensitive color.

Demo 12

In order to be able to reproduce the results in polarization light microscopy, a defined illumination color temperature of 3200 K or 5500 K must be used. Depending on the light source, there are various filters which only function correctly when used in conjunction with neutral density filters.

The birefringent sample examined can be an unstained histological section of a mouse embryo, a nylon fiber embedded in IMMERSOL W, or the permanent sample of potato starch.

2. Practical Demonstrations of How a Microscope Works

Demo 1

Image Formation in a Microscope

The trainer demonstrates the intermediate image using a histological section of a mouse embryo stained with AZAN (LIEDER), under 10× magnification in brightfield by removing the eyepiece and inserting a long, thin piece of paper held at a slant or a small piece of frosted glass on a stem.

Demo 2

Color Correction of Various Classes of Objective

The trainer demonstrates the various color-correction stages using a histological section stained with iron haematoxylin (LIEDER). Both samples have no color of their own and all visible color bands demonstrate the chromatic response of the objectives. A good comparison can be drawn between the simpler achromat objective (such as A-Plan 40×/0.65) and a semi-apochromat objective (such as EC Plan-NEOFLUAR 40×/0.75).

Demo 3

Cover-Glass Thickness

The trainer demonstrates the effects of using the wrong cover-glass thickness by directly comparing it with the correct one. A too-thick cover glass can be created for this purpose by stacking two cover glasses on top of one another with a drop of IMMERSOL in between. N-ACHROPLAN 50/1.0 Oil is recommended as the immersion objective for these demonstrations.

Demo 4

Objective with Correction Collar

The trainer demonstrates correct use of an objective with a correction collar. The function of the correction collar is best demonstrated using an LD EC Plan-NEOFLUAR 40/0.6 Korr Ph2 objective in phase contrast with an unstained histological section such as that of a rabbit tongue.

Demo 5

The Effects of the Aperture

The trainer presents a prepared slide of a diatom shell (Klaus Kemp, UK, diatoms.co.uk) under brightfield transmitted light using a small and a large illumination aperture to demonstrate the differences in detail rendering and depths of field.

Demo 6

Parfocality

The trainer demonstrates the parfocality adjustment for the entire zoom range and harmonization between the visual image and the camera image. The eyepieces must be adjusted in the same manner as for any conventional microscope. Since Stemi 305 is not equipped with a dioptric eyepiece scale, each eyepiece is adjusted to the height at which the field diaphragm of the eyepiece appears at maximum sharpness without chromatic aberration. To adjust the parfocality, Stemi 305/Stemi 508 are focused on a sample detail with the greatest possible contrast. Then the zoom magnification is set to maximum. The image is focused again. When zoom magnification is reduced, the image should remain in focus.

At maximum zoom magnification, the depth of field is lowest and the numerical aperture greatest. Since the accommodation depth is also low, it is easy to exactly focus on a fine detail with the eye. At a minimal zoom magnification, the depth of field is small and the camera image can be precisely focused using parfocal stereo microscopy.

Demo 7

Alignment for Koehler Illumination

It is recommended to use a stained histological section such as slides of the "mouse embryo" or the "snail" *"Helix pomatia"* (LIEDER). The microscope should not be set up near a bright window, as this can result in loss of contrast and other imaging problems such as eye floaters. The optics of the microscope also suffer under changes in temperature near unprotected windows.

Before the demonstration of the Koehler illumination begins, adjustment of the eyepiece/tube and the light source is demonstrated. The pupil distance of the tube is set to a value at which only a single, unified circle displays the image field of the microscope simultaneously for both eyes. First, the eyepieces are set to the zero mark ("white dot" = without crosshairs, "red dot" = with crosshairs). When using crosshairs such as provided by an ocular micrometer (Pol crosshairs), the image can be focused with the eye lens of the eyepiece most easily in front of a light background outside the microscope. After both eyepieces are inserted, the focus knob of the microscope is used to focus on a fine structure for one eyepiece at medium object magnification. The other eyepiece is focused in correspondence with the focus on such a focused object using the focus for the eye lens of the eyepiece.

First, the image of the HAL 100 lamp is projected onto a homogeneous wall. The trainer explains that for optimum homogeneity of illumination the two images from the lamp (direct image or mirror image) must have a particular orientation to one another. When the HAL 100 image is projected onto a wall, both images should be focused such that they both produce the clearest image possible. These images should be nearly identical in size. They should overlap by approx. 50%. The next step in adjusting the lamp is to insert the lamp housing into the microscope. Remove any diffusing lenses from the light path. Focus on the sample image and open the condenser aperture. Observe the light source in the rear focal plane of the objective using the Bertrand lens slider (optional: remove one eyepiece; auxiliary microscope).
Use the SW 3 set screws of the lamp housing to shift the images of the filaments until the rear focal plane is homogeneously filled with light and filament structures from both lamp images completely and uniformly cover the rear focal plane.
This is easiest to see at objective magnifications of 40× or higher. After the lamp has been adjusted, the diffuser lens is reinserted in the light path.

Next, the alignment steps for Koehler illumination are performed as described.

In this exercise, the position of the following planes must be demonstrated: light-source image planes (front focal plane of the condenser, observed by inclining an empty slide under the closed aperture diaphragm, rear focal plane of the objective with aperture diaphragm open, use of the Bertrand lens system, exit pupil of the eyepiece with diffuser glass), object, and object image planes (focused sample plane, intermediate image plane with diffuser glass plate inside the tube, final camera or retina image with additional magnifiers/eyepieces and diffuser glass).

It is recommended to demonstrate the effect of the field diaphragm on stray light reduction by asking the participants to observe the inside walls of the tube (without looking directly into the beam of light) with the field diaphragm fully open, in contrast to those field-diaphragm diameters as used in the Koehler method. When the field diaphragm is fully open, rings of stray light are visible. These are blocked when the field diaphragm is correctly adjusted.

First, all participants practice the Koehler alignment method using already adjusted microscopes and a stained histological section. In a second step, the microscope settings are changed by a participant and must be corrected by another participant without changing the objective. Then, each participant can demonstrate the correct adjustment step and the objective is changed. The participants are given the task of describing the effects of changing the objective on the diameters of the field and aperture diaphragms. It is helpful here to prepare a schematic drawing together with all participants. The aperture cones from the light source can best be examined using a cube of frosted glass.
The participants now change the aperture diaphragm diameter in small steps and observe the effect of changing diameter on image contrast and the rendering of details in fine structures in a transparent, unstained histological section.

Demo 8

Darkfield Transmitted Light

Darkfield ring light is centered by observing the rear focal plane of the objective. Using the centering tools, the darkfield ring is aligned concentrically with the edge of the pupil of the rear focal plane.

If possible, the darkfield mirror image should be demonstrated. Adjustment of the darkfield mirror image can be demonstrated most easily using the paraboloid dry condenser 0.8/0.95 together with the condenser mount Z. There are two options for adjusting darkfield: The first method consists of centering the image of the field diaphragm at the same height of the condenser at which the field diaphragm is imaged in the sample plane. This works best with an immersion objective. After centering the field-diaphragm image, open the field diaphragm completely and alter the condenser height to darkfield. It is often the case that the field-diaphragm image is not easy to detect. For this reason, many microscopists favor the second method: Use a 20× dry objective. Alter the height of the darkfield condenser until a dark brownish spot appears in the center. This is the cross above the point of the rays of the darkfield mirror image.

If necessary, the dark spot can be centered using the x/y adjustment screws of the condenser. Now, move the condenser to the height at which the darkfield image appears to be most homogeneous. When using an iris-diaphragm immersion objective for darkfield, the iris diaphragm is closed until an optimal darkfield is achieved. This requires a strong light source (microLED, HAL 100). Use a conversion filter.

Darkfield microscopy of plankton samples, hay infusions of soil, and pond water (one or two weeks old); in particular the organic surface film of such cultures, when removed with a cover glass and observed in darkfield, does a nice job of showing bacteria and small protists.

To achieve good results with store-bought slides and cover glasses in darkfield microscopy, these must be cleaned with ethanol or dishwashing liquid, rinsed with a large amount of demineralized water, and wiped dry.

Demo 9

Phase-Contrast Microscopy

This can be demonstrated using live yeast cells which do not yield their internal structure to viewing unless they have been perfectly compacted and affixed to the underside of the cover glass. When they are pressed flat, their nuclei and other organelles become visible. The cells of the onion are perfectly suited for a demonstration of the organelle inventory of the fuel cell. The onion must be fresh because old onions are unsuitable for use under phase-contrast illumination due to an incorrect refraction behavior (similarity/congruence of the index of refraction between the structure and the surrounding medium).

Demo 10

Phase-Contrast Microscopy: Adjusting the Phase Ring

Adjustment of the phase ring is demonstrated and practiced by the participants using the unstained histological section of a rabbit tongue.

The trainer explains and demonstrates the alignment of the phase annular diaphragm. Each participant carefully repeats the steps of the alignment procedure.

The trainer also demonstrates the phenomenon that phase-contrast images have a large depth of field. This is one of the reasons why phase contrast is unsuitable for thicker samples.

