

Microphotography

General Rules

In research, microphotography is often the most important proof of new findings. The photograph needs to convince the most critical of experts. For didactic purposes, the picture needs to be «typical». That requires a very detailed knowledge of the subject. The selection of the best site of the preparation requires most of the time. The photographic part itself typically causes fewer problems and can be done in a relatively short time.



Illumination

For microphotography, the skilled use of the microscope and a good understanding of its optics are further preconditions. Adjustment of illumination according to Köhler's principles is of special importance. It ensures highest contrast with uniform illumination. Critical illumination, as usual for course

microscopes, images the incandescent filament within the preparation plane. This disturbs the uniform illumination. The good adjustment of the condenser aperture is very important. If it is too closed, contrast seems to be improved. But the structural details of the preparation are bordered by dark lines, that result from optical artifacts.

Preparations

Many microscopic objects are quite low-contrast, with the exception of dark field or fluorescence microscopy. A colored histological preparation seems to be shining like a colored window of a cathedral. This is a delusion. The thin microtome section takes up only a small amount of the dye and thus the light absorption is not so significant. This is why the difference in brightness between colored section and surrounding is not large. For the preparation, a high-contrast staining is necessary. Within the microscope and during taking the picture everything which could reduce contrast has to be avoided. Low-contrast pictures seem to be blurred, even if they are well-focused. Perfect preparations are crucial for good microphotography. If an acceptable preparation is unavailable, one should not take a picture.

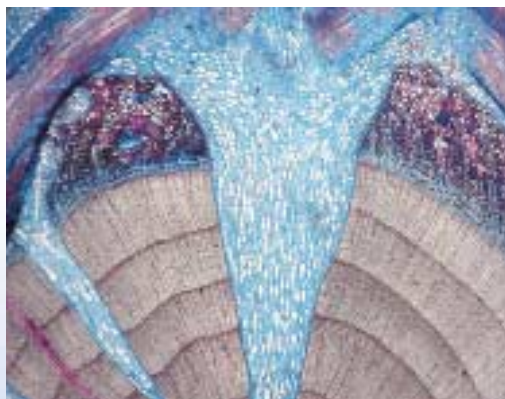


Figure 1:
Suction root from mistletoe,
transmission light microscopy,
Etzold staining.
(Friedrich Thormann,
Sulzbach, Germany)

Fluka Microphotography Competition 2001

- **Take your Best Picture and Enter Today!**
- **Request Protocols for Common Staining Procedures**

This is a strong indication of an unprofessional adjustment. In addition, all dust particles, which are anywhere within the light beam, impair the quality of the image. In most cases it is sufficient to follow the $\frac{2}{3}$ rule, which says, that condenser aperture should be $\frac{2}{3}$ of objective aperture. Thus an inexpensive dry condenser with maximal aperture of 0.95 is good enough. Some problems, which one can tolerate for visual microscopy, e.g., dust in the visual field, are very disturbing on a microphotograph and have to be strictly avoided. At Köhler's illumination, disturbing dust can be located easily. Dust particles, which appear sharp on the preparation structures, lie in a plane conjugated to the preparation plane, mostly on the field lens of the ocular or near to the field stop. If the dust becomes diffuse while shifting the condenser in height, the dust is below the condenser. If it does not become diffuse, it lies onto the field lens.

Optics of Microscope

It is recommended to combine objectives with oculars from the same supplier. A wrong combination can lead to chromatic differences in enlargement. This is obvious if borders of structures appear in orange, yellow or blue (color picture) or diffuse (black and white picture). The beneficial enlargement should not be exceeded. The over-all enlargement should not be more than 1000 times as big as the numeric aperture of the objective. If the over-all enlargement is chosen below the beneficial enlargement, e.g., not more than 500 times as big as the numeric aperture of objective, the pictures seem to be more focused.

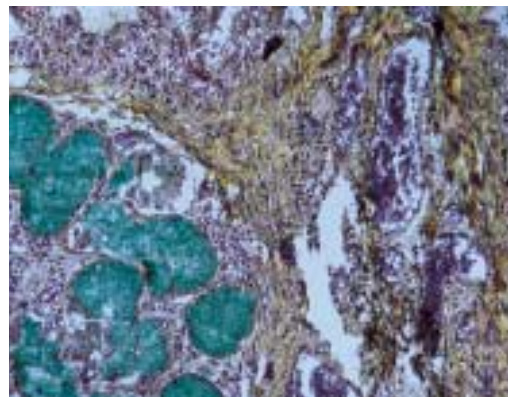


Figure 2:
Lung tissue of an asthmatic patient, Nomarski contrast, (Dr. Norbert Lange, Siegen, Germany)

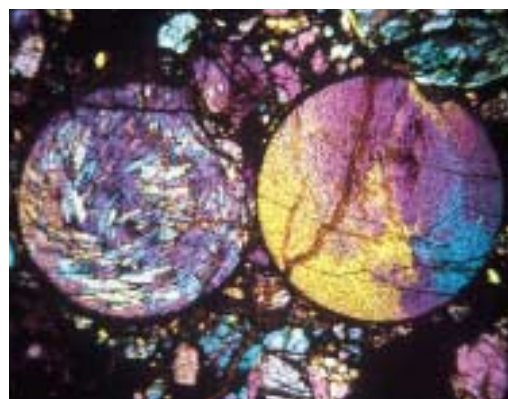


Figure 3:
Chondroitin (Meteorite) thin section, polarized light (Prof. Erich Steiner, Vienna, Austria)

Older objectives often have a lower contrast and a higher curvature of image field. If possible, one should use newer objectives from well known suppliers. These objectives work with high contrast and sufficiently low curvature of image field. Even the cheaper objectives of these suppliers are sufficient for most applications. Special planar objectives are required for special issues only, e.g., for taking pictures from blood smears. In this case, the curvature of image field is very disturbing. Only newer planar objectives should be used, as the older ones lack in contrast. If dry objectives with numeric apertures over 0.65 are used, wrong thickness of cover glasses or too thick layers of mounting media will cause low contrast and blurred pictures. This can only be avoided with objectives that have a correction collar, but these are very expensive and their correct adjustment requires a lot of experience. The depth of focus is very low in microphotography. It is higher for objectives with smaller numeric apertures. Objectives with enlargements below 10:1 are difficult to focus. Thus the adjustment is controlled with a Galilean telescope focused at infinity.

Preparation of Photographic Pictures

Making of black and white pictures is more difficult than making colored pictures, as color nuances of the preparation need to be transformed to appropriate shades of gray. Lower sensitivity films (ISO 25-50/15°-18° ISO) are fine-graded and better suited for microphotography. As microscopic pictures are mostly low in contrast, standard development results in too soft, i.e. low contrast negatives. Black and white films need to be developed with high contrast. This makes it difficult to keep sufficient shades of gray to distinguish the different colors. Interesting structures can be set off by contrast filters, e.g., using a green filter for red stained chromosomes will result in deep black imaging. A little bit shorter illumination time than standard conditions gives sharper borders for black and white pictures and deeper colors for color pictures. Colored microphotographs are mostly prepared as slides. Due to their brilliance, projected slides seem better than positive prints of the same quality. Color transparency films give more contrast, which is better for microphotography. For normal microscope illumination, one uses artificial light transparency films or day light films combined with conversion filters. To avoid yellow tinge, one has to work with high lamp voltages, which would give too intense light for visual microscopy. Thus one uses lower voltage for adjusting the preparation, but increases voltage to the highest value before taking the picture. Regarding the determination of illumination, for most pictures integrating measurement is good enough, whereas spot measurement is only needed if the range of brightness is very high, e.g. for dark field or fluorescence microscopy.

Critical Inspection of Pictures

For selection of pictures, one should first get a general impression from projected pictures, asking if the preparation is really interesting and does not show any irregularities. These selected pictures may be put onto a transilluminator and investigated through a 10 x magnifying glass to their quality, especially sharpness. Only the important section of the picture should be used. Unimportant parts can be cut off or masked. A correct legend is crucial.

Fluka your Competent Partner for Microscopy

Fluka is proud to offer a wide range of reagents for both light and electron microscopy, including mounting and embedding media, fixatives, and stains. Fluorescence microscopy, which is rapidly gaining importance, is a focus of our innovation. We offer some 200 fluorescent stains for microscopy as well as special mounting media for fluorescence microscopy. Please ask for your copy of our fluorescent probes brochure to get a full picture.

Because of our extensive experience, commitment to quality assurance and first class service, we are ready to give you full support in achieving the best results.

Protocols for common staining procedures are available on request. Please contact Pierre Nording at pnording@sial.com or send a fax +41 81 756 54 49.

Fluka Microphotography Competition

This biannual competition is organized by the Association of German Biologists and Fluka with the intention to encourage public interest in biological science. The eighteenth competition closes 31 March 2001 and everyone is invited to enter. We have included several interesting examples of microphotographs from previous competitions in this AnalytiX in order to show you the broad field of microphotography.



Figure 4:
Daphnia, just after the birth of one young, the second remaining in the brood pouch. An aspheric condenser with a blue patch stop provided Rheinberg Illumination, the light source being opal perspex in front of an electronic flashtube. (Spike Walker, Penkridge, England)



Figure 5:
Sodium glutamate crystallization, polarized light (Helmut Gatzel, Berlin, Germany)

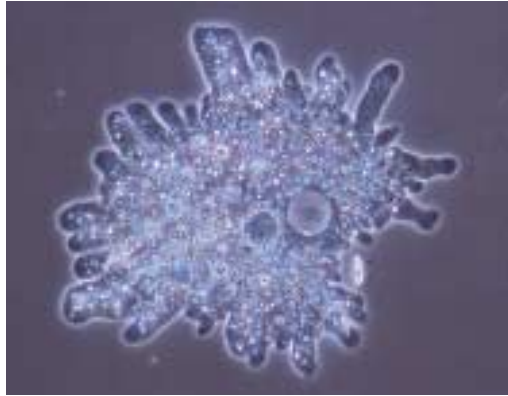


Figure 6:
Amoeba proteus, living; phase contrast microscopy. (Rudolf V  th, Bellenberg, Germany)



Figure 7:
Spider, inclusion in amber, approx. 40 million years old, reflected polarized light. (Odine Lang, Braunschweig, Germany)

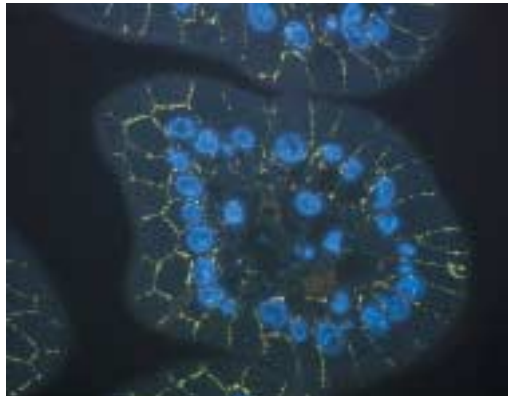


Figure 8:
Rat intestine, fluorescence microscopy, using labeled antibody for β -Catenin (yellow) and DAPI for staining nuclei (blue)

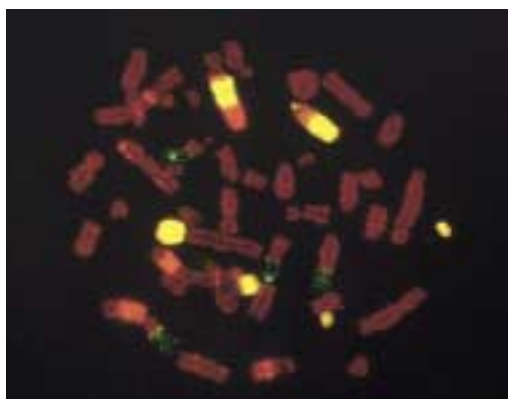


Figure 9:
Metaphase chromosomes of human leukocytes taken from an irradiation sensitive patient after in vitro irradiation. Fluorescent in situ hybridization of human chromosomes to demonstrate translocations. (Susann Neubauer, N  rnberg, Germany)

Fluka – your convenient source for fluorescent probes



Get your free copy

- **Fluka Fluorescent Probes Catalogue**
- **New Mounting Media**
- **Embedding Media Kits**

Sigma-Aldrich

Your favorite analytical research associate

More than 20,000 analytical products and 2,000 applications from all the Sigma-Aldrich brands have been brought together in one place. They have been organized by analytical technique.

If you browse the on-line catalogue or search by keyword, name, CAS#, or application, you will be able to find the products you need.



www.sigma-aldrich.com/analytical

Fluka – A Member of the Sigma-Aldrich Family



Biochemicals and
Reagents for Life
Science Research



Organics and
Inorganics for
Chemical Synthesis



Speciality Chemicals
and Analytical
Reagents for Research



Laboratory Chemicals
and Reagents for
Research and Analysis



Chromatography
Products for Analysis
and Purification