Josefine Neuendorf

Urine Sediment

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Josefine Neuendorf
Neuendorf Labordiagnostik
Wiesloch
Germany

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Foreword

The advantages of urine diagnosis are undisputed:

- Readily obtainable test material
- Fast diagnosis
- Fast results
- High differential diagnostic reliability
- Cost-effective diagnosis

Although medical knowledge has advanced in terms of diagnostic urine sediment analysis, current knowledge is not sufficiently integrated in modern diagnostic methods. It is essential that precision be reintroduced. What is seen using microscopy can be identified in a more differentiated manner, and thus better interpreted. General statements such as "urine sediment contains epithelial cells and/or erythrocytes" are unhelpful. It is important to consider the characteristic morphological features of a urinary sediment constituent, as well as classify and name it accordingly. Only in this way is it possible to formulate a well-founded urine sediment finding suggestive of renal or post-renal disease.

The central aim of this book is to reappraise sediment diagnosis with an up-to-date naming system and an evaluation of urinary sediment constituents from a differential diagnostic perspective. Furthermore, detailed instructions on the precise method for processing urine into urine sediment are provided.

The knowledge imparted here on microscopy techniques, as well as on servicing and maintaining the microscope, is crucial for ease of work and facilitates the morphological determination of cell components. All aspects of correct urine processing discussed here guarantee reproducible results.

Autodidactic acquisition of cell morphology is highly challenging and time-consuming, since microscopy specimens are fresh native specimens that cannot be fixed and, as such, cannot be archived.

Therefore, precise photographs of the constituents of urine sediment are required in order to be able to more readily compare and classify what is seen microscopically. For this reason, the book contains a multitude of digital photographs from both bright-field microscopy and phase-contrast microscopy. Furthermore, numerous examples provide the reader with the opportunity to practice evaluating and diagnosing microscopic images.

In the context of my lecture activities, I encounter a considerable need for information on urine diagnostics among nephrologists, urologists, gynecologists, medical students, medical technical laboratory assistants, and medical specialists. The extremely positive feedback from seminar participants prompted me to compile the main contents of my lectures in book form.

Heidelberg, Germany
2019

Josefine Neuendorf

Preface

The inspection of urine has been of particular relevance in the identification of diseases for almost 2000 years. The urine glass, known as the matula, was even the status symbol for physicians in the Middle Ages. As a result of the automation of laboratory diagnostics and the introduction of urine test strips, a considerable portion of urine diagnostics can be standardized now. However, when it comes to the analysis of urine sediment, an experienced diagnostician is by far superior to automated urine sediment analysis.

In her book, Josefine Neuendorf presents all the information needed for urinary sediment analysis, from obtaining urine, pre-analytics, and analysis to urine sediment diagnosis in a clear and concise manner. Each of these analytical steps on the way to reliable diagnosis based on urine sediment are made clear and convincing thanks to the easy-to-understand presentation of the basic principles on the one hand, and the detailed explanations on differentiating morphological details on the other. The 3rd edition of the book has gained considerably from the clear depiction of urinary sediment constituents using high-resolution images of urine sediment.

Well performed urinary sediment analysis can make a valuable contribution to the differential diagnoses of acute kidney diseases, especially when the arguments for the various differential diagnoses need to be weighed up in a timely manner. Properly obtained and correctly interpreted findings of urinary sediment analysis can support the indication for kidney biopsy or the decision to initiate treatment immediately.

Questions on urine sediment are also part of exams. Thus, according to the catalog of learning targets issued by the German Institute for Medical and Pharmaceutical Examination Questions (IMPP), students should be familiar with performing urinary sediment analysis. In particular, the differentiation of glomerular from non-glomerular erythrocyturia is an important examination topic, which is tested by asking students to identify acanthocytes on images of urine sediment. Moreover, the German Specialty Training Regulations for Nephrology call for experience and skills in the performance of—and reporting of findings from — microscopic analysis of urine sediment, including phase contrast microscopy. This book provides a clear explanation of all these aspects.

Therefore, the book “Urine Sediment” represents not only a valuable aid in daily practice, but also during studies and continuing education.

Prof. Andreas Kribben, MD
President of the German Society of Nephrology (DGfN)
Head of the Department of Nephrology University Hospital Essen
Germany

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Particular thanks go to my husband and children. Without their understanding, I would never have found the time or the peace to complete this work.

I would like to thank Dr. Norbert Günther, Mr. Werner Kietzmann, Mrs. Sarah Müller, and Prof. Dr. Rüdiger Waldherr for their expert input.

My special thanks go to Mrs. Margit Schmude (Nephrological Laboratory, University Hospital Mainz, Germany). Due to her many years of experience, our professional exchanges were of great value to me.

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Abbreviations

aHPF	All (analyzed) high power fields
Acantho	Acanthocyte/s
Amph	Amorphous phosphates
Bact	Bacteria
Biconcave Ec	Biconcave-shaped erythrocyte/s
Bright field	Bright-field microscopy
Ca-Oxa	Calcium oxalates
Ca-oxalates	Calcium oxalates
cm	Centimeter
Disc-shaped Ec	Disc-shaped erythrocytes
dUc	Deep urothelial cell/s
DysEc	Dysmorphic erythrocyte/s
dysmorphic Ec	Dysmorphic erythrocyte/s
Ec	Erythrocyte/s
Ec ghosts	Erythrocyte ghosts
EcCa	Erythrocyte Cast/s
EpiCa	Epithelial cast/s
EumEc	Eumorphic erythrocyte/s
Eumorphic Ec	Eumorphic erythrocyte/s
FC	Fatty cast/s
GranCa	Granular cast/s
HPF	High power field
HyalCa	Hyaline cast/s
Lc	Leukocyte/s
LcCa	Leukocyte cast/s
mm	Millimeter
NR	Normal range
OFB	Oval fat bodies
OFB casts	Oval fat body casts
PhaCo	Phase-contrast microscopy
SqEc	Squamous epithelial cell/s
Thornapple Ec	Thorn apple-shaped erythrocyte/s
TransEc	Transitional Epithelial Cell/s
UTI	Urinary tract infection
YC	Yeast cell/s

About the Author

Josefine Neuendorf MTLA, lecturer in medical laboratory diagnostics, gives lectures and practical seminars for physicians, MTLAs, and medical staff and teaches at the Heidelberg Academy for Health Professions at the Heidelberg University Hospital, Germany.

Part I

The Microscope

1

1.1 Structure of the Microscope

Figures 1.1 and 1.2 show the magnification calculation and the structure of a microscope.

1.2 Cleaning and Maintaining the Microscope

- Vibrations should be avoided when the light source is on, since the lamp responds highly sensitively to this.
- Protect the microscope from exposure to dust, e.g., by using a dust cover/plastic cover, and by closing openings that dust could get into (eyepieces should always be pushed in). All positions on the objective turret (revolving nosepiece) must be fitted either with an objective or with a dust protection plastic plug (Fig. 1.4).

Remove dust from objectives and eyepieces by blowing or dabbing with a fine microfiber cloth and then cleaning with a high-quality paper tissue (swabs, linen cloths, cotton buds, and eyeglass cleaning cloths should not be used) soaked in rinsing solution or glass cleaner solution. Dry paper tissues should never be used.

- A good cleaning solution for all optical glass surfaces and the tripod consists of a mixture of 1 l glass cleaner and 20–30 ml odorless methylated spirits.
- A particular technique is recommended for cleaning glass lenses (Fig. 1.3):

  Only ever wipe the glass lens in a circular motion, otherwise dirt on the glass lens collects at the edge of the lens. Avoid using cotton buds, since these cause renewed smearing. Only ever clean external glass surfaces. The cleaning of internal glass surfaces can only be performed by a professional specialist.
- In order to clean the outer objective lens more effectively and thoroughly, the objective can also be unscrewed from the microscope from time to time.
- Never oil the precision guides, drive mechanisms, screws, or moving parts.
- If an objective is missing from the nosepiece, the holder must be closed with a plastic dust cap (Fig. 1.4).

1.3 Servicing the Microscope

- Depending on its use, the microscope needs to be serviced by a specialist at regular intervals. This includes checking the microscope for functionality, cleanliness, resin, etc.
- It is a legal requirement in Germany for the power cable and all electrical parts be checked by a specialist once a year. Following inspection, the electrician affixes a sticker to the microscope.

1.4 Light Bulb Replacement

- When replacing the microscope lamp, it is essential that the halogen lamp is not touched with the naked hand under any circumstances.
- A lint-free linen cloth must be used.

Magnification is calculated as follows:

10x Eyepiece · 40x objective = 400x magnification

8x Eyepiece · 40x objective = 320x magnification

Fig. 1.1 Calculating magnification

J. Neuendorf, *Urine Sediment*, https://doi.org/10.1007/978-3-030-15911-5_1

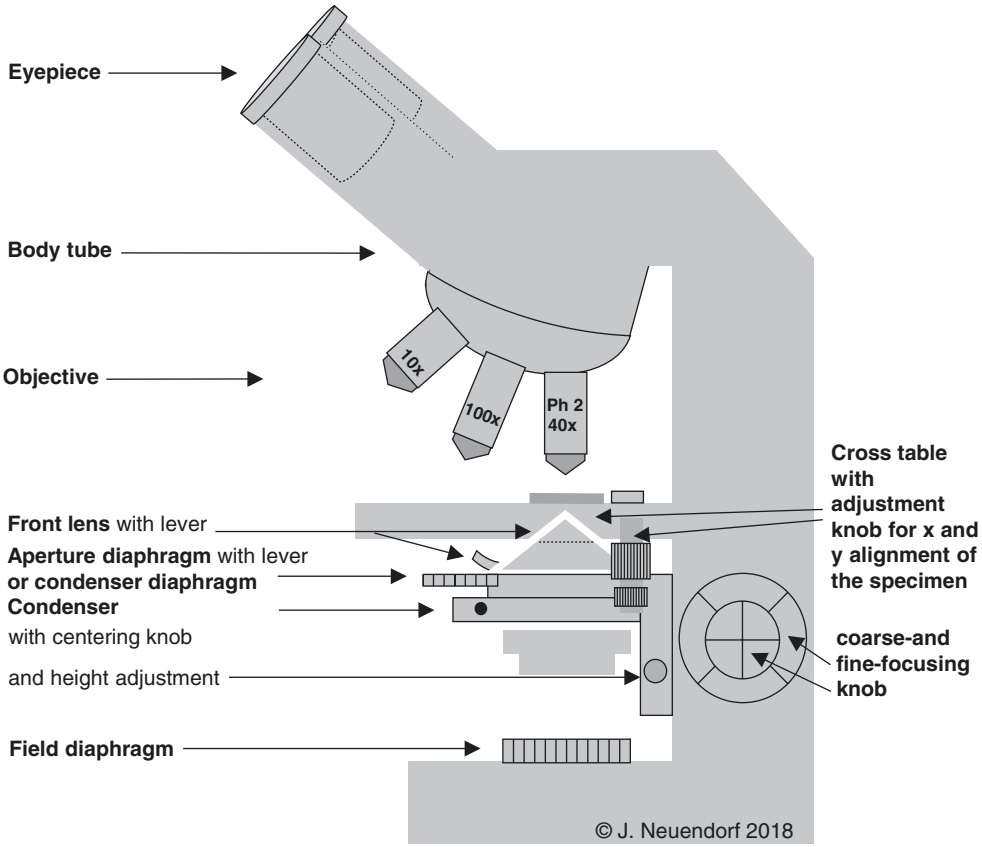


Fig. 1.2 Structure of the microscope

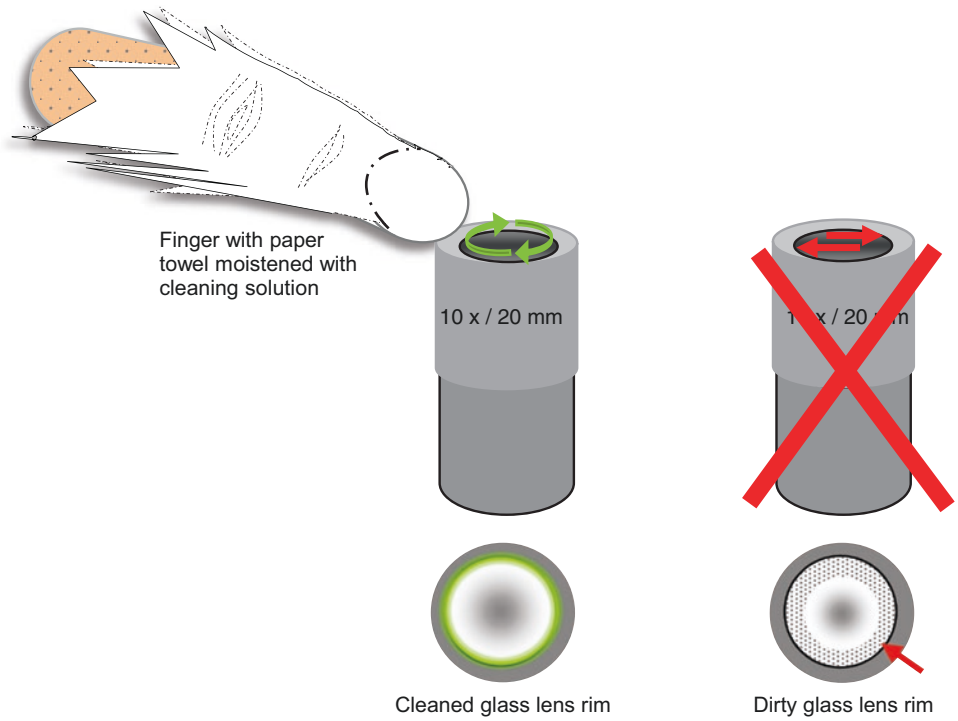


Fig. 1.3 Cleaning glass lenses

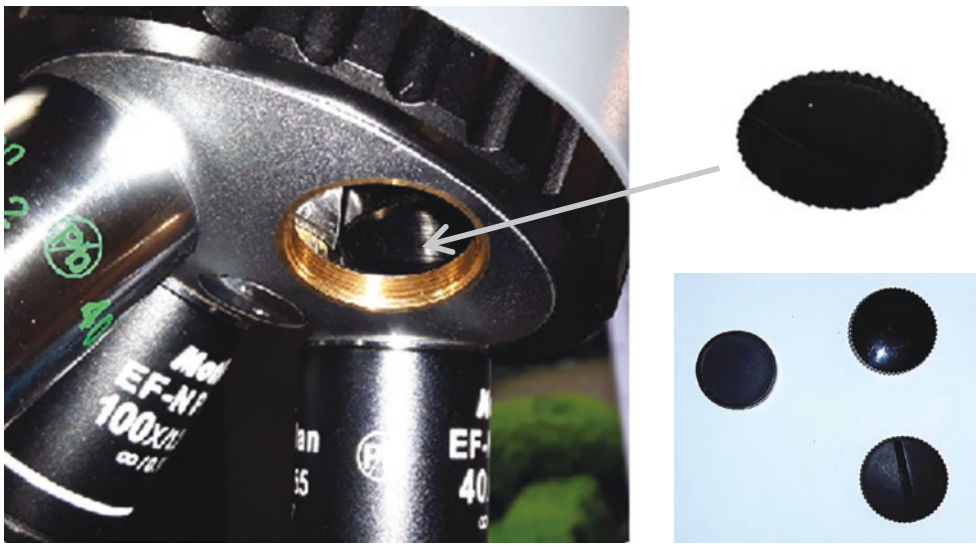


Fig. 1.4 Nosepiece without microscope plastic dust cap, various plastic caps

Setting-Up Köhler Illumination

2

2.1 Setting-Up Köhler Illumination or Aligning the Microscope

Professor August Köhler (1866–1948) worked for Carl Zeiss in Jena, Germany and, in 1893, published a set of rules for the correct illumination of microscopic specimens.

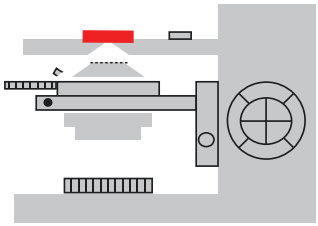
The aim here is to achieve uniform illumination of the microscopic image while at the same time increasing resolution capacity by using a condenser. Unwanted reflections and contrast-reducing overexposure are largely eliminated (Zeiss 1997).

Köhler illumination has been described for bright-field and phase-contrast microscopy (Figs. 2.1 and 2.2). In order to be able to better set the microscopic plane of the slide, one uses a stained specimen (Köhler specimen) to set-up Köhler illumination. This can be a stained blood smear from the hematology department or simply a slide marked with a felt-tip pen.

> Important: The Köhler specimen should be of the same thickness as the specimens to be subsequently examined microscopically. Attention should be paid to this if one is working with KOVA® slides/counting chambers.

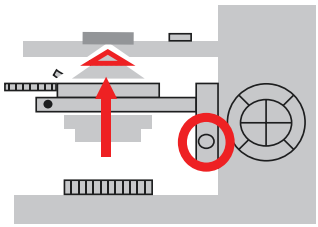
J. Neuendorf, *Urine Sediment*, https://doi.org/10.1007/978-3-030-15911-5_2

1



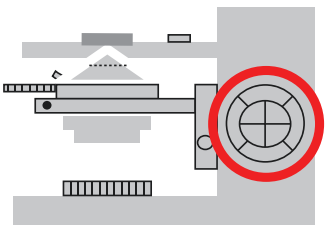
Place the slide or Köhler specimen on the stage.

2



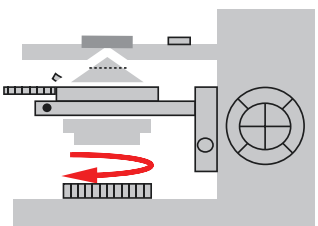
Turn the front lens (if there is one) into the beam.
Turn the condenser upwards.

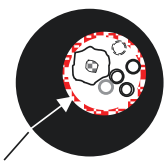
3



Using the 10x objective, bring the microscopy plane of the slide into sharp focus.

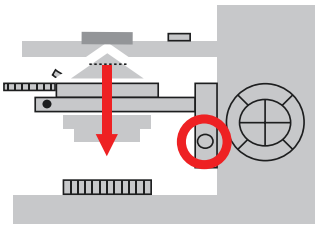
4





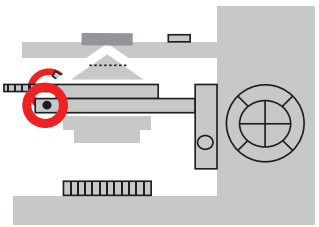
Close the field diaphragm completely while viewing. A bright circle (or hexagon) with an unsharp border will become visible against a dark background.

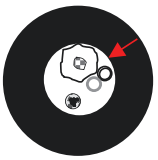
5



Lower the condenser slightly while viewing until the edge of the bright circle comes into sharp focus.

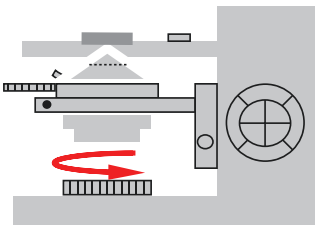
6





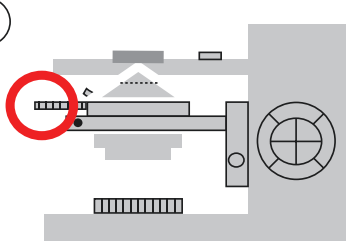
While still viewing, turn the bright circle to the center using the two condenser-centering knobs (left and right).

7



Again while viewing, open the field diaphragm only so wide that the entire field of view is illuminated; readjust slightly if necessary as described in 6.

8



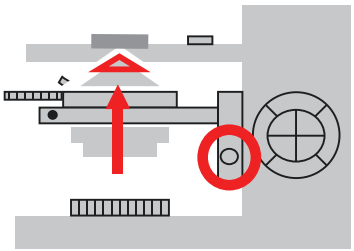
To adjust image contrast, close the aperture diaphragm lever approximately two thirds.

- Set-up Köhler illumination also using a 40x objective!

Fig. 2.1 Setting up Köhler illumination or aligning the microscope

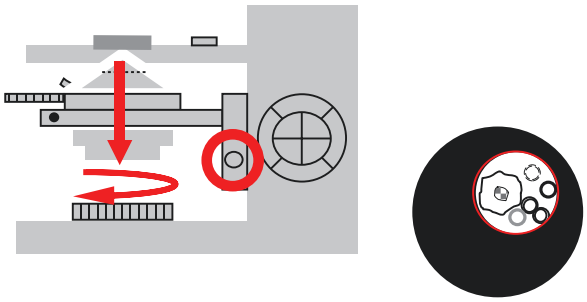
2.2 Quick Guide to Setting-Up Köhler Illumination

1. Place the Köhler specimen on the stage.
   Place the condenser on the highest setting.
   Push the condenser front lens (if there is one) away from the stage.



2. Using the 10x objective, bring the specimen into sharp focus using the coarse- and fine-focusing knob.

3. Close the field diaphragm in the foot of the microscope and slowly lower the condenser until the field diaphragm image comes into focus (hexagon or circle).



4. Turn both condenser-centering knobs until the image of the field diaphragm appears at the center of the field of view.
   The condenser is now centered.

5. While viewing, open the field diaphragm only wide enough for the entire field of view to be illuminated.
   Adjust image contrast using the aperture diaphragm (condenser diaphragm).

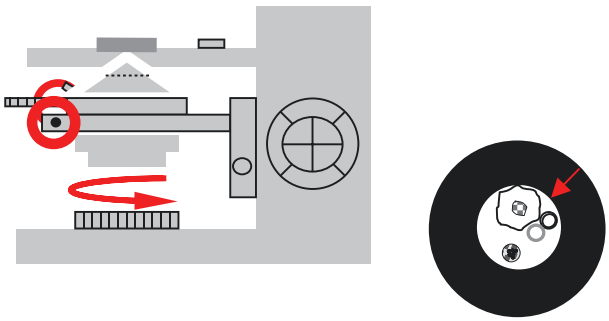


Fig. 2.2 Quick guide to Köhler illumination

Reference

1. Zeiss C (1997) Mikroskopieren von Anfang an! Was heißt Köhlern? http://www.meditec.zeiss.de/C1256B5E-00496AB1/Contents-Frame/2BEEE02E723F-BA45C1257346003FB23C?opendocument. Accessed on: 23rd January 2015.

Phase-Contrast Microscopy

3

3.1 Application

Phase-contrast microscopy is used to better examine unstained specimens, native specimens, or low-contrast elements of urine. Originally transparent structures are made visible by virtue of the fact that they are surrounded by a dark and a light border.

3.2 What Is Required for Changeover?

By swapping a special **phase-ring objective** and inserting a phase annulus in the **condenser**, a normal bright-field microscope can be rapidly converted into a phase-contrast microscope. It is important here that the phase annulus in the condenser and the phase ring in the objective are centered relative to each other. This is done using an **auxiliary microscope** or viewfinder **diopter**.

What is practical is that both microscopy techniques (bright-field and phase-contrast microscopy) can be used effortlessly in parallel.

3.3 The Light Pathway of Phase-Contrast Microscopy

See Fig. 3.1

3.4 Phase-Contrast Microscopy Equipment

This includes a PhaCo objective and various PhaCo condensers:

3.4.1 PhaCo Objective

See Fig. 3.2

3.4.2 PhaCo Condensers

See Figs. 3.3 and 3.4

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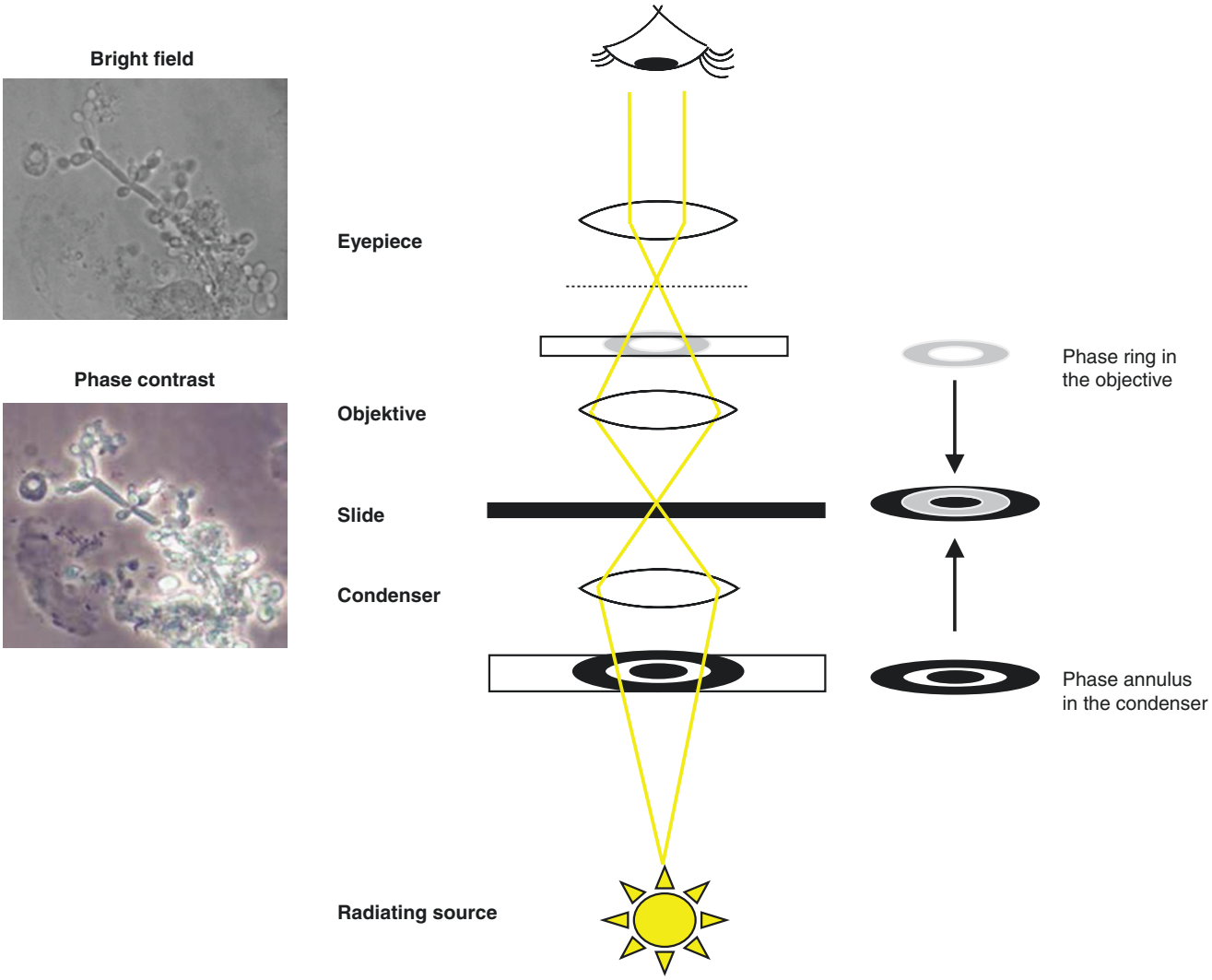

Fig. 3.1 The light pathway of phase-contrast microscopy

Fig. 3.2 PhaCo 40× objective with phase ring, marking: Ph 2

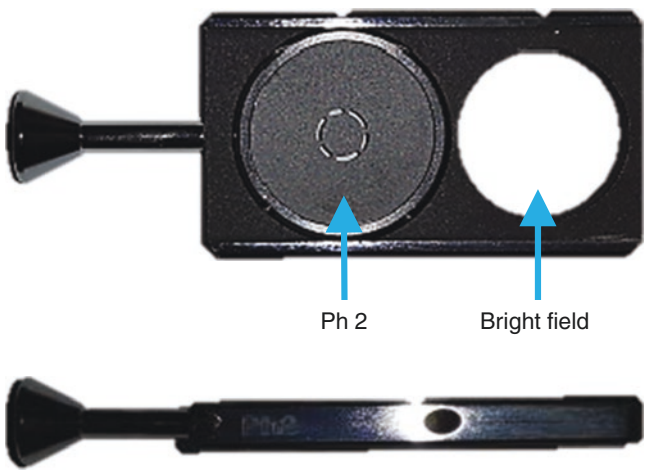

Fig. 3.3 Phase-contrast slider for the condenser with phase annulus Ph 2 and bright-field setting (top and side view)

Abbé Condenser

2

Phase annulus (Ph 1, Ph 2, Ph 3) for the corresponding lens or bright-field setting
E.g.: 40x- PhaCo-objektive = phase annulus 2 in condensor

1

Centering knob for the condenser phase annulus

3

Dial for adjusting the respective phase annulus or bright-field setting

Simple phase-contrast attachment for the Leitz condenser

Fig. 3.4 Zeiss Abbé condenser and simple phase-contrast attachment for the Leitz condenser

3.5 Centering the Phase Rings

Centering the phase annulus on the condenser is performed using the auxiliary microscope (Figs. 3.5 and 3.6). To achieve this, the auxiliary microscope is inserted in the observation tubes rather than the eyepiece (Fig. 3.6). Alternatively, a simple **diopter** can be used for centering rather than an auxiliary microscope.

In order to guarantee the phase contrast effect, centering needs to be performed and checked from time to time (as soon as phase-contrasting becomes weaker).