In a further step, it is demonstrated that phase-contrast objectives with a magnification of 40× and more destroy image contrast when used for critical brightfield tasks such as those in hematology. This is best demonstrated using a 40× phase-contrast objective (e.g. A-Plan 40× Ph2).
The trainer also demonstrates the advantage of a strong green filter for increasing the contrast of fine structural details in phase contrast. Preparation of the upper epidermis of the skin (inside surface) of an onion is recommended. Thin leaves of the waterweed *Egeria densa* or the ruffled Aponogeton *Aponogeton crispus* can also be used.
The sample should be mounted so that upper surfaces of the leaves are facing the objective.

Demo 11

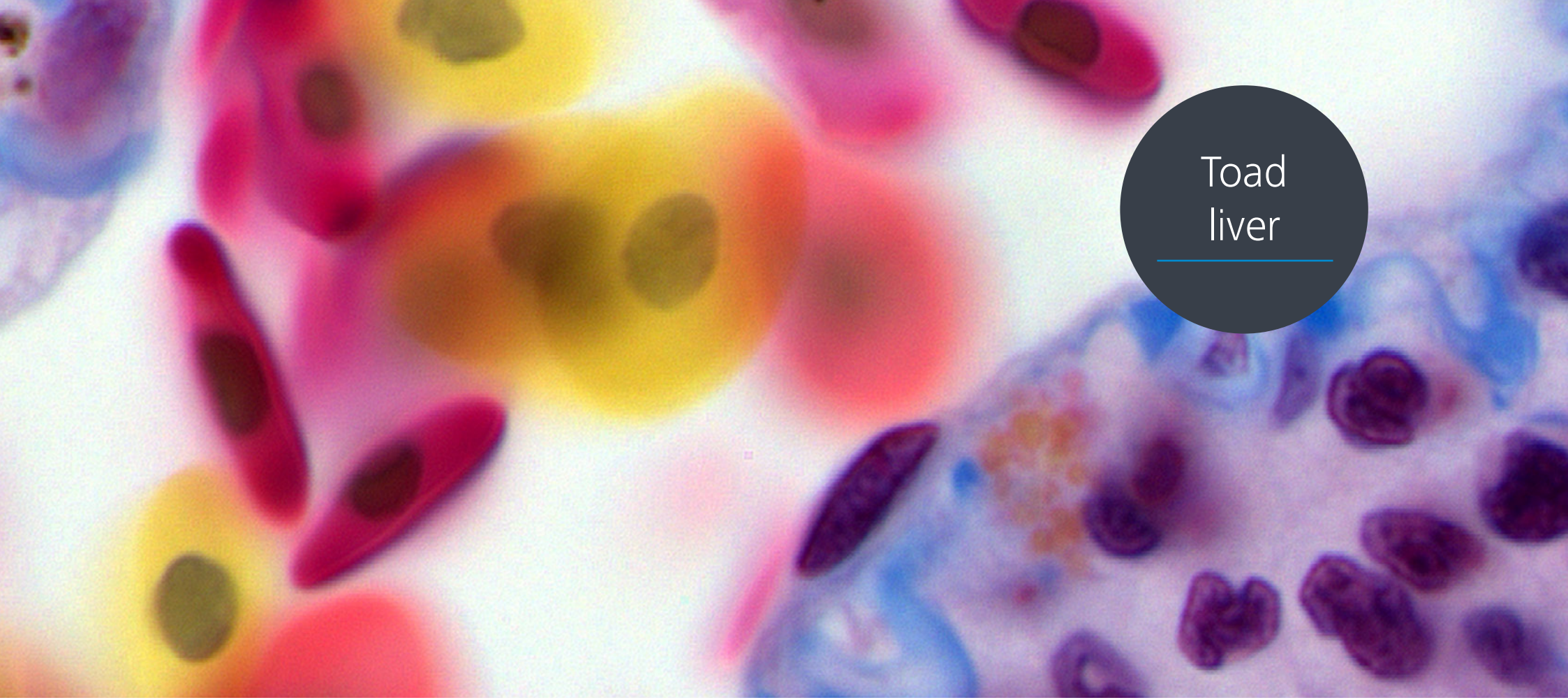
Oblique Illumination

Oblique illumination is demonstrated using a 20× objective magnification and a transparent biological sample (such as the unstained histological thin section of an embryo/plankton sample). Remove the eyepiece to show the focal plane of the objective. By turning the condenser turret, adjust the aperture diaphragm of the universal Ph condenser (brightfield position) to a position in which the image of the completely closed aperture iris diaphragm is displaced to the outer edge of the rear focal plane. A phase-contrast annular diaphragm which is too large for a given objective aperture (in this case Ph 3) also produces a somewhat more uniform but softer oblique illumination.

Demo 12

Polarization Contrast

When a birefringent detail is rotated between crossed polars using a lambda plate, it can be seen that most structures appear four times each in blue and yellow. If the long morphological axis of a cylindrical crystal appears blue in what is referred to as its positive quadrant position (SW–NO), this structure has a positive optical character. If its blue polarization color appears in the NW–SO quadrant, it has a negative optical character. With this knowledge, it is possible to distinguish between optical objects in a brightfield environment which otherwise would not be distinguishable.
The trainer demonstrates the adjustment of the crossed polars and the use of the lambda plate.



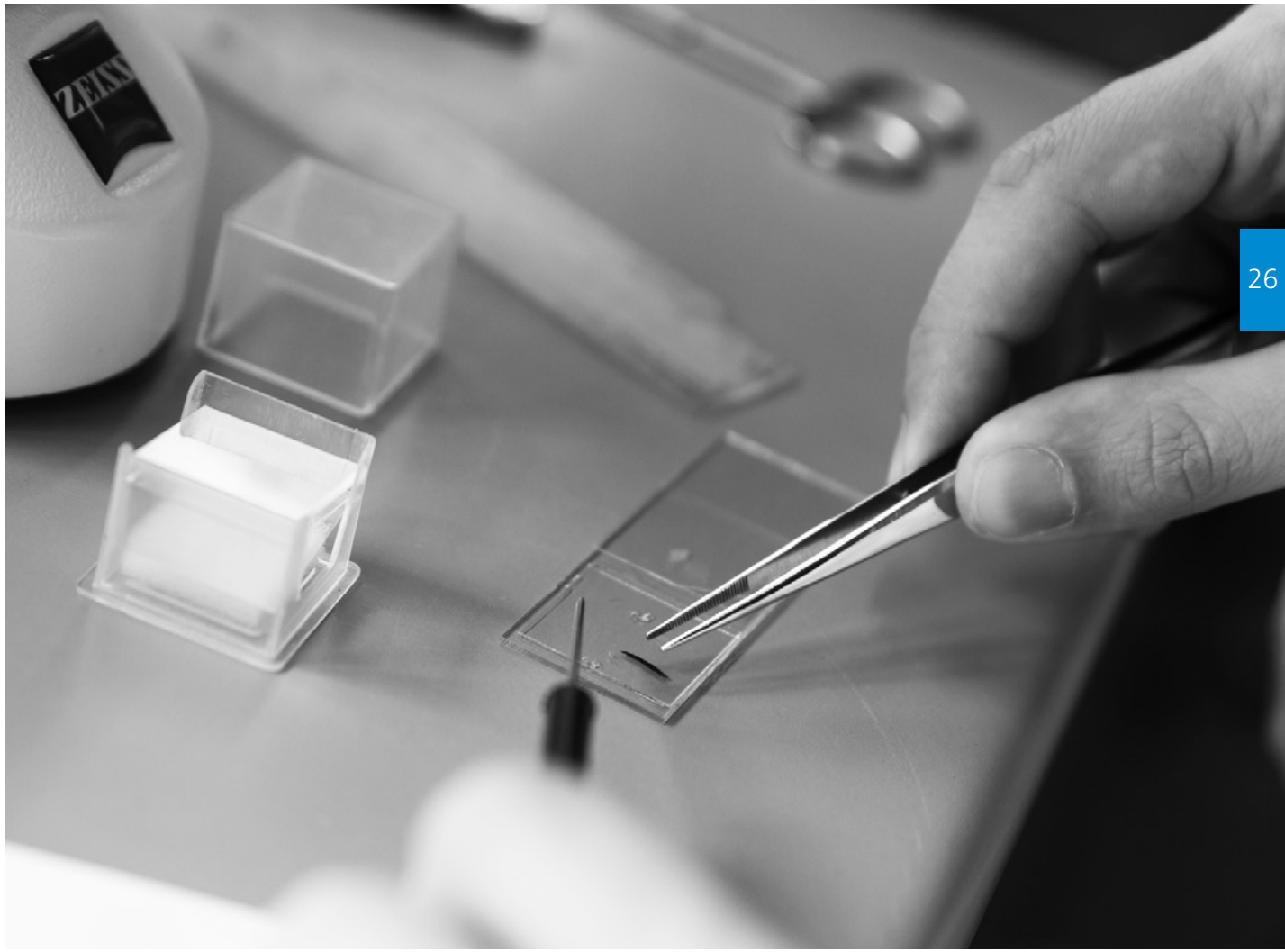
Part II

Transmitted Light Microscopy in Biology

3. Sample Preparation

In the early days of light microscopy, every interesting sample was observed directly with the microscope.

Brit Robert Hooke (1635–1703) and Dutchman Antony van Leeuwenhoek (1632–1723) discovered that most objects reveal their structures only if they are transparent and are thin enough to allow light to pass through. For this reason they began to section plant tissue with a razor blade in order to increase its transparency.
It was from these beginnings that the techniques of sample preparation have developed.



Specimens[1]

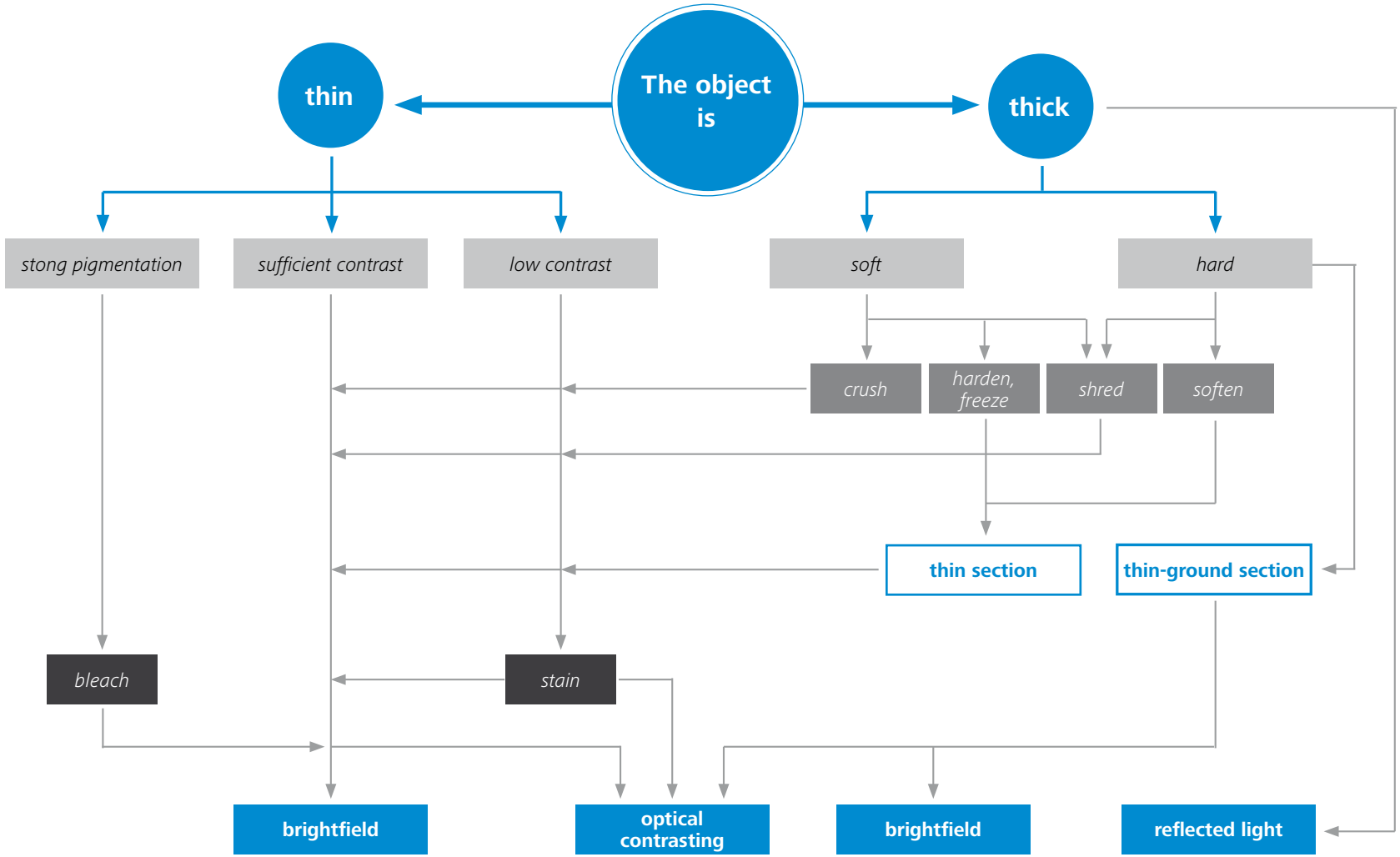
All microscopic specimens consist of four components, three of which must always be transparent: the slide, the embedding medium, the object to be observed, and the cover glass.

A distinction is made between permanent specimens and fresh specimens.

Fresh specimens are wet specimens in which water normally acts as the embedding medium. These are prepared for immediate observation and are generally disposed of immediately after. Otherwise, they must be made semipermanent by replacing the water with a quick-hardening resin. Permanent specimens are stained or unstained microscopic specimens which are enclosed in a special medium between the slide and cover glass. This preserves them for a prolonged period and they can be viewed under the microscope at any time. Examples of permanent specimens, which are on hand at any school, are blood smears, moss leaves, giant chromosomes, blood vessels, intestinal villi, or cross sections of various roots, leaves, and shoots.

[1] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 10

Overview of Preparation and Illumination Techniques



[8] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 11

Overview of Important Stains and Dyes

Stain / dye	Method; staining time	Area of application	Rinsing; counterstaining	Notes
Neutral red	Vital staining; a few minutes up to three hours	Suitable for zoological and botanical specimens (cell nucleus, organelles, tissue, plankton, small aquatic animals)	Not required	■ Vital staining should be performed under dark conditions if possible. ■ The staining effect is checked under a microscope. ■ As the staining effect is rapidly lost, examination should take place as soon as possible. ■ Not well suited for preparation of permanent specimens.
Methylene blue	Fixative solution (progressive one-phase staining); a few minutes (heat above a flame)	Suitable for zoological specimens (rapid nucleus staining during cell division, shredded and squeezed specimens)	Not required	
Acetocarmine	Fixative solution (progressive one-phase staining); a few minutes (heat above a flame)	Suitable for zoological specimens (rapid nucleus staining during cell division, shredded and squeezed specimens)	Not required	■ Staining and fixing often incomplete. ■ The staining effect is checked under a microscope.
Ethyl green acetic acid	Fixative solution (progressive one-phase staining); a few minutes	Suitable for zoological and biological specimens (rapid nucleus staining for delicate objects and protozoans)	Not required	■ Well suited for quick diagnostics, excursions, and student exercises. ■ Not suited for preparation of permanent specimens.

[3] Schlüter, Werner, 1973, p. 127 (revised and amended by the author)

Stain/dye	Method; staining time	Area of application	Rinsing; counterstaining	Notes
Safranin	Staining of pieces and sections; a few minutes up to 24 hours	Suitable for zoological and biological specimens (staining of the nucleus and of lignified plant membranes)	Wash out with 70% ethyl alcohol; Counterstaining can be performed using acid green, chrysoidine, methylene blue, and aniline blue	Before counterstaining, perform a differentiation using 96% HCL alcohol.
Fehling 1 + 2	Progressive one-phase staining; a few minutes	Suitable for zoological and biological specimens (glucose, fructose, lactose, maltose, formaldehyde, acetaldehyde, ascorbic acid)	Not required	■ Mix equal parts of Fehling 1+ 2. ■ Add to sample solution drop-wise. ■ Wait a few minutes. ■ Observe whether reaction takes place without heating. ■ If no reaction occurs, heat the solution in a water bath (60°C). ■ Initially CuOH (yellow) is formed, then Cu_2O (red).
Iodine/potassium iodide/Lugol's iodine	Progressive one-phase staining; a few minutes	Suitable for botanical specimens (amylose)	Not required	■ Add to sample solution drop-wise. ■ An iodine/starch complex is formed (blue/brown-violet). ■ Coloration fades upon heating and returns after cooling.

4. Representation of the Microscopic Image[4]

Microscopic observations can be evaluated using suitable methods of representation. The following overview is tailored primarily to the needs of the school and is intended as an initial orientation.

Method of representation	Contents
Oral description	Explanation or labeling of a depiction Criteria to be noted: objectivity, brevity, precision of expression, focus on the essentials
Microscopic drawing	Sketch or detailed drawing; accurate depiction of object, semischematic or schematic depiction
Microphotography	Small image format for slide projection, large format for overhead projector, digital for presentation using data projector
Microprojection	Projection with demonstration attachment for a small group of observers or large-scale projection for larger groups
Film	Original objects and processes, possibly complemented by animation

[4] Schlüter, Werner, 1973, p. 70f., (revised by the author)

When selecting one of the above methods of representation, keep in mind the following:

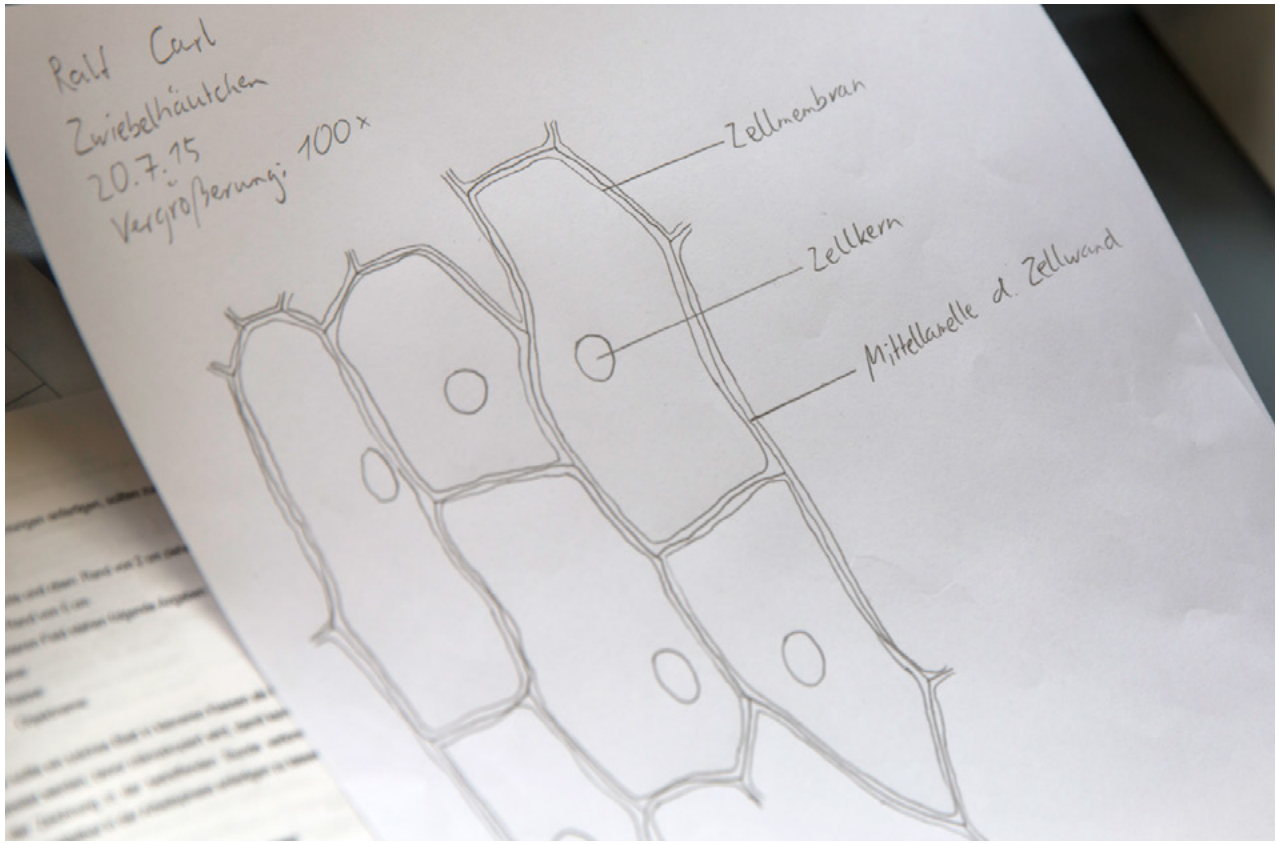
- The ability and knowledge, the experience and inner bearing of the presenter determine the quality of presentation far more strongly than the scope of effort applied and the modernity of technological equipment used.
- The method of presentation is selected depending on the aim to be achieved. There is no generally preferable method, as each has its inherent advantages and disadvantages.
- The quality of every presentation is strongly defined by the quality of the microscopic specimens on which it is based.
- The current level of knowledge and ability achieved by the students should be taken into account when selecting a method.
- Selection of a method may be limited due to unavailable or insufficient materials and technical prerequisites.
- A suitable combination of several methods can increase the gain in knowledge. The combination of microphotography and microscopic drawings of the same object provides a great amount of information.

Microscopic Drawings[5]

Before students prepare microscopic drawings, they should first receive instructions on the basic page layout:

- Left, right, and top: leave 2 cm margin.
- Bottom: draw in 5 cm margin.
- Within the bottom margin, the following information is written at the left or right margin:

Name:	Date:
Class:	Magnification:
Name of object:	Staining:



Younger students should prepare a sheet such as this beforehand, either as homework or in the lesson prior to using the microscope; this leaves the entire class period for working with the microscope and preparing the drawing. Students with more experience can integrate this task directly into the work phase.

[5] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 18

Preparation of Microscopic Drawings[6]

When drawing, students should abide by the following rules in order to achieve a result which fulfills the requirements of a microscopic drawing:

- Draw and label everything using only a well-sharpened pencil.
- Work neatly, using thin lines.
  Do not erase unless necessary.
- Write the name of the object (title) centered below the upper margin. Underline the title using a ruler.
- Draw the object so that it fills about ⅔ of the available space.
- Draw only what you see. To this end, compare your drawing again and again with the image in the microscope.
- Label the parts of the object in printed letters. Write all names of parts to the right of the object. Avoid slanting or crossing the lines used to connect names with parts.

Criteria for Evaluating and Grading Microscopic Drawings[7]

Presentation and overall impression (2 points)	Page layout
	Size of drawing (⅔ to ¾)
	Neatness
	White paper
Script (2 points)	Clear lines (no dotted lines, no shading, no coloring in)
	Variation in line thickness achieved through use of pencils of different hardness (never colored pencils!)
Information on the drawing and scientific character of the drawing (12 points)	Labeling appropriate to the task ■ Labeling lines parallel (must not intersect) ■ Labeling to right of drawing (all lines of text begin at the same indentation) ■ Neat printed letters
	Labeling is complete and correct
	Proportions reflect those in the microscopic image
	Drawing shows biological structures actually observed
	Appropriate section of image
Total points: 16	

[6] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 18

[7] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 19

Advantages and Disadvantages of Microscopic Drawings[8]

The advantages:

- Close observation of the object promotes memory retention.
- Development of essential drawing skills.
- Training in clean, precision work.
- Few technical requirements.
- Easy archiving.
- Easy reproduction.
- Important characteristics of the object can be selectively highlighted, less important ones sketched in only lightly.
- Schematization of characteristics possible.

The disadvantages:

- Errors in shape, size, and relative placement.
- Subjective rendering; drawings are not objective natural documents.
- Time-consuming.
- Drawings of living specimens are insufficiently exact.
- Risk of sloppy work.

[8] Schlüter, Werner, 1973, p. 72

5. Selected Experiments

Experiment 1

Fertilization Experiment with the Sea Urchin as Example[9]

Duration of experiment:

approx. five hours

Equipment/materials:

- Glass beakers/transparent plastic cups
- Small glass bowls
- Pipettes
- Slides
- Cover glasses
- Microscope
- Binoculars
- (Possibly) potassium-chloride solution (0.5 mol/l)

Sea urchins *(Echinoidea)* are referred to as open-water spawners. Females deposit their egg cells and males their sperm cells into open water. After a time, sea-urchin larvae can be found in the plankton. Sea urchins have been important objects of reproductive and developmental biology since the works of zoologists Oscar and Richard Hertwig. Using the method described below (Part 1), the brothers Oscar (1849–1922) and Richard (1850–1937) Hertwig obtained egg and sperm cells from sea urchins in the year 1875. This gave them an excellent opportunity to investigate the school of thought (Part 2) on the origination of progeny which prevailed at that time. It would be possible to perform this experiment as part of a field excursion to the Mediterranean.

A visit to a marine-biology station is another possible means of performing the experiment. The animals must be collected just prior to use (e.g. while snorkeling) In the Mediterranean, the black sea urchin (*Arbacia lixula)* and the purple sea urchin (*Paracentrotus lividus)* are the most common types. About three hours should be scheduled for work in the laboratory.

[9] Gemballa S., Hoßfeld U., Bogner W., 2012, pp. 20–26

Procedure

Part 1: Obtaining egg and sperm cells from sea urchins

After being removed from the sea, the animals should be held in a sufficient amount of preferably cool seawater. Glass beakers (or transparent plastic drinking cups) filled with seawater are necessary for the experiment. The opening diameter of the containers must be sufficient to allow the sea urchins to support themselves.

Techniques

1. Carefully grasp a sea urchin with both hands. Make sharp, sudden movements with your lower arms. After this is repeated a few times set down the sea urchin, anus downward, onto a glass beaker.

2. If the first technique is unsuccessful, leave the sea urchin in the glass beaker and use a pipette to drip some tap water into the mouth opening between the jaws. If this also does not lead to release of germ cells, replace the tap water with potassium-chloride solution in a concentration of 0.5 mol/l (MKCl = 74.5 g/mol).

3. If neither of these techniques produces the desired results, inject the potassium-chloride solution through the mouth membrane into the body cavity using a cannula.

Part 2: Where do the embryos come from?

Spermatists (e.g. van Leeuwenhoek)

All future generations are nested inside one another within the sperm cells; this means they are already preformed. With each new generation, the outer sheath begins to develop. The egg cell is there only to give the sperm cell a "developmental impulse."

Ovolists (e.g. Malpighi)

Only the egg cells are of importance in the development of a new generation. They already contain the precursors of following generations. The sperm cell is there only to give the egg cell a "developmental impulse."

Work with the two historical schools of thought (spermatists vs. ovolists) offers a good opportunity to practice with students some important building blocks in the attaining of scientific knowledge. This example involves the need for control experiments and the refutability of statements. The students will typically select the fusion of egg and sperm cells as an experiment with which to refute both schools of thought. However, they overlook the approach of performing two control experiments (only egg cells and only sperm cells). The trainer should provide suitable impulses to do so. The control experiments demonstrate that one type of cell alone is insufficient for development to occur. But they do not exclude the possibility that the potential for development could be present in only one of the cell types. At second glance, then, refuting the old schools of thought remains problematic. Could the counterpart of the second germ cell not simply provide a stimulus (for example by emitting a chemical substance) for the development process which is laid down only in the second type of cell?

Clear refutation is a challenging task. At this point, if the students do not initiate this themselves, the trainer should stimulate and conduct discussion in small groups. For example, a few students could be charged with defending the schools of thought to be refuted.

Make certain during this process to require clear recording and documentation (for example in the form of drawings of some of the multicellular stages indicating the time elapsed). The fertilization experiments are evaluated with the aid of microscopic observations. This allows observation of the formation of a fertilization membrane and the movement of the male prenucleus or the karyogamy.

As the microscopic specimens are highly susceptible to drying out or overheating (under continuous illumination), it is strongly recommended that the experiments be carried out in small glass bowls and adequate amounts of seawater and viewed with binoculars. These conditions allow excellent observation of the multicellular stages at 60× to 80× magnification without interfering with their development.

Successful execution of fertilization experiments can then lead to the discussion on the concept of biological species in an experimental context. As it is often possible to obtain germ cells from both species of sea urchin, cross-species fertilization experiments could be performed. The negative results can be explained only through the existence of reproductive barriers between species. The concept of biological species can thus be reinforced on an experimental basis.

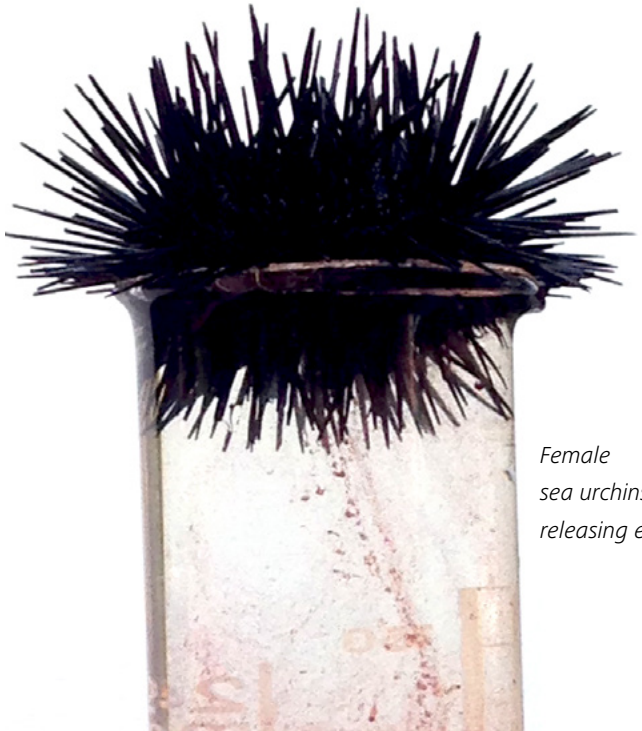
Tasks

1. Obtain adequate numbers of egg cells and sperm cells from the species black sea urchin *(Arbacia lixula)* and purple sea urchin *(Paracentrotus lividus)*. Note: The samples obtained must be clean and be supplied with adequate amounts of fresh water. Ensure that no "foreign" cells are carried over from other samples (by using the same pipette, for example).

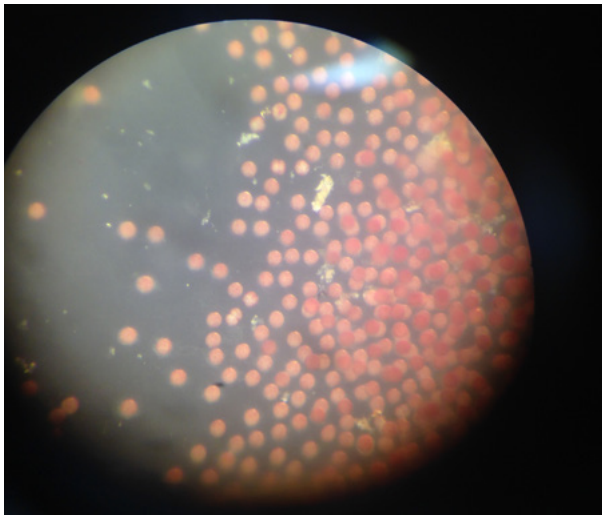
2. View selected samples under the microscope (10× and 40× objective) to clearly distinguish between egg cells and sperm cells.
The large egg cells can just be made out with the naked eye. They also differ in color from the whitish reproductive cells of the males.

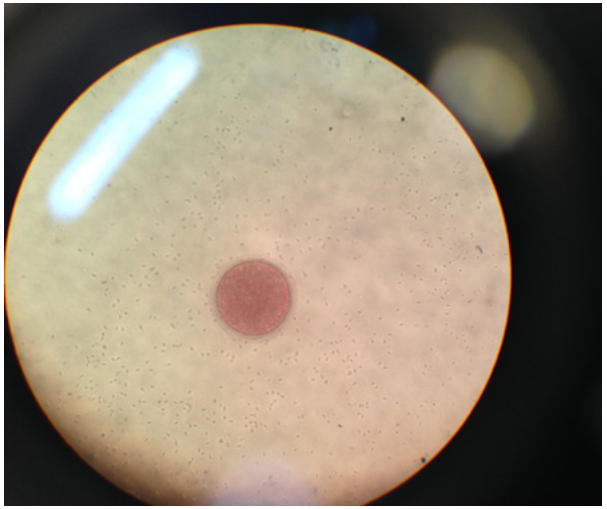
3. Conduct a series of experiments to test the schools of thought named in part 2. Observe the experiment through binoculars for a few hours. Document your methods and results.

4. Perform further experiments to test whether the black sea urchin and purple sea urchin can be crossed. Document your methods and results.



Female sea urchins releasing egg cells





Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, Kirsten Gesang

Experiment 2

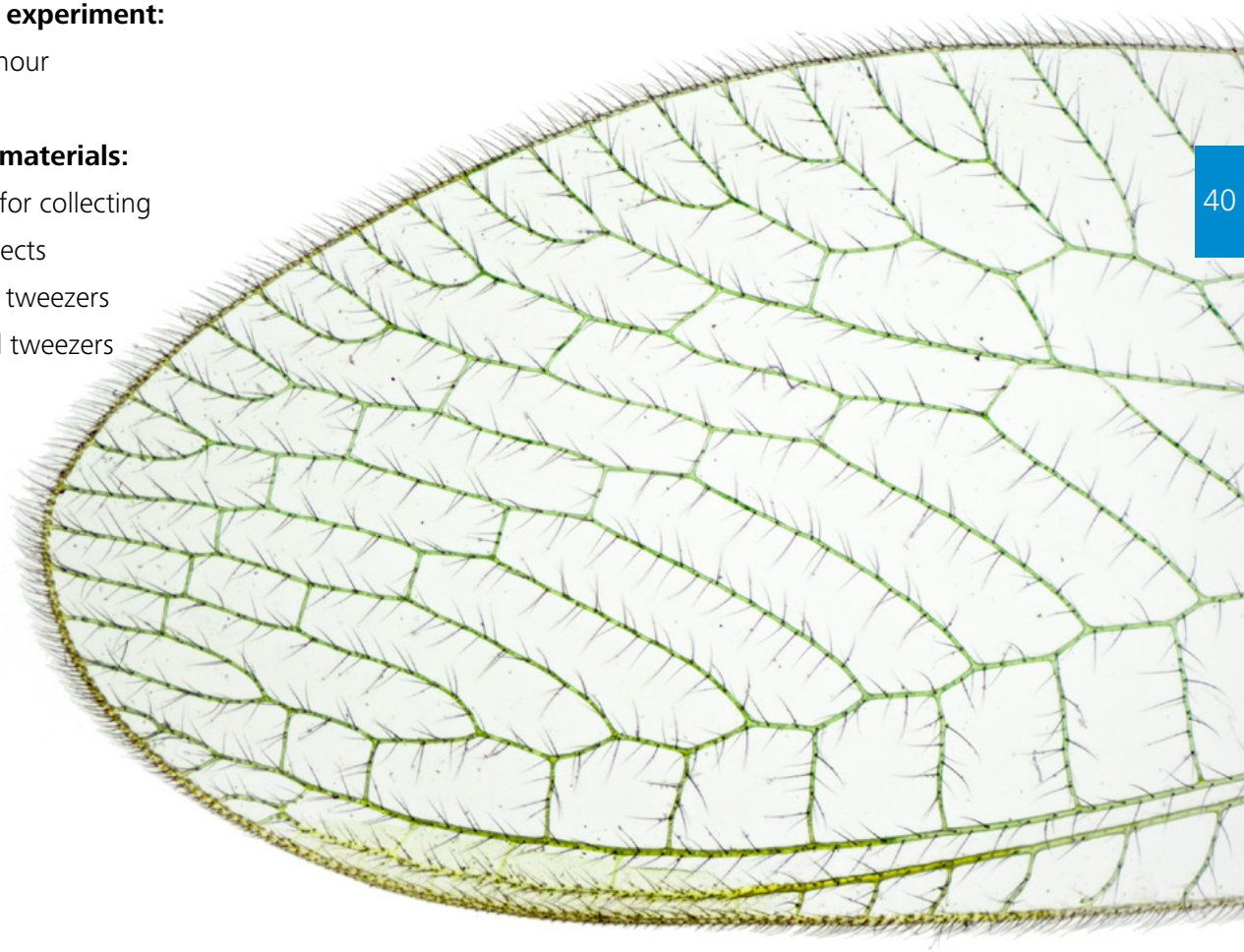
Dry Specimens of Insects[10]

Insect wings are counted among the dry objects and can be investigated microscopically without preparation. This is performed by laying air-dried objects on a slide without using a cover glass. Only external characteristics can be observed and drawn under low magnification. Binoculars are thus usually sufficient.

Duration of experiment:
approx. one hour

Equipment/materials:

- Containers for collecting suitable objects
- Anatomical tweezers
- Spring-steel tweezers
- Slides
- Microscope
- Binoculars
- Bees
- Gnats
- Beetles
- Flies



[10] Schlüter, Werner,1973, p.139

Procedure

First, dead insects are collected. This task can be given to the students as homework or be done inside or outside the school just before work with the microscopes begins. Most suitable are flies, bees, beetles, and gnats. The first step is to observe the objects without a microscope and to correlate the terms with the relevant images. As gnats are particularly easy to find, the second part of the experiment focuses on this family. Particular emphasis is placed on preparing a microscopic drawing of the two types of wing.

Tasks

1. First, observe the specimens without a microscope. The illustrations show the four main specimens. Determine the family each illustration belongs to. (Solution: gnat, blowfly, honeybee, dung beetle)

2. Observe the objects using binoculars. Look for important body parts on each insect (head, eyes, antennas, wings, legs). Name the similarities and differences in shape, color, and number of these elements.

3. Observe the gnat using binoculars. Work very carefully. Use spring-steel tweezers as this insect is not as sturdy as the other specimens.
The gnat has four wings. Locate the second pair of wings. Describe the appearance of the wings and establish a reasonable presumption as to their function.

4. Prepare a microscopic drawing of the two types of wing.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[11] Gäbler, M., In: www.commons.wikimedia.org/wiki/File:Tipula_oleracea_female_%28Linnaeus_1758%29.jpg (Access: April 29, 2013)
[12] Vogel, U., In: www.oldskoolman.de/bilder/plog-content/images/freigestellte-bilder/natur-tiere/fliege-mit-ruessel.jpg (Access: April 29, 2013)
[13] Mayer, M., In: www.bz-berlin.de/multimedia/archive/00372/biene_37207828.jpg (Access: April 29, 2013)
[14] König, P., In: www.duden.de/_media_/full/K/Kaefer-201100285695.jpg (Access: April 29, 2013)

Experiment 3

Insect Leg Types[15]

The extremities of insects can vary considerably in appearance. Phylogenetically, these emerged as biramous legs providing locomotion on solid ground. Each of these legs consists of various members (coxa, trochanter, femur, tibia, tarsus). The aim of this experiment is to observe different types of leg. It is intended to demonstrate that the basic structure of the legs is the same even though they differ in function and shape.

Procedure

Before the experiment begins, dead insects are collected as in experiment 2. Most suitable are various types of beetle, cockroaches, grasshoppers, bees, and flies. The objects collected are observed at low magnification using binoculars. The main emphasis is on the structure of the legs. These may be detached from the insect if necessary to facilitate observation.

The tasks present the basic structure of an insect leg which is then detected and carried over to the various leg types. Finally, the correlation is drawn between the structure and function of each type of leg and described in the students' notebook.

Duration of experiment: approx. one hour

Equipment/materials:

- Glass containers
- Small glass bowls
- Spring-steel tweezers
- Binoculars
- Various insects
- Five colored pencils

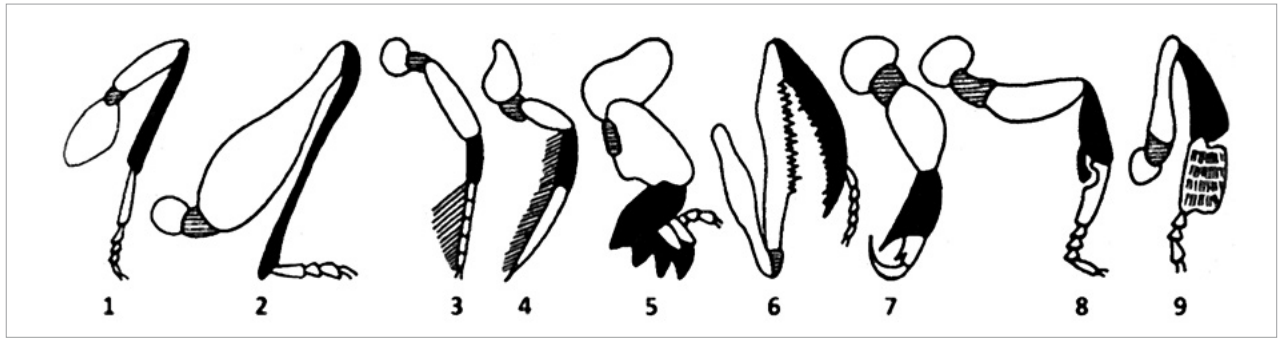
[15] Schlüter, Werner, 1973, pp. 253 ff.

Tasks

1. Use the binoculars to examine the insect legs collected. In order to obtain a better view of the legs, you can remove the extremities during preparation.

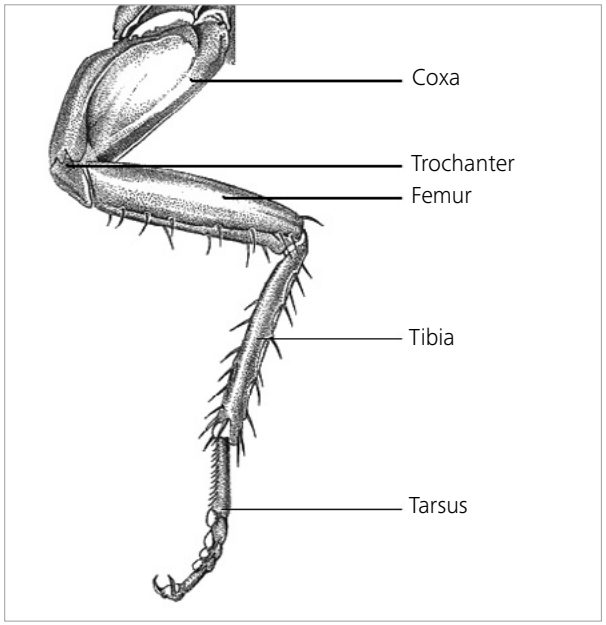
2. The first figure illustrates the basic structure of an insect leg. Color the various sections of leg in different colors. Continue with the following figures. Always color the same leg part using the same color.

3. Describe the deviations in the various legs (1–9) from the basic structure.
Establish a reasonable presumption as to the functional tasks of the legs.



Leg Forms of Invertebrates[17]

(Solution: 1 Stepping leg, 2 Takeoff leg, 3 Swimming leg, 4 Paddling leg, 5 Digging leg, 6 Preying leg, 7 Grasping leg, 8 Cleaning leg, 9 Collecting leg; hatched area: trochanter, black area: tibia)



American cockroach (Periplaneta americana)[16]

[16] Storch, V., Welsch, U., 2009, p. 253 (revised by author)
[17] Stresemann, E., 1969, p. 4 (revised by author)

Experiment 4

Onion Cells and Their Component Parts[18]

The cellular structure of plants is demonstrated using easy-to-obtain objects. This involves investigating the most important cell organelles: cell wall, cytoplasm, mitochondria, and cell vacuole.
To illustrate specific cell organelles, it is necessary to apply a microscopic staining technique which affects the microscopic object. This effect can lead to alterations (artefacts) which are not present in living cells.

Procedure

First, cut the onion in four pieces and remove one scale of the onion. Now, use a razor blade to cut a square in the convex surface of the onion and peel off a piece of skin using the tweezers. Place this in a water drop on the slide and cover it with a cover glass. Now, observe the specimen under lowest magnification. For further observations, 100× magnification is suitable. Small air bubbles are often present on parts of the onionskin. These should also be examined in order to avoid confusing these with cell organelles later.

Of the cells in the onion, only the cell walls are clearly visible. The other cell organelles have the same index of refraction as the water in which the cells are being examined. These are only vaguely visible due to the lack of contrast and can be stained by means of various techniques.
In this experiment, methylene blue, eosin, and neutral-red solution are used. The cell components treated with these stains are compared to one another.

Duration of experiment: approx. one hour

Equipment/materials:

- Microscope
- Simple phase-contrast setup
- Slides
- Cover glasses
- Tweezers
- Razor blade
- Lancet needle
- Pipette with bulb
- Square glass bowls
- Three staining vials
- Distilled water
- 0.1% aqueous methylene-blue solution (X_n)
- Neutral red
- Eosin
- Sugar
- Onion

[18] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 22

Observation of the mitochondria can continue with the addition of sugar solution. The examination requires a microscope equipped with a simple phase-contrast setup.

Tasks

1. Examine the onionskin under lowest magnification. Look out for the presence of entrapped air bubbles to rule out confusing these with other cell organelles later.

2. Place a piece of onionskin in a staining vial with methylene blue for five minutes. Then rinse the specimen with tap water. Transfer it back to the slide and examine it.

3. Then stain two pieces of onionskin, one in a staining vial with eosin and one in a staining vial with neutral-red solution. Prepare one specimen with each of these.

4. Use the microscope to examine the three stained specimens. Name the stained cell components of each.

5. Prepare another specimen of onionskin. In doing so, replace the drop of water with a drop of sugar solution. Examine the specimen in phase contrast at high magnification under a microscope. In the tapered corners of the cells in the cytoplasm, small, grained structures are visible: the mitochondria.

Stain/dye	Methylene-blue solution	Eosin solution	Neutral-red solution
Stained cell components			

Experiment 5

Chloroplasts in Waterweed[19]

Using the waterweed (*Elódea* MICHX.)[20] as specimen, it is very easy to examine chloroplasts under the microscope. These cell organelles form the center of photosynthesis and contain the green pigment chlorophyll. After a short time, the chloroplasts also begin to move, which is easy to observe.

Procedure

This experiment deals with chloroplasts and the movement of chloroplasts. In general, any green plant parts can be used for this experiment, but the waterweed is particularly well-suited for examination. At suitable magnification, the chloroplasts and their movement can be observed.

Tasks

Transfer a waterweed leaf onto a slide. Add a drop of water and cover the leaf with a cover glass. Under low magnification, find the midvein of the leaf and then focus on the long cells under higher magnification.

The green oval bodies in the cytoplasm are chloroplasts. Normally, the chloroplasts move after a short time. In the case of older leaves and of plants that have been held under unfavorable conditions, movement begins much sooner than it does in strong, healthy plants.

Duration of experiment: approx. 30 minutes

Equipment/materials:

- Microscope
- Slides
- Cover glasses
- Tweezers
- Lancet needle
- Pipette with bulb
- Distilled water
- Waterweed (*Elódea* MICHX.)

[19] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 22
[20] Rothmaler, W., 1972, p. 464

Experiment 6

Chloroplasts in the Tomato[21]

Chromoplasts are plastids which contain pigments such as xanthophylls or carotenes. These stain the relevant plant parts yellow, orange, or red. Among other functions, these serve to attract animals or to reflect the ripening process as in the case of tomatoes.

Procedure

In this experiment, a few cells are scraped from the freshly cut surface of a tomato. Microscopic examination of the specimen allows the observation of chromoplasts which become visible as yellow or red bodies under higher magnifications.

Tasks

Scrape some flesh from under the skin of a freshly halved tomato. Prepare a fresh specimen using distilled water. Even at low magnification, isolated, balloon-shaped cells are recognizable. Under further magnification, yellow-red bodies become visible in the cytoplasm; these are referred to as chromoplasts. Prepare a microscopic drawing of a few cells. Focus mainly on the area surrounding the cell nucleus.

Duration of experiment: approx. 30 minutes

Equipment / materials:

- Microscope
- Slides
- Cover glasses
- Lancet needle
- Pipette with bulb
- Distilled water
- Tomato

[21] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 22

Experiment 7

Preparation of Fresh Specimens of Human and Animal Origin[22]

It is simple to prepare a specimen from human mucous membranes. After staining with methylene blue, the isolated epithelial cells in the nucleus become visible. The structure of striated muscle cells can be seen in a specimen of shredded meat fibers. The striations become even more readily visible when the specimen is viewed under polarized light.

Duration of experiment: 30 minutes

Equipment/materials:

- Microscope
- Slides
- Cover glasses
- Polarizing filter
- Tweezers
- Two lancet needles
- Scissors
- Glass rod
- Pipettes
- Filter paper
- Wooden tongue depressor (or teaspoon)
- 0.1% alcoholic methylene-blue solution (X_n)
- 2% acetic acid (C)
- 0.9% NaCl solution
- Small piece of beef

Procedure

Part 1: Epithelial Cells from Oral Mucous Membranes

Use the wooden tongue depressor (or the handle of a teaspoon) to scrape some mucous membranes from the inside of your cheek. Mix this material with a little water on a slide. Now place a small drop of 0.1% alcoholic methylene-blue solution next to the water before placing the cover glass on top. The best observation results are obtained from the cells on the diffusion boundary between the water and the methylene-blue solution. This method of preparation isolates the cells from the surrounding tissue.

[22] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 24

Part 2: Shredded Muscle Tissue Specimen

From a piece of beef, cut a small sample along the grain. Transfer this sample to a large drop of 0.9 % NaCl solution on the slide. Shred the meat fibers using two lancet needles until there are no raised areas left in the specimen.

Remove the thick, opaque material from the slide and use a pipette to draw off the clouded sodium-chloride solution. Now, add fresh 0.9 % NaCl solution and place a cover glass on top. First, observe the specimen at 100× magnification and then at 400× to 500×. It can be seen that muscles consist of single fibers which are formed in turn from myofibrils. The most obvious feature is the striation. This is visible because the myofibrils are made up of alternating zones which are single and double refractive. Examine the specimen in polarized light. This makes the striations even more obvious.

Use a strip of filter paper to absorb the NaCl solution from under the cover glass; then use a fresh pipette to place a drop of 2 % acetic acid next to the edge of the cover glass. Use another piece of filter paper to draw this drop underneath the cover glass. When viewing under the microscope, look at the edge of the muscle fibers. Several elongated to lenticular cell nuclei become visible here after the acetic acid has been added. The striated muscle tissue is therefore made up of several cells. But no cell boundaries are visible.

Tasks

1. Examine a few isolated mouth-mucous-membrane cells at high magnification and draw them.

2. First, observe the muscle fiber specimen at 100× magnification and then at 400× to 500×. Observe the object under the microscope again after adding 2 % acetic acid. Describe your observations.

Experiment 8

Detection of Intracellular Components with Starch as Example[23]

Detection of intracellular components is particularly simple in the case of starch. The detection reaction is characterized by a change in color due to the deposit of iodine ions in the starch molecules. A blue to brown/violet color appears.

Duration of experiment: 15 minutes

Equipment/materials:

- Lancet needle or knife
- Razor blade (possibly)
- Slides
- Cover glasses
- Pipette
- Filter paper
- Potato
- Lugol's iodine (I2KI)
- Water

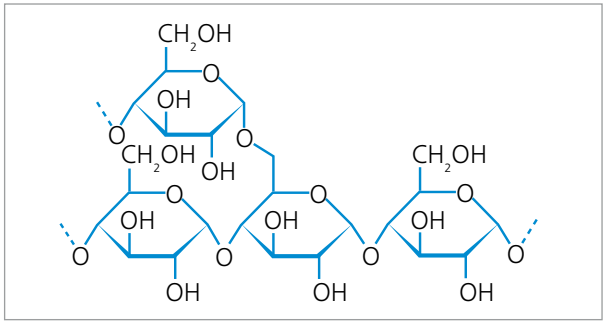
Procedure

Scrape some flesh from the potato with the knife or lancet needle and place it in a drop of water on the slide. After placing a cover glass on top, observe under the microscope. Better results can be achieved if you cut a wafer-thin slice of the potato using a razor blade and place it in a drop of water on the slide. Then, place a drop of very dilute Lugol's iodine next to the cover glass and use a piece of filter paper to draw it through the specimen from the other side. Observe the object under the microscope once again and prepare a microscopic drawing.

Tasks

1. First, observe the fresh specimen under a microscope without stain.

2. Stain the flesh of the potato with one drop of very dilute Lugol's iodine. Observe the object under the microscope again. Prepare a microscopic drawing.



[23] Friedrich-Schiller-Universität Jena, Arbeitsgruppe Biologiedidaktik, 2011, p. 26

Experiment 9

Paramecia[24]

Paramecia (*Paramecium* O. F. MÜLLER) belong to the ciliate group (*Ciliata*) and can be successfully cultivated in an infusion of hay. Such an infusion is best made of hay from wet, swampy meadows, and of straw and withered leaves. The dead plant material is placed in a preserving jar together with pond water. Tap water may also be used, but this slows the development of the protozoic fauna. The infusion should be placed near the window in a warm room. Direct sunlight should be avoided, however. After a few days, a bacterial layer forms on the surface of the water (scum layer). Under these conditions, the protozoa emerge from their cysts and quickly reproduce.

After about one to two weeks, paramecia and other microorganisms appear in large numbers. The temporary putrid smell disappears around the third or fourth week because the water undergoes a biological cleansing. Slowly, a natural balance is established between the number of species and the population density. If the ciliates are needed for a longer time period, a new infusion must be set in the third week and then inoculated with water from the existing infusion.

Duration of experiment: 30 minutes (preparations must be made one week ahead of time)

Equipment/materials:

- Preserving jars
- Slides
- Cover glasses
- Pipettes
- Pond water
- Dead plant material (hay, leaves, straw)
- Gelatine

[24] Schlüter, Werner, 1973, pp. 198 ff.

Procedure

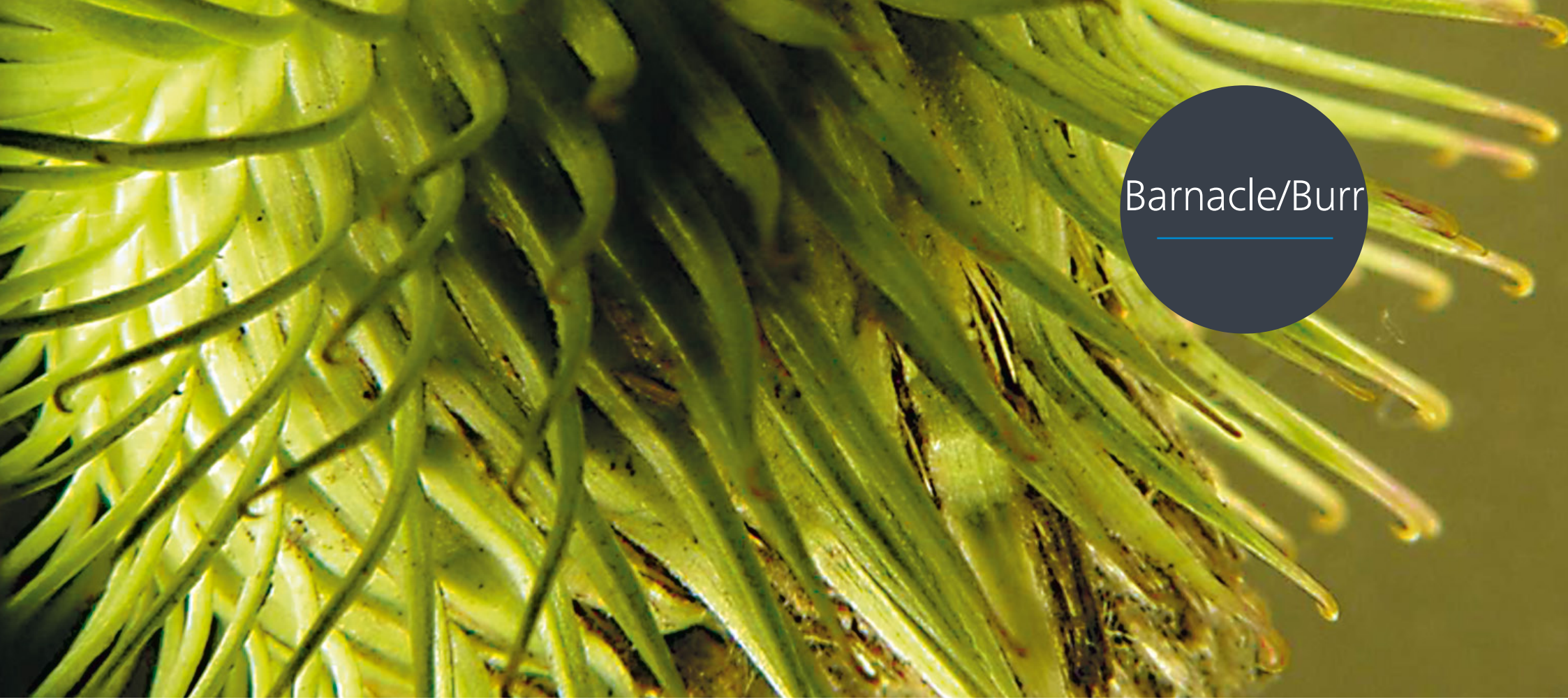
As described above, several hay infusions are prepared one to two weeks before the experiment is planned to take place. Samples are taken by pipette from the scum layer, the middle layer, and the sediment of the infusions for examination under the microscope. Pure cultures of paramecia are difficult to obtain. For this reason, it is recommended to discuss the variety of species during microscopic examination. Frequently found species include *Colpidium, Euplotes, Vorticella, Paramecium*, and life-forms such as amoebas. The paramecia are first examined live in order to observe their movement and feeding behaviors. This is performed by placing a drop of culture liquid on a slide, placing a cover glass over it, and observing it first at low magnification and then at higher magnification. Particularly revealing images are achieved using darkfield and contrasting colored illumination. Gelatine can be added in order to slow down the very rapid movement of the cilia.

Tasks

1. Observe the life-forms present in the drop of water. Prepare sketches of three individuals. Pay particular attention to their shape.

2. Observe the movements of the paramecia. How do they behave when they come upon an obstacle? Describe your observations.

3. Add a drop of gelatine to the liquid being examined. The movements should now slow down. Observe the structure of a paramecium. Prepare a microscopic drawing.



Part III

References and Notes

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- JOHANNES LIEDER GmbH, Ludwigsburg, Germany, www.lieder.com
- Klaus Kemp, Somerset, England (test slides of diatoms, www.diatoms.co.uk). ZEISS recommends “Test slide with 8 forms.”
- Living plant material such as yeast cells, onions *Allium cepa,* thick-leaved waterweed *Egeria densa*, or ruffled or crinkled Madagascar Aponogeton *Aponogeton crispus, A. longiplumulosus*
  www.tropica.com, www.dennerle.com

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- Braune, Wolfram, Leman, Alfred & Taubert, Hans: Pflanzenanatomisches Praktikum.
  Vol.1, 9. ed. Spektrum Akad., published 2007.
  ISBN-10: 3827417422
  This richly illustrated book optimally conveys the basic principles for preparing simple living plant samples for microscopic examination.
  A second volume describes microscopy of the lower cryptogamic plants (algae, fungi, mosses, and dyes) with photos and drawings of their life-cycle stages.

- Balbach, Margaret, and Bliss, Lawrence C.: A Laboratory Manual for Botany. 7. Ed.
  Brooks/Cole Thomson Learning, published 1991. ISBN-13: 9780030301841
  This book describes all necessary botanical specimen-preparation techniques and contains many good, practical exercises for the participants.

- Kubitschek, Ulrich: Fluoreszenzmikroskopie From Principles to Biological Applications.
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  18. edition, Spektrum Akad., published 2010.
  ISBN-10: 3827416760
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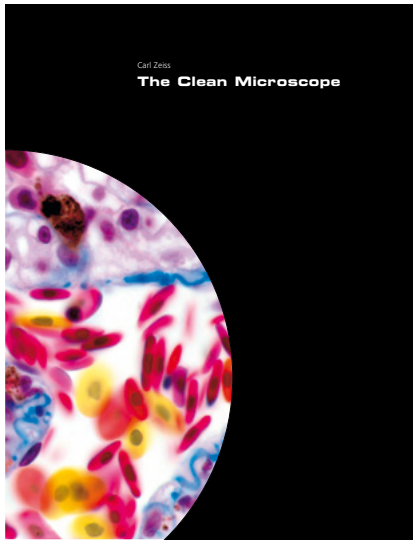


- The ZEISS brochure The Clean Microscope helps you in optimizing the practical performance of your microscope.
  The brochure can be downloaded free of charge as a PDF file **here**[1].

- Microscopy from the Very Beginning.
  Carl Zeiss Microscopy GmbH. The PDF file can be downloaded free of charge **here**[1] .
  It contains easily understandable technical drawings and explanations on all important methods of light microscopy.

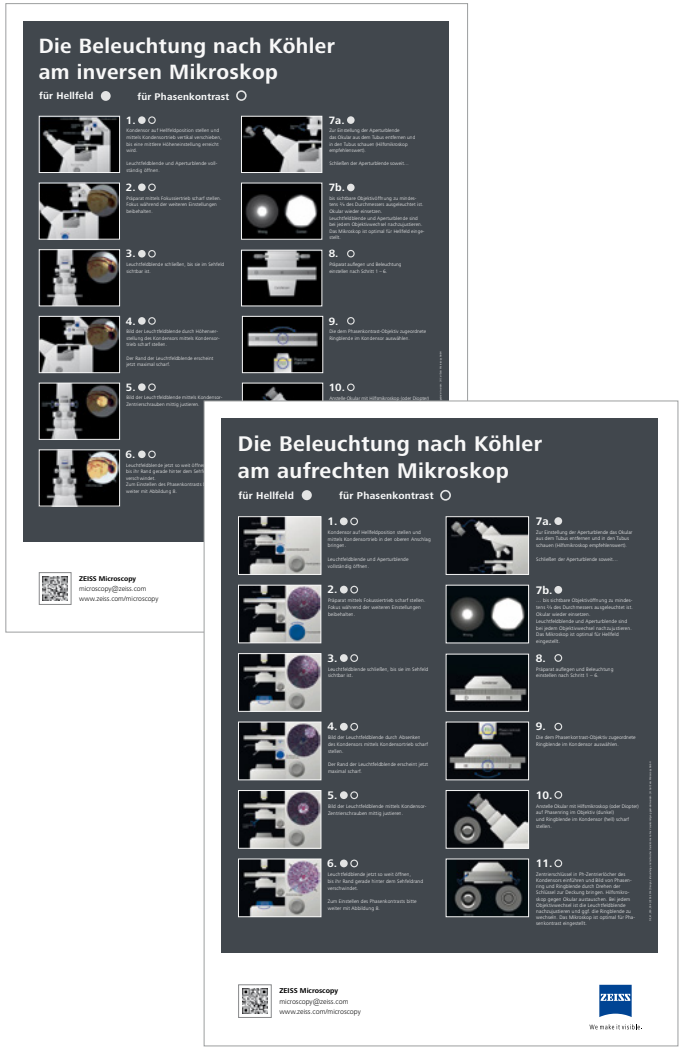
[1] www.zeiss.com/micro-brochures

Posters

ZEISS offers teaching materials and posters for downloading which illustrate the method of

Koehler illumination for upright and inverted microscopes.

The posters can be downloaded free of charge as PDF files **here**[1].



[1] www.zeiss.com/micro-brochures

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