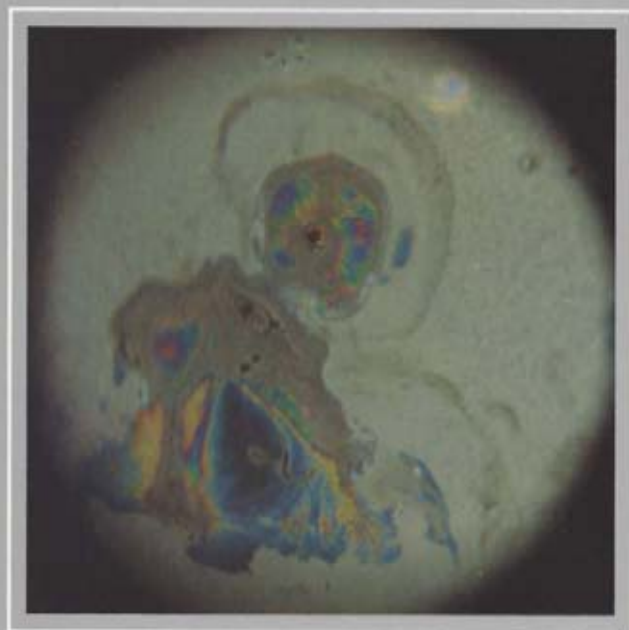


# Specular Microscopy of Intraocular Lenses

Atlas and Textbook for Slit-Lamp and Specular Microscopic Examinations



Martin Wenzel

Foreword by David Apple

Thieme

Specular Microscopy  
of Intraocular Lenses

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**Kabi Pharmacia Ophthalmics**

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Atlas and Textbook for Slit-Lamp and  
Specular Microscopic Examinations

Martin Wenzel, M.D.

Ophthalmology Department  
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## Foreword

It is an pleasure and an honor to write a foreword to this excellent monograph authored by Martin Wenzel. This work is unique because it does not merely represent a review of other work, but contains extensive original material based on his years of research in this field. The author's presentation of specular microscopy of intraocular lenses (IOLs) is also very timely, as this research tool is used in connection with an important aspect of cataract surgery and IOLs, namely, long-term fixation and biocompatibility, as well as pathogenesis of various complications such as uveitis.

Although IOL implantation is now an extremely safe procedure, the remaining nagging complications include decentration, posterior capsular opacification, and uveitis. The most common of these is actually pos-

terior capsular opacification. It is highly probable that a major cause of this condition is related to a breakdown of the blood-aqueous barrier and/or inflammation. Specular microscopy of IOLs and other such studies address this possibility directly and are essential.

With its exhaustive list of references, this volume is an example of fine scholarship on a topic that at first glance appears limited, but actually has real clinical significance in terms of improving the results of IOL implantation.

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## Preface

*It is obvious that inflammation and its sequelae are still the most common underlying or ultimate reason for IOL explantation or enucleation (Apple, 1989)*

In industrial countries about one out of every ten persons undergoes a cataract operation and has an artificial lens implanted at some point in his or her life. Thus, 250,000 lenses are implanted every year in Germany, while in the USA the figure is more than 1,300,000.

Side effects due to lens incompatibility occurring after implantation have retarded the clinical acceptability of such interventions for decades. The problems encountered were due to fixation, to injuries to the corneal endothelium, and to inflammatory complications. It was only with the introduction of the flexible posterior chamber lens at the end of the 1970s that the implant found a place in the eye, thus largely solving the problems arising from fixation. Parallel to this development a renaissance occurred in the use of specular microscopy of the corneal endothelium and this drew the attention of surgeons to this sensitive cell layer. It is now general practice to do everything possible to avoid damaging the endothelium, resulting in a significant reduction of bullous decompensation. Thus, only inflammation remains as the single most serious biological problem arising from lens implantation. Overall, severe inflammatory complications occur only very infrequently. Nevertheless, vigilance in the effort to further limit the

incidence of this rare problem is necessary. Findings based on clinical experience alone have sometimes led to misleading interpretation in cases of postoperative inflammation.

From 1985 onward, the reintroduction of lens specular microscopy as developed by Vogt made available a new method of investigation, which, supplementing the standard slit-lamp examination with focal illumination, opened the path to extending the knowledge on the inflammatory processes that may arise after IOL implantation. Not only can such complications now be differentiated with more certainty, but specular microscopy enables us to recognize developing foreign body reactions at early stages and to prevent adverse effects. The apparatus required for specular microscopy is minimal. It may be carried out with any commercially available slit-lamp and is thus accessible to all ophthalmologists.

The present volume is intended for both research and for clinical practice. The first chapter essentially consists of a survey of the literature previously published on the cytology of IOLs from explanted lenses. The next two chapters give a practical guide on how to learn the technique of specular microscopy and on the evaluation of findings. The fourth chapter then provides examples of clinical application. The aim here is to include also theories which have been derived from case observations or from long-term studies yet under way.

## Acknowledgments

It would have been impossible to complete this volume without the support of others. I would especially like to thank the following persons who provided me with invaluable advice after reviewing the manuscript: Marc D. de Smet, M. D., National Eye Institute, Bethesda, Professor J. Reimer Wolter, M.D., Departments of Ophthalmology and Pathology, University of Michigan Medical Center, Ann Arbor and Emily Hindman, Department of Ophthalmology, University of South Carolina Medical Center, Charleston.

Photos provided by colleagues often demonstrated relevant details better than mine, so why not use them? I want to thank the following colleagues for offering their material: K. M. Saari, M. D., Professor, and K. Nummelin, Department of Ophthalmology, University of Turku, Finland (Figs. 20, 21, 23, 24, 48); J. Ygge, M. D., Ph. D., Department of Ophthalmology, Karolinska

Institutet, Huddinge, Sweden (Figs. 26, 34, 35); P. Hayes, M. D., P. Condon, M. D., P. Tormey, M. D., and M. Kinsella, M. D., Ardkeen Hospital, Department of Ophthalmology, Waterford, Ireland (Fig. 33); A. Böcking, M. D., Professor, Department of Pathology, University of Aachen, Germany (Fig. 5); G. Hollweg, Ph. D. and J. Richter, Department of Pathology, University of Aachen, Germany (Figs. 2, 3); W. te Poel, Department of Ophthalmology, University of Aachen, Germany (Figs. 4, 17, 18, 47a).

Of invaluable help in the making of this book was G. Davis, Ph. D., who translated the original German manuscript.

I feel particularly indebted to my teacher, Professor M. Reim, M. D., who inspired the investigations leading to the writing of this book. My thanks are also due to all those many people whose teachings, suggestions, and ideas have been incorporated into this book.



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# 1. Why Specular Microscopy?

*The benefit of specular microscopy studies is that the lenses are examined in eyes that are normal and the IOLs are clinically well tolerated. This is in contradistinction to those IOLs that are examined after removal because of clinical intolerance and whose surface deposits may reflect a grossly altered physiological state. In order for us to gain a greater understanding of this foreign-body response to an IOL, several aspects of the sequence of events occurring intraoperatively and in the early postoperative period require examination (Obstbaum, 1990).*

## What is Specular Microscopy?

### Optical Reflections

Our knowledge of the reflection of light is ancient. The law of reflection according to which "the incident angle is equal to the emergent angle" was first proposed by Euclid (330–280 B.C.). In daily life, however, we receive most visual information from diffuse reflected light, but specular reflections are usually felt to be disturbing. In strabismology, however, reflected images off the cornea or the lens play a useful role (Purkinje, 1823; Hirschberg, 1886; Effert, 1986). With the aid of the image reflected on an implanted lens, the iris can be observed behind the edge of the pupil or information can be obtained on the degree of lens tilt (Meyner, 1990).

Otherwise reflected images cause confusion particularly in beginners learning retinoscopy or biomicroscopy.

### Specular Microscopy: the Examination of a Reflecting Surface

Specular microscopy is a variant of light microscopy. By means of specular microscopy, even surfaces on which light is reflected are optically examined. The "mirror" is not part of a device, but the surface to be examined itself.

On this surface the most minute irregularities may be examined, even if they are smaller than 1  $\mu\text{m}$ . Punctiform deposits appear as elevations on the specular area and become black spots, irrespective of their pigmentation, because they cannot reflect the light in the same direction as the rest of the surface. If a completely even surface includes areas of varying optical density, then the behavior of the reflection will also change, allowing the appearance of structures visible by specular microscopy. In specular microscopy of the corneal endothelium it is probably not irregularities of the interface between endothelium and aqueous humor which are responsible for the cell borders, but the varying refractive behavior of cell surface and cell border. Otherwise the cell outline would also be visible when using "indirect specular microscopy" (Hartmann, 1987).

Specular microscopic examinations can be carried out using any type of microscope that can examine reflected light. Other forms of reflected light microscopy can also be carried out with the "specular microscopes" commercially available (Thaer and Geyer, 1985).

The examination technique was first described in detail by the Swiss ophthalmologist Alfred Vogt. His description of the technique as "examination in the specular area" is more exact than "specular micros-



copy," the usual term today. A "specular microscopic" examination is also possible using a slit lamp, which from an optical point of view is more of a telescope than a microscope. Be that as it may, we shall mainly employ the term in current usage, "specular microscopy," since in the ophthalmic literature the slit lamp is often described as a "microscope."

## The Development of Specular Microscopy

### Alfred Vogt

Alfred Vogt's contribution to the development of specular microscopy as a part of slit-lamp microscopy was important.

He was born on 31 October 1879 in Burg near Menziken in the Swiss canton of Aar-



Fig. 1 A. Vogt (1879-1943) (FSGA Zwickau)

gau. After studying medicine in Basel, he took his doctorate there in 1905 working with Carl Mellinger, professor of ophthalmology. In 1906 he opened an office as an ophthalmologist in Aarau, and in 1909 he was named head of the Department of Ophthalmology at the canton hospital. He was refused permission to apply for his habilitation at the Basel Medical Faculty. On account of his outstanding scientific work, however, he was given an associate professorship in 1918 after Mellinger's death and was later appointed to the chair in Basel. In 1923 he accepted a chair in Zürich, and it was there on 10 December 1943 that he died after a long illness. He had many areas of interest, including lens pathology, retinoscopy, retinopathy and retinal surgery, genetic research, and slit-lamp microscopy. For Vogt, specular microscopy, together with examination in focal and regressive light, was one of the three major applications of the slit lamp (Bider, 1964; Niederer, 1989).

While examinations of the anterior segment of the eye using microscopes had long been practiced, it was the invention of Gullstrand's nernst slit lamp in 1911 together with the development of the binocular Zeiss-Czapski corneal microscope which revolutionized the study of the anterior sections of the eye (Vogt, 1930, p. VI, 1). Vogt's *Atlas of Slit-Lamp Microscopy* (1921) as well as his *Textbook and Atlas of Slit-Lamp Microscopy of the Living Eye* (1930-1942) remain important works even today. As he was not able to document his findings with the slit lamp photographically, the illustrations were produced by the painter Rudolf Bregenzer.

It is unclear who was actually the first to use specular microscopy to examine optical interfaces in ophthalmology. Vogt commented on this in his *Atlas* (1930, p. 28): "Historically speaking, the lens chagrin (that is, the anterior specular area of the lens) was first seen by Tscherning in 1898, and later by C. Hess (1904); it was then Stähli who examined the anterior surface of

the cornea "in reflection." Koeppe claims to have seen the back surface in reflection".

The fact that Vogt was already carrying out specular microscopic lens examinations in 1917, when he described color shimmering of the posterior capsule of the lens, has been largely ignored in the literature. It was not until 2 years later that he described the examination of the cornea using specular microscopy.

Using specular microscopy, Vogt was able to identify inflammatory cells, such as those on the anterior surface of implanted artificial lenses described in the present volume, as deposits on the corneal endothelium and the human lens (1930, p. 202). Some of his observations also apply to the precipitates seen on IOLs. He also described the spontaneous movement of small cells ("lymphocytes"), which at 10 to 20  $\mu\text{m}$  per second is, however, significantly faster than the spontaneous movement of cells on lens implants (1930, p. 204). He observed that the color of the giant cells gives some indication of their age: that recent deposits tend to have a grayish white color, while older ones are brownish (1930, p. 219); that the morphology of such deposits does not allow one to draw any conclusions as to the cause of the disease (1930, p. 222); that the precipitates may originate from the iris without the iris showing evidence of transillumination by the slit lamp (1930, p. 225); that the branches of spindle-like cells can unite to form straight lines and that this seems to occur particularly frequently as the cells become senescent (1930, p. 225).

Precipitates on the human lens, unlike those on artificial lenses, occurred more frequently on the posterior than on the anterior surface (1931, p. 391). Inflammatory deposits on the anterior surface of the lens usually took the form of small filamentous and star-shaped cells, which, morphologically speaking, recall the remnants of the tunica vasculosa lentis (1931, p. 368,384). A cotton thread which had been left in the eye by mistake after an extracapsular cataract extraction caused a lasting inflammatory

reaction with precipitates on the anterior capsule but did not require an operation to remove it (1942, p. 910). It is not entirely easy to understand the distinction Vogt made between an "infective" iritis, where precipitates are formed, and a "toxic" iritis, the most significant of which is diabetic iridocyclitis, where there is protein exudation in the anterior chamber and an absence of precipitates (1930, p. 279; 1942, p. 876).

### Photomicroscopy of the Corneal Endothelium

In 1919 Alfred Vogt was the first to describe how the human corneal endothelium could be rendered visible in the specular area. Unfortunately, examination in the specular area became increasingly rare outside Zürich, especially after Vogt's death, since it is a difficult technique to learn without personal guidance. In the second half of the century it appeared that his work on specular microscopy would be completely forgotten by the international community.

When Maurice introduced a method of photographing the corneal endothelium in untreated enucleated cat's eyes in 1968, he began his paper with the sentence: "It is well known in ophthalmology that the outlines of the endothelial cells of the cornea may be seen by using the reflected beam of the slit lamp." He simplified the old, descriptive term "observation in the specular area," used by Vogt, to "specular microscopy," the term usually used today. In so doing he was not claiming to be inventing specular microscopy; but he was the first to describe specular microscopic *photography*.

Laing, who in 1975 became the first to photograph the human corneal endothelium in vivo, started his publication with the line: "In 1968, Maurice described a new specular microscope suitable for photographing the corneal endothelium in situ." Since he was using an endothelium camera as developed by Maurice, he did not need to refer to Vogt in this context either.



Finally, in 1976, Bourne and Kaufman were the first to describe in vivo how a cataract operation causes varying degrees of irreversible damage to the corneal endothelium, a fact which is important for present-day ophthalmic surgery. Thus the endothelial injuries described by pathologists in individual cases (Naumann and Ortbauer, 1969; Bresnick, 1969) could be confirmed in vivo for the first time. But in their opening remark: "In 1968 the specular microscope was described by Maurice for the observation of corneal endothelial cells in situ," they too made no reference to Vogt.

In the following years numerous publications in the field of endothelial diagnostics appeared in reaction to these three articles. After the papers by Maurice, Laing, and Bourne the term "specular microscopy" is sometimes used as a synonym for "endothelial microscopy." But the term "observation in the specular area" as used by Vogt refers to much more than just endothelial diagnostics. In addition to its use in the diagnostics of the corneal epithelium and the tear-film (Rao et al., 1987; Tsubota, 1991), the specular microscopy of lens surfaces is becoming widespread.

### Specular Microscopy of Lens Implants

Although specular microscopy is one application of slit-lamp examination, there were in fact no specular microscopic examinations until long after the invention of artificial lenses. Kraff et al. (1980) published slit-lamp photographs of severe inflammatory reactions after the implantation of lenses which had been coated with polyvinyl alcohol. They also took photographs in the specular area (Fig. 4 of their article) without specifically saying they had done so.

It was only with the appearance of "specular microscopes" for use in endothelial diagnostics that examinations of lens implants using specular microscopy increased. However the installation of "contact elements" made many of these devices suitable for corneal exams, but not for ex-

amining the surface of the lens. Thus, these contact elements had to be developed or modified for lens examination.

In 1983 S. Oak, R. Laing, and their colleagues were the first to report on such examinations. Laing and Bursell had already reported on specular microscopic examination of the human lens in 1981, but now cellular precipitates on implants were described in vivo. To do so they installed a new W 10X objective lens in their Bio-Optics LSM 2000C specular microscope so as to obtain greater object distance. They presented the following case: 1 month after lens implantation and a later reoperation they found on the implant pigment deposits and ameboid cells which they suspected to be leukocytes. They did not, however, direct their examination to the specular area itself, but instead they photographed the cells in the vicinity of the specular area with focal illumination. These appeared as bright shadows against a dark background. The authors concluded the article by suggesting that specular microscopy of the lens was destined to become just as important clinically as endothelial diagnostics using specular microscopy. In 1984 McDonnell et al. described cells on the posterior lens capsule using specular microscopy in three cases of secondary cataract.

In 1984, too, Mayer published in his atlas a photograph of an implanted lens taken by specular microscopy, but there were no deposits on it. He had assumed that it was possible to make out deposits on lenses too.

In 1984 K. Ohara described specular microscopic examinations of cells on implants. His paper was published in Japanese only without an English summary. One year later he published his technique in a much-quoted article in the *American Journal of Ophthalmology*. He presented specular microscopic examinations of three patients whom he had examined several times. The resolution of such photographs is better than it is with focal illumination and it paved the way to new kinds of examination. Ohara divided cells into two groups, a differentia-



tion which is still accepted today. The first group consists of small, round, bipolar, or amoeboid cells with branches; the second has large, round, oval or bizarrely shaped cells with central pigmented deposits, whose diameters measure between 150  $\mu\text{m}$  and 400  $\mu\text{m}$ . Ohara began his examination by first surveying the surface of the lens in the specular area with the slit lamp. He then examined the lenses under greater enlargement using a Keeler-Konan contact microscope. Later he preferred to examine lenses without the use of a contact element (1986a). Using specular microscopy, material defects and deposits on the lens can be detected before implantation (Ohara and Shimizu, 1988; Ohara et al., 1989).

Specular microscopy of lens implants has generated considerable interest, especially in Japan. The Pocklington microscope is particularly suited to generating visible interference patterns in the form of Newton's rings on lens implants. In 61 out of a series of 133 patients who had undergone lens implantation, Okada (1986) found—in addition to the known cells—an amorphous film on the cell-free areas of the lens surface. This film first became visible in the form of separate gray spots, which were slowly amalgamating. Sometimes the film also became visible due to the formation of Newton's rings. He was also able to differentiate them from the irregular deposits he had seen after administering oily indomethacin eye-drops and which he suspected contained precipitation from the vehicle (Okada et al., 1988). Twenty patients were examined repeatedly. The Newton's rings appeared for the first time at the beginning of the 2nd week after the operation and disappeared again between the 2nd and the 9th post-operative weeks (Okada and Sagawa, 1989b).

Using monochromatic light with a wavelength of 564 nm he determined the thickness of the periphery of giant cells at 2  $\mu\text{m}$ . Within half an hour the patterns of interference changed significantly, particularly along the periphery of the cells (Okada

and Abe, 1989). On "surface-passivated" lenses he found a similar cellular reaction, and he therefore concluded that such lenses are biologically just as compatible as are regular lenses made of PMMA (Okada and Tobari, 1991). In 1991 he also published a summary of the physical principles on which he had based the calculation of interference patterns.

T. Hara (1987) attempted as far as possible to leave the anterior lens capsule intact during the operation and implanted lenses in to the capsular sac. After the operations, clouding of the anterior capsule usually occurred. Using the specular microscope in such cases he found "various kinds of metamorphosed masses" on the lens. Although in morphological terms they suggest the inflammatory cells described above, he thought that they originated from lens epithelial cells. In a later series of 77 patients (Hara, 1988) he used specular microscopy to describe the anterior surface of the lenses of patients whose anterior capsules had either been left in or removed. He worked on the notion that in both cases the majority of cells deposited on the surface of the lens originated from lens epithelial cells. He believed that these were the causative cells because most of the cells contained no pigment, did not move quickly, fused more readily with the anterior capsule than with the implant and that a transformation of the cells could not be suppressed with steroids. However these arguments are somewhat suspicious. They contradict the data discussed below, which was obtained from explanted lenses; also, morphologically similar cells are found on anterior chamber lenses after intracapsular extraction (Wenzel et al., 1987; Tamura et al., 1990), and these can hardly be cells of the epithelium of the lens.

### Why Do Inflammations Occur after Lens Implantation?

*Our knowledge of the causes of the inflammatory processes we observe after op-*

erations, particularly cataract operations, is still quite incomplete (Schirmer, 1899).

*The inflammatory response to surgical manipulation is still little understood but evidently is essential for normal healing (Jaffe, 1990).*

These two quotes from competent ophthalmologists one hundred years apart remind us of the need to be modest in our undertakings. This is not the place for a comprehensive survey of the present state of our knowledge on inflammatory reactions occurring after cataract operations. We may, however, refer the reader to recent surveys (e.g. Apple, 1989; Jaffe, 1990; Obstbaum, 1992; and others). The emphasis in what follows lies more on the cytological findings provided by explanted lenses.

The reasons for the inflammatory processes which develop after lens implantation are many and various, and it is often difficult to distinguish them clinically. The major contribution to the inflammatory response is probably the cataract operation itself rather than the lens implantation. Inflammation was always a dreaded complication of cataract surgery even before the advent of artificial lens implantation. Thus, the term "iritis" was originally introduced only to describe inflammatory processes after cataract extraction (Schmidt, 1801)! It was later that the term was broadened to its present day use. As early as the end of the last century, the possible causes of postoperative inflammation were being described in terms of infection, phacogenic or toxic genesis, and trauma (Wenzel 1990a). Clinical symptoms often give no indication of the possible cause and even histological examinations do not always provide clarity in the case of enucleation. Table 1 lists the conditions which, according to current thinking, may bring about postoperative inflammation. Among the consequences of such inflammations may be: the collapse of the barrier between the blood and the aqueous humor; the emigration of granulocytes, macrophages, or other inflammatory cells; the formation of syn-

Table 1 Causes of postoperative inflammations after lens implantation

- 1) Trauma
  - Intraoperative tissue damage
  - Autoimmune reaction (especially to residual lens material)
  - Wound incarceration
  - Posterior synechias of the lens capsule
  - Rupture of the anterior membrane of the vitreous
- 2) Foreign matter
  - Infection (including hypersensitive reactions to bacterial products)
  - Foreign-body reaction (especially to the implant)
  - Toxic (or immunological) reaction
- 3) Individual risk factors
  - Systemic disease (e.g., diabetes mellitus)
  - Inflammation or degenerative eye disease (e.g., iritis)
  - Unknown etiology

echias; and scar tissue. Specular microscopy is particularly suited to the examination of the later stages of inflammation (macrophages, synechias).

## Trauma

Every injury to the eye causes an inflammation. Even during the preparation of a cataract extraction and up to the performance of a corneoscleral incision there is a noticeable increase in cyclooxygenase-dependent inflammatory mediators within the aqueous humor (Struck et al., 1991). A particular risk of severe inflammation may be provided by a severe intraocular hemorrhage, a wound incarceration, or a rupture of the hyaloid membrane (Jaffe, 1990). Posterior synechias of the iris to the lens capsule may appear some months after an operation and facilitate direct protrusion of macrophages from the iris to a lens implant.

## Autoimmune Reaction to Residual Lens Material

Fragments of a lens left in the eye can cause inflammation of varying degrees of severity, serious inflammations being the exception. A severe reaction often does not set in until several weeks after the operation. Histologi-



cally, in the case of a phacogenic iritis a granulomatous inflammation is located in the area of a retained lens nucleus. A central reaction of polymorphonuclear granulocytes is surrounded by concentric layers of various inflammatory cells including epithelioid and giant cells. In the adjacent choroid both lymphocytes and plasma cells can be found. The clinical picture is also known as "phacoanaphylaxis," even if IgE is not involved (Verhoeff and Lemoine, 1922; Nissen et al., 1981; Jaffe, 1990; Apple et al., 1984a, 1984e, 1989; Abrahams, 1985). Smears of anterior chamber and vitreous body taps of phacogenetic uveitis may resemble those of a chronic infective uveitis (Meisler et al., 1986). Phacoanaphylaxis might in fact be facilitated by a superinfection with *Propionibacterium acnes* which can act as an adjuvant as described in animal experiments (Smith, 1989). Apple (1989) does not exclude the possibility that Verhoeff and Lemoine described such a synergistic inflammation due to bacteria and residual lens material in 1922.

### Foreign Matter

During a cataract operation a variety of foreign particles can get into the eye and stay there. Lenses, irrigation solutions, or viscoelastic materials are sometimes contaminated by foreign particles (Meltzer, 1980; Bene and Kranias, 1986; Apple, 1989; Jaffe, 1990; Smith and Eiseman, 1990; Kwitko and Belfort, 1991). Among these, two are particularly noteworthy as possible triggers of postoperative inflammations, bacteria and the lens implant.

### Infection

A cataract operation can never take place under sterile conditions (Jaffe, 1990). Realistically all preoperative endeavors can only reduce the potential pathogens at the site of the operation as much as possible. Bacterial infiltrations which are not clinically significant may be assumed to be present

in over 40% of the operations (Kohn, 1978; Clayman et al., 1986; Dickey et al., 1991) (cf. Fig. 3). Postoperative infections can be present with differing degrees of severity. As a diagnosis, "*benign endophthalmitis*" is a difficult entity to define (Schirmer, 1899). This syndrome is characterized by intermittent inflammation which does not begin immediately after the operation. It may relapse and may occur at the same time as the formation of a fibrinous membrane or even of a hypopyon. It often appears 2 to 4 days after the operation, but in cases where the healing process has initially gone well, it may only break out months later (Piest et al., 1987; Wenzel and Reim, 1988). A positive culture and histologic finding would be needed to prove the diagnosis; the microbiological examination becomes more reliable the more material is cultured, but this then decreases the amount available for histologic examination. Negative microbiological findings cannot, however, exclude the possibility of an infective origin. It seems that for a long time there was some reluctance to discuss the possibility of infection as a cause of postoperative iritis. Often the diagnosis given—largely as an excuse—was "toxic lens."

Bacteria appear to have a particular affinity for plastics, particularly for haptic made of polypropylene (Peters, 1988; Dilly and Sellors, 1989). Coagulase-negative staphylococci may be the predominant causative organisms in infections associated with implanted foreign bodies. The underlying pathomechanisms are complex. These bacteria are able to adhere to and grow on polymer surfaces. In the course of polymer colonization they produce an extracellular mucosal matrix, in which they become completely embedded. The slime protects the staphylococci against phagocytosis and against antibiotics, and is thus responsible for the maintenance of the infectious focus (Peters, 1988; Faschinger, 1990; Hartmann et al., 1991).

The picture of a fulminant panophthalmitis, on the other hand, is quite unmistak-

able, characterized as it is by an immediate massive cell infiltration in the anterior chamber of the eye, by hypopyon, chemosis, and severe pain (Allen and Mangiarcine, 1974; Forster, 1974; Zaidman and Mondino, 1982; Ormerod et al., 1987).

### *Foreign-Body Reactions*

A foreign-body reaction characterized by a granulomatous response is frequently found on cytological examination of explanted lenses. Foreign-body granulomas may form in the absence of immunologic modulation (Epstein and Fukuyama, 1989; Smetana et al., 1990). Insertion of any foreign body into the eye causes a cellular reaction in the anterior chamber. It is for this reason that animal eyes were used as a model for inflammations as early as the last century. In 1863 von Recklinghausen described the star-shaped appearance and the active mobility of the "pus corpuscles." He also found that they contained pigment granules. Conheim (1867) described inflammatory processes which occurred when a silk thread was passed through the eye or when aniline blue was injected into the anterior chamber. He discovered that the pus corpuscles originated in the blood. V. Humboldt (1877) introduced small pieces of tissue into the anterior chamber and described the ensuing inflammations. He reported that seeing into the eye offered ideal conditions for the examination of inflammatory reactions—but he also found that bacterial contamination often affected the result. This approach is still in use today (Höh et al., 1985) and offers an alternative to animