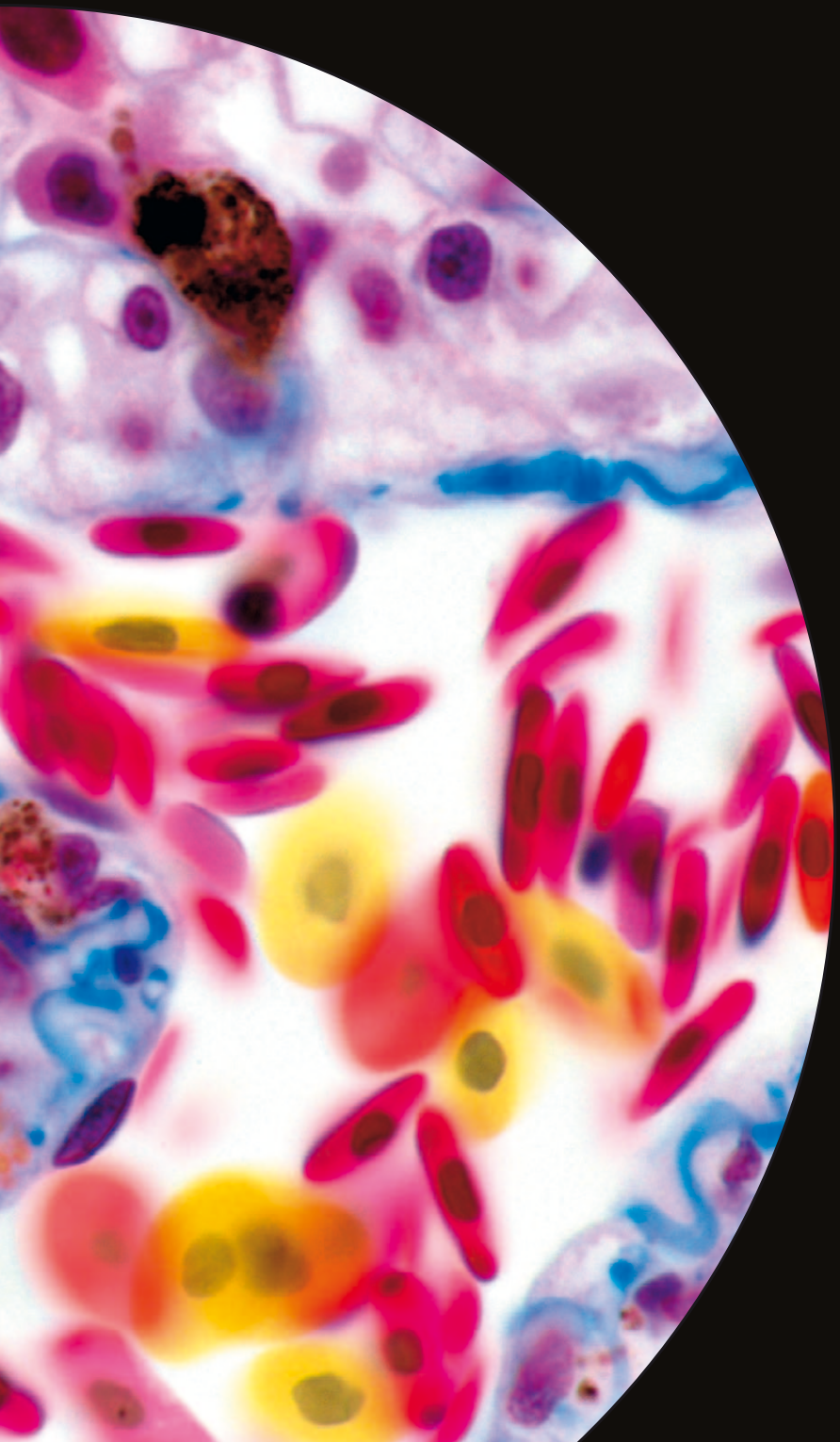
Carl Zeiss

The Clean Microscope



We make it visible.

The Clean Microscope

Dr. Michael Zölffel

Carl Zeiss MicroImaging GmbH · Carl-Zeiss-Promenade 10 · 07745 Jena · Germany

Contents

Title photo:
Toad liver, stained with Azan
Plan-APOCHROMAT 63/1.4, brightfield

Recognizing and Cleaning Soiled Optics

Clean optics are essential for successful microscopy and perfect images.

A variety of cleaning procedures have been recommended in the course of decades, leaving many users uncertain as to which method will yield the best results.

The choice of the best cleaning method depends on the nature of the optical surface concerned, and the type of dirt to be removed.

The Effect of Dirt on the Image

The closer any dirt is to the object or to a camera sensor, the greater is its effect on the visual or recorded image. The following surfaces are critical:

- The external surface of the front lens of the objective
- The surface of the camera sensor and its protective glass cover
- Both surfaces of the cover slip
- The surface of the specimen slide
- The surfaces of the camera adapter optics
- The surfaces of the condenser front lens
- The outer and inner surfaces of the eye lens of the eyepiece and the surfaces of graticules
- The outer surface of the protective glass covering the light exit opening of the illuminated field diaphragm
- Other glass surfaces in the light path, e.g., the bulbs of halogen or high-pressure lamps, fluorescence filters and beam splitters, collector lenses, contrast and heat filters.

Some optical surfaces are more sensitive to dirt than others. The front lens of the objective is particularly critical; therefore, it is discussed in greater detail herein.
For any dry objective, the smaller the free working distance and the smaller the surface area of the concave front lens, the greater is the danger of the front lens being soiled with embedding media, immersion liquids or dust.
Examples of such objectives are:
EC Plan-NEOFLUAR 40x/0.75
EC Plan-NEOFLUAR 63x/0.95 corr.
N-Achroplan 63x/0.80, 63x/0.95 o.D.
Fluar 20x/0.75
Plan-APOCHROMAT 20x/0.80
Plan-APOCHROMAT 40x/0.95 corr.
All EC Epiplan and EC Epiplan-NEOFLUAR dry objectives
EC Epiplan-APOCHROMAT objectives with 20x, 50x, 100x and 150x magnifications.

Exposure of the front lens of any objective to dust is greater in case of an inverted microscope than an upright microscope. In particular, all LD dry objectives with magnifications of 32x, 40x and 63x need to be checked regularly.
The front lens of an immersion objective should be cleaned to remove residue both after use and, additionally, before fresh immersion liquid is applied. The mixing of different immersion media or different batches of the same medium (e.g., the immersion oil IMMERSOL F™) may result in blurred images.
Microscope cameras must always be handled with utmost care and protected from dust by all available methods.

Before any critical application,
check the objective front lens for any dirt.

How to Recognize Dirt

To recognize dirt on optical surfaces, you should have an idea of the best result you can expect from a specific microscopy method and a specific application. If you then compare your expectation with the visual image in terms of maximum definition, best contrast and cleanness, you will immediately recognize whether or not your microscope is soiled anywhere.

If the sharpness or contrast of the image is less than optimum, there is a high probability that your microscope optics are not clean.

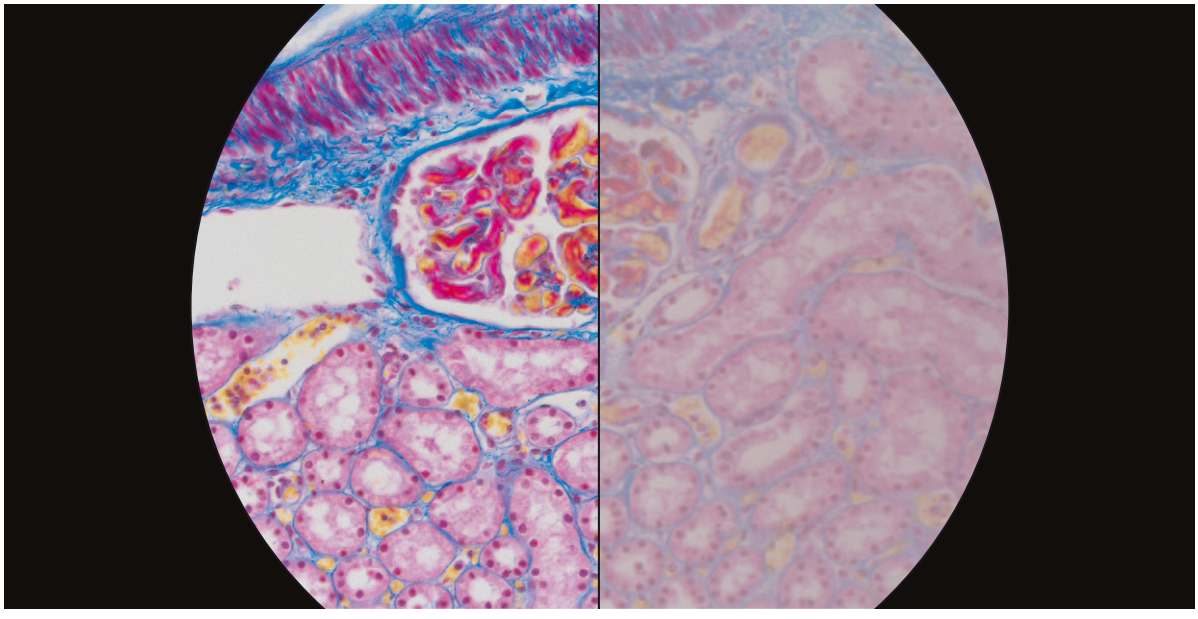
To locate the dirt, proceed as follows:
Carefully rotate objectives or cameras by a small amount within their thread.
Check specimen slide and cover slip by moving the specimen while focusing on the upper and lower surfaces in succession.
Check the condenser by moving it up and down and, if possible, slightly swiveling or turning the front lens.

The affected optical surface is identified when the dirt follows the movement of the suspected optical component. The camera is the only exception to this rule: dirt within the camera will not move when you move the camera.
A macroscopic check for larger dust particles and scratches on optical surfaces can be carried out with a 3x to 6x magnifier, or an eyepiece held the wrong way up.

Dirt on the front lens of an objective is easily detected if you look at an evenly lit surface from the rear end of the objective. The inner lens components produce an enlarged image of even the smallest bit of dirt on the front lens.

The final check should always be an assessment of the image quality improvement achieved.

Clean (left) and oil-soiled (right) front lens of a Plan-APOCHROMAT 20/0.80 objective. Toad kidney, trichrome staining, brightfield



Different Types of Soiling

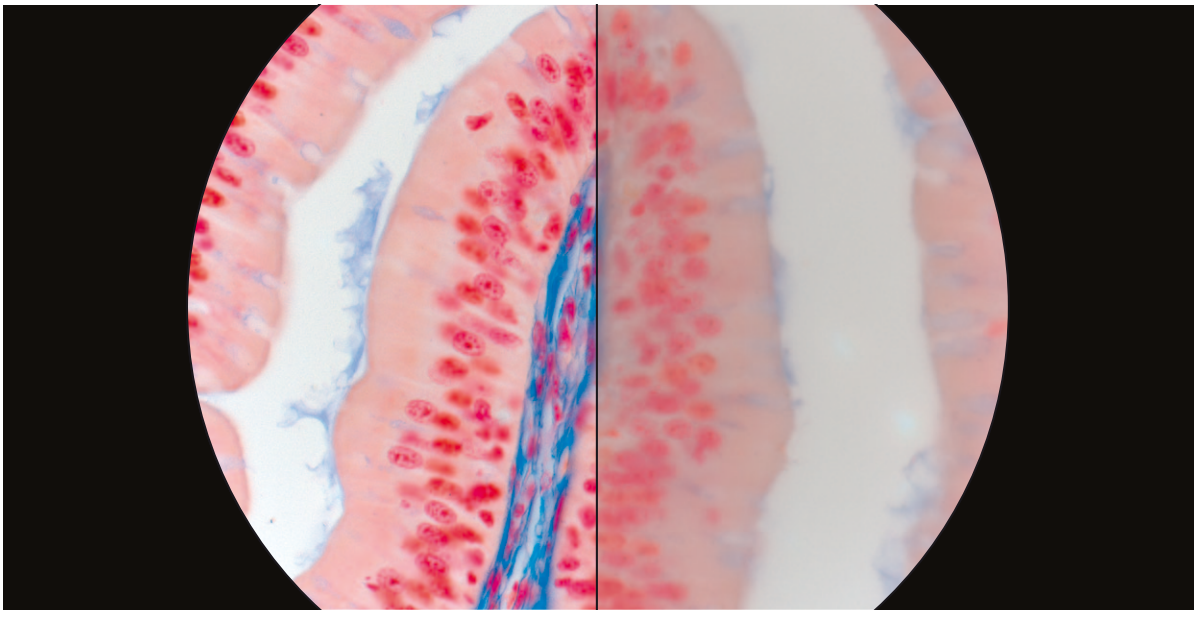
We must differentiate between dust particles (e.g., glass abraded from specimen slides, flakes of the microscopist's skin, fluff from clothing, pollen) and other kinds of soiling (e.g., liquid or dried-up embedding or immersion media, culture solutions residue from improper cleaning attempts, fingerprints, grease).

Dust may either rest loosely on optical surfaces or more or less stick to them. Other dirt may be soluble in water or need organic solvents for complete removal.

A blurred image may not always be due to dirt:
Using an objective with a large numerical aperture in conjunction with a cover slip of the wrong thickness may result in blurred images (spherical aberration).

Dry objectives of this type normally have a correction collar, which permits compensation for spherical aberration.
Turn the correction collar (while continuously adjusting the focus) until the best image contrast and sharpness is achieved.
Many highly corrected immersion objectives also require specially selected cover slips of a thickness of 0.17 mm, if the best image is required.
Immersion objectives should only be used in conjunction with a suitable, bubble-free immersion liquid. Use oil immersion objectives with IMMERSOL™ from Carl Zeiss only. For the C-APOCHROMAT water immersion objective, ideally use distilled water or IMMERSOL W™ only.
The occasionally recommended use of anisole as an immersion medium results in loss of sharpness and contrast. Moreover, anisole may attack the cement of front lenses, especially of objectives of older make.

A dull image despite clean optics is caused by spherical aberration: Correction collar of the Plan-APOCHROMAT 40/0.95 objective adjusted correctly (left) and incorrectly (right). Small intestine of a frog, stained with Azan, brightfield



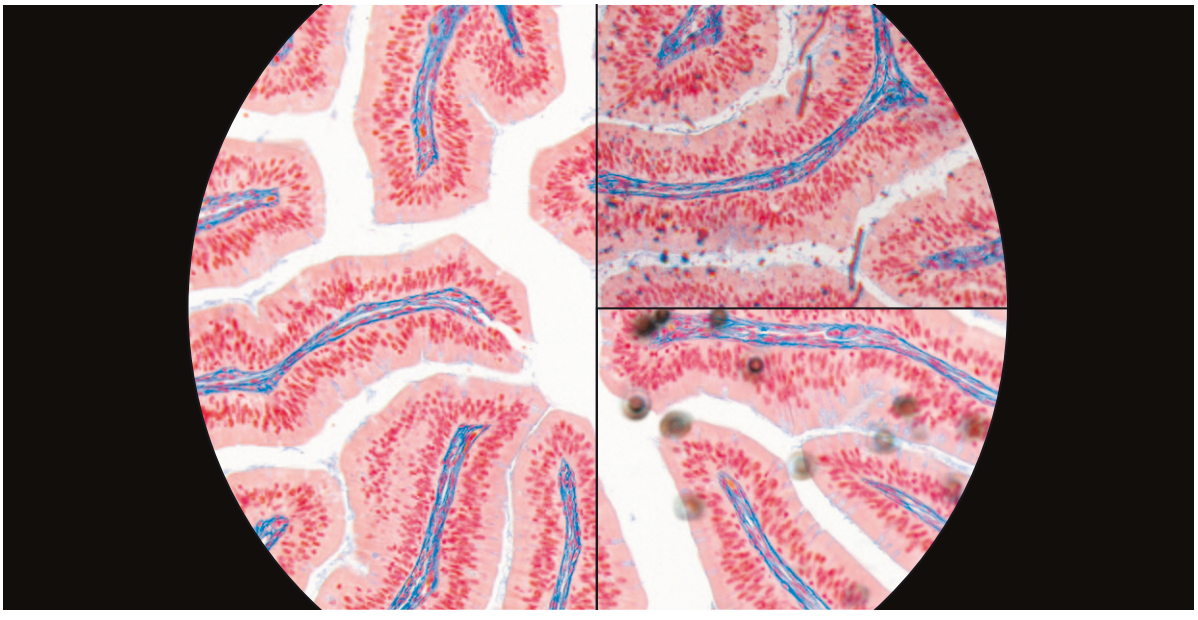
Different Optical Surfaces

Concave or convex surfaces (e.g., front lenses of dry objectives and dry condensers, eye lenses of some eyepieces) should be distinguished from plane or plane-parallel surfaces (e.g., front lenses of most immersion objectives and condensers, filters, the protective glasses covering camera sensors or light exit openings).
Clean concave or convex surfaces using the familiar cotton swabs or the new polyester ones as described on page 8.
Clean easily accessible flat surfaces with similar means or simply with soft facial tissue.

Microscope optics may consist of optical glass, fused quartz or polymers. The surfaces of all of them are coated to minimize stray light. Some antireflection coatings are wipe-resistant (e.g., the eye lenses of eyepieces), while others are too soft to be wipeable. Most antireflection coatings are composed of layers of magnesium fluoride and should only be cleaned with agents free from ammonia and acids.
Sometimes, household glass cleaning agents (e.g., SIDOLIN, SPARKLE, Blue WINDEX) are recommended. However, as they contain diluted ammonia, they should not be used routinely.

Some optical components are surrounded by black antireflective lacquer surfaces, which are sensitive to organic solvents. The plastic and rubber parts of eyepieces will likewise be attacked by some organic solvents (e.g., acetone, chloroform).
The lenses of older microscopes are cemented with alcohol-soluble cements such as Canada balsam. Most lens cements used today are polyacrylic synthetic resins, which do not have this problem.
The internal optical surfaces of the microscope, components of the fluorescence filter sets, cameras and camera adapters should never be cleaned by the user but by experienced service staff of the original manufacturer only. The user may clean the external surface of the objective front lens, the condenser front lens, the eyepiece eye lens, full-glass color and conversion filters, and the external surface of the protective glass covering the light exit opening of the illuminated-field diaphragm.

Clean optics (left), dust on the cover of the light exit opening of the illuminated field diaphragm (top right), and extremely soiled camera (bottom right). Small intestine of a frog, stained with Azan. Plan-APOCHROMAT 10/0.45, brightfield



Cleaning Agents and Methods

The goal is the complete removal of dust and dirt without leaving any residue of the cleaning agent and without damaging the surfaces.

The following utensils and agents are required:

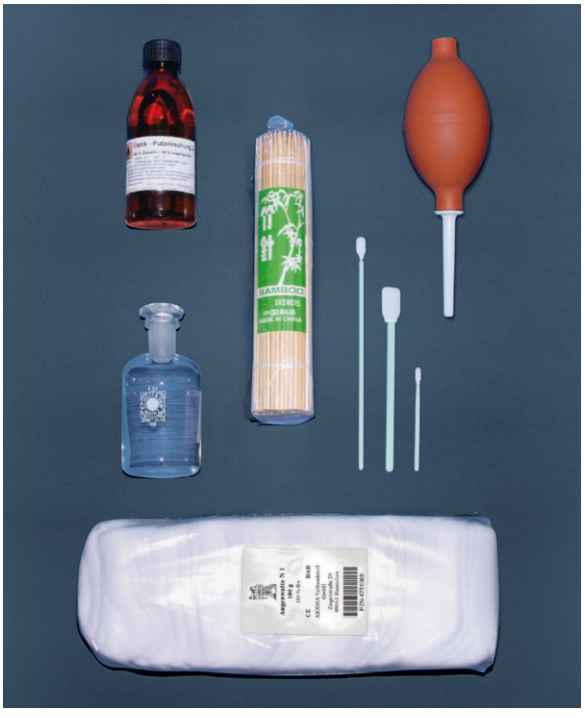
- Long, thin wooden sticks, preferably of bamboo (obtainable from Chinese restaurant suppliers) or a comparable, not too flexible material
- High purity cotton (cotton wool) such as used, e.g., in ophthalmology (available from KERMA, Germany), or WHATMAN Lens Cleaning Tissue 105
- Absorbent polyester swabs for optical cleaning (ITW Texwipe CleanTips®). They are a very good alternative to cotton swabs and can be re-used
- Soft facial tissues (e.g., Kim Wipes soft, KLEENEX).
- Dust blower (available from labware suppliers or pharmacies)
- Distilled water
- Freshly prepared solution of 5-10 drops of a dishwashing liquid (e.g. Fairy Ultra, Fit) in 10 ml distilled water
- Solvent for the removal of greasy or oily dirt, such as the in-house Carl Zeiss recipe (Optical Cleaning Solution), or analytical-grade n-hexane.
  Only for cover slips: Pure acetone.
  Note: Cleaner's naphtha, white spirit, petroleum ether and others are trivial names of benzine (light gasoline) fractions containing n-hexane

For the quick-and-easy cleaning of flat surfaces (e.g. removing immersion media from cover slips or the front lenses of immersion objectives), use soft facial tissue (e.g. Kleenex) soaked in diluted dishwashing liquid.

Caution: The commonly available lens tissue, also sometimes called Joseph paper, is not intended for cleaning but only for the dust-free storage and protection of optical components. With the single exception of WHATMAN Lens Cleaning Tissue 105, such lens papers are too harsh for cleaning; also, they do not absorb the dirt efficiently or quickly enough.

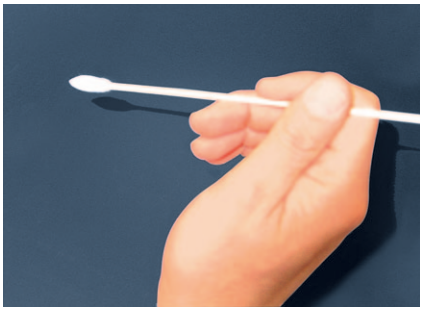
For cleaning all other optical surfaces, use either freshly made cotton swabs or the new polyester swabs (ITW Texwipe CleanTips®). These absorb dirt through their microfiber surfaces. Their cleaning capacity is limited in time, though.

Cleaning devices



Preparation of cotton swabs

- Wash your hands (powdered latex gloves are not suitable).
- Dip the bamboo stick into the (aqueous or organic) cleaning solution. This makes the cotton fibers attach better to the stick.
- Get the stick into contact with the cotton.
  Do not press the cotton; otherwise it will not as easily wind around the stick.
- Turn the stick to wind up a few cotton fibers at first, then gradually more, so that an evenly growing, approximately elliptical cotton bud forms at the tip.
- To keep the cotton tip clean, the stick should be kept in a polyethylene bag until it is used. Do not touch the tip with your fingers, as perspiration and grease from your skin will considerably affect the cleaning capacity.
- Remove the cotton tip after every stroke and replace it with a freshly made cotton bud.
- The stick can be used for a long time. Use separate sticks for water-based solutions and organic solvents.



If you prefer the use of WHATMAN Lens Cleaning Tissue 105, fold the sheet around the stick so that a sharp point is formed. Do not touch the point. Replace the tissue after every completed stroke. The ITW Texwipe CleanTips® polyester swabs can be used until their cleaning power starts to diminish.

Cleaning Procedure

- Blow all **loose dust** particles away with a dust blower.
- Remove all **water-soluble dirt** with distilled water. If this is not successful, repeat using diluted dishwashing liquid.
  Remove any remaining residue with a dry cotton swab, but breathe on the surface first to generate a film of moisture. Be careful not to spray droplets of saliva onto the surface.
- To remove **oily dirt,** first use diluted dishwashing liquid. If the result is not satisfactory, repeat using a solvent (Carl Zeiss Optical Cleaning Solution, or n-hexane).
- **Greasy dirt** must always be removed with a solvent.
- After cleaning, inspect the surface (see section „How to Recognize Dirt", page 5).

Place the objectives, eyepieces and cameras on a dust-free surface (e.g., fresh aluminum foil). All other components to be cleaned should be accessible in the best possible way.

Dip the cotton swab or ITW Texwipe CleanTips® swab into the cleaning solution and shake off excess liquid. An excess of liquid in a cotton bud will flow over the rim of the lens and attack the lens cement. This may cause the cemented components to finally come apart.

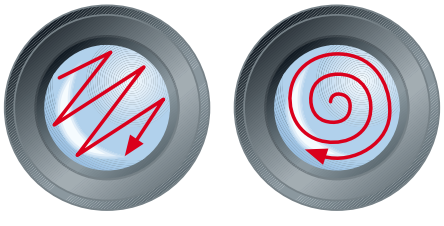
The solvent should take up as much dirt as possible.
In order to increase the retention time of volatile organic solvents in the cotton bud, some users chill the solvent (-10°C to -20°C). Chilled solvents have a disadvantage, though: Due to their low temperature, atmospheric moisture may condense on the lens surface and leave a residue. A more suitable way to improve the retention time of a solvent is to add isopropanol, for example.

Not all solvents can be recommended for cleaning microscope optics. Among those that clean very efficiently, some are toxic (e.g., chloroform, acetone); others are unfriendly to the environment (e.g., Freon®, carbon tetrachloride); still others will leave residues on the surface (e.g., xylene, toluene, diethyl ether).

Residue forms particularly with the use of xylene and non-analytical grade ethanol and, above all, if the dirt contains water-soluble components.

Acetone can be recommended when oil and grease are to be removed from cover slips. Acetone attacks most types of plastic as well as rubber, so its use for cleaning, e.g., eyepieces can be problematic. One cannot exclude the possibility that acetone attacks cemented optical components (e.g., objectives, camera adapters, eyepieces) when used frequently.
Acetone may also dissolve special organic coatings.

For cleaning, move the utensil in a **spiral motion from the center to the rim.** Never wipe in a zigzag pattern, as this will merely spread the dirt.
With larger optical surfaces (e.g., tube lenses), first wipe in a **spiral motion starting at the rim** and proceeding toward the center; then execute another spiral movement from the center to the rim.
As a rule, several spiral wipes will be required.
We recommend pure, volatile n-hexane or the Optical Cleaning Solution from Carl Zeiss.



wrong **correct**

Do not wipe in a zigzag pattern, but in a spiral movement!

Cleaning External (Non-Optical) Microscope Parts

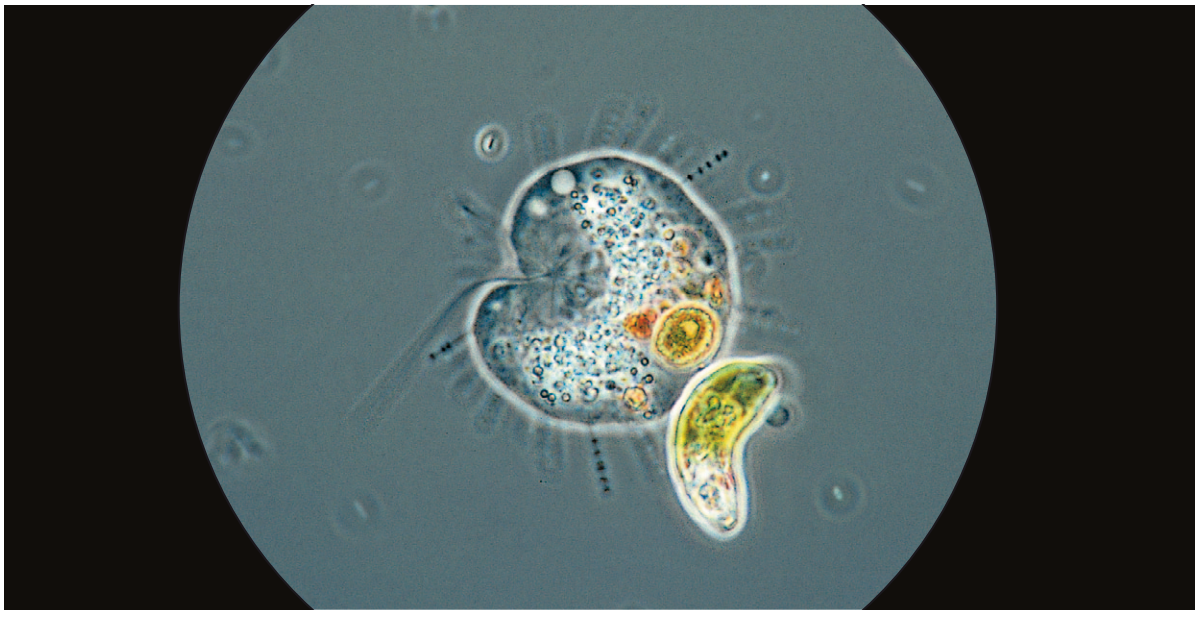
The painted surfaces of microscopes from the AXIO range are powder-coated and extremely durable. They can be cleaned with a very slightly moistened microfiber cloth. Loose dust and other dirt can be removed with a soft hair brush (marten hair) used exclusively for that purpose.

! Perfect Preparation

Optimum results in microscopy depend not only on the cleanness of the microscope optics but also on perfect specimen preparation:

- Thickness of the specimen
- Staining intensity
- Refractive index and dispersion of the embedding or immersion medium
- In high-resolution microscopy: distance of a live cell from the cover slip
- Correct cover slip thickness (e.g., 0.17 ± 0.01 mm) to avoid spherical aberration

Thin, clean live specimen of the freshwater protozoan Dimorpha mutans. Plan-APOCHROMAT 63/1.4, phase contrast



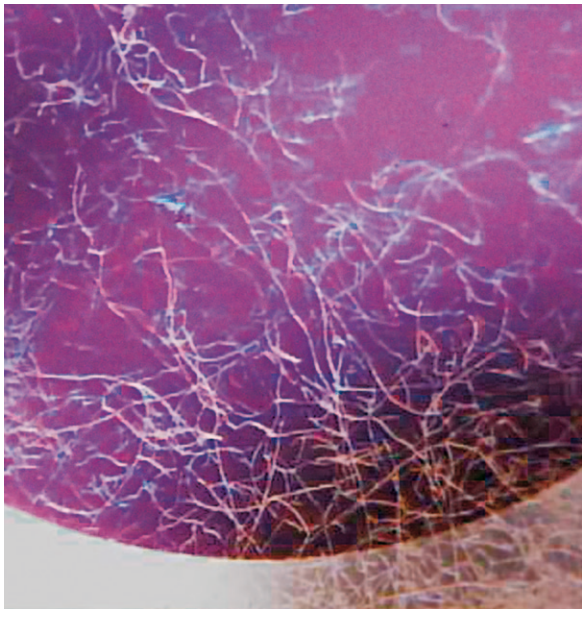
How Soiling Can Be Avoided

The openings of the binocular viewing tubes must always be closed. Unless the eyepieces are in, use dust stoppers. If no dust stoppers have come with the microscope, wrap bits of aluminum foil around the apertures.
The best fundamental method against **dust accumulation** is to first cover the microscope with two plastic bags of suitable size and then with the dust cover supplied by the manufacturer.

In tropical climate regions, this method is not recommended, as it can frequently lead to the grows of fungus. **Fungus contamination** can best be avoided by dry room air produced either by air conditioning or installing an infrared lamp above the microscope (minimum distance 150 cm or 5 feet).
Carl Zeiss microscope optics are impregnated with an approved antifungus agent (manufacturer: Bayer). However, there is no 100% protection against fungus. Also, there is hardly any remedy once fungus infestation has happened.

! Never locate the microscope in a place where it could be affected by corrosive **acidic or alkaline vapors.**

Fungus mycelium on coated glass, reflected light illumination.



Suppliers and Recipes

Kerma ophthalmic cotton N 1, DAB 6
The cotton wool used in ophthalmology is 100% pure cotton (DIN 61 640-A, Ph.Eur., DAB). It is absolutely pure, highly absorbent and soft. The fibers can be blown away from optical surfaces.
www.kerma.de

WHATMAN Lens Cleaning Tissue 105
10 cm x 15 cm lens tissue in packs containing 25 wallets of 25 sheets each, Catalog No. 2105 841.
The only lens tissue recommended by Carl Zeiss. It is chemically pure and silicon-free, and contains absolutely no additives. This product is also sold by other suppliers, e.g. KODAK.
www.whatman.com

Rubber blower
This is a particularly powerful blower:
Giotto's, Type GTAA 1900.
www.giottos.com

ITW Texwipe CleanTips
Absorbent polyester swabs for
cleaning optical components
(Alpha, Clean Foam or Absorbond series)
Available in different sizes and absorption grades, e.g., from Basan under designation TEXWIPE TX743B.
www.texwipe.com
www.basan.com

Carl Zeiss optical cleaning solution
Recipe: 85% n-hexane, 15% isopropanol.
The solution is not sold by Carl Zeiss MicroImaging GmbH.

The n-hexane should be analytical grade. Some benzine fractions (petroleum ether) are not suitable, as they leave an insoluble film on the optical surface. Acetone, which is recommendable exclusively for the occasional cleaning of oil-contaminated cover slips, should also be analytical grade.

Cover glasses of specified thickness D = 0.17 mm
To avoid spherical aberration use cover glasses D = 0.17 mm. They are available from Carl Zeiss.
Item discription: High-precision cover glasses, D = 0.17 mm, 18 x 18 mm, 1000 Pieces,
Order-Nr. 474030-9000.

SAFETY WARNING
When working with chemicals, solvents and other possible hazards, be sure to follow the current safety regulations applicable in your country and/or institution.

Subject Index

Please contact the local Carl Zeiss representative on all questions regarding maintenance and service.

Carl Zeiss MicroImaging GmbH
07740 Jena, Germany

Phone : +49 3641 64 3400
Telefax: +49 3641 64 3144
E-Mail : micro@zeiss.de

www.zeiss.de/micro

Information subject to change.
Printed on environmentally friendly paper bleached without chlorine.
50-1-0025/e – printed 06.11
ISBN: 978-3-940885-03-6

What to Watch Out for when Cleaning Microscope Optics

1. When starting to clean, don't forget to use a dust blower first, except when liquids (such as immersion oil) have to be removed.

2. Never wipe lenses with dry swabs or tissue – this will cause scratches!

3. Don't use abrasive materials such as dry leather wipers, dry linen cloths or polystyrene sticks as recommended by some manufacturers.

4. Don't apply any solvents before trying distilled water, except when grease has to be removed. (A film of distilled water can be created by breathing on the surface.)

5. Don't use ethanol, diethyl ether or acetone for cleaning older microscopes
(e.g., the STANDARD line from Carl Zeiss Oberkochen, or the MIKROVAL and JENA-Microscopes 250 CF lines from Carl Zeiss Jena).

6. Don't use any disposable cotton swabs (e.g., Q-Tip®) instead of the cotton or ITW Texwipe Clean Tips® swabs recommended herein, as the former are not free from contaminations.

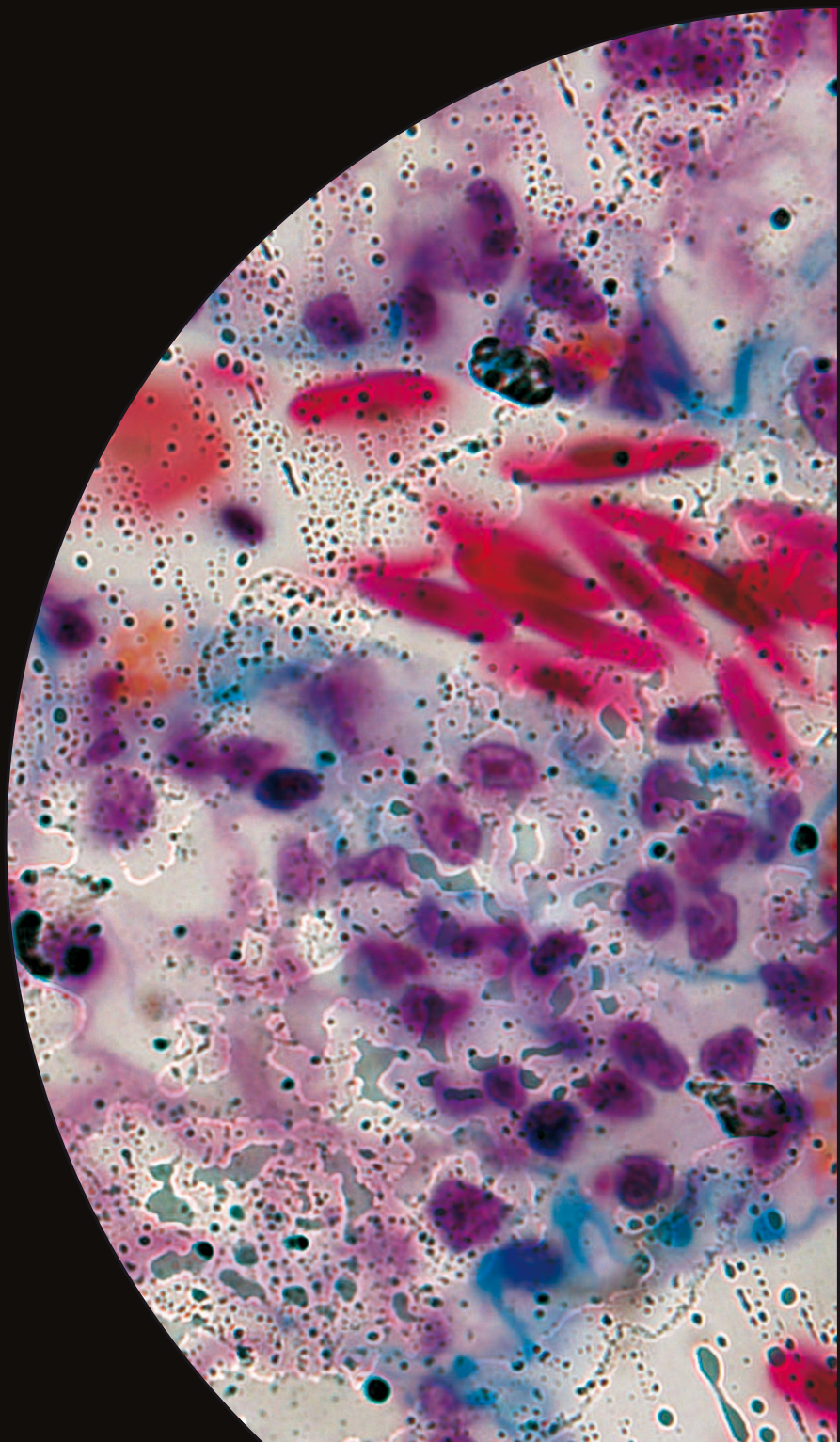
7. Beginners should not use any of the occasionally recommended metal rods instead of the wooden (bamboo) sticks, as the front lenses may easily get damaged by the former.

8. Don't use any of the optical spray cans containing pressurized liquid air. The pressurized air from these cans may easily leave a residue that is difficult to remove, if at all.

9. Never use acids or ammonia to clean objective front lenses, which are sensitive to acid and alkaline vapors.

10. Never try to clean internal optical surfaces, cameras or adapter optics.

Clean optics are essential
for successful microscopy and
perfect images.



Methods and Principles

The Clean Microscope

ZEISS

We make it visible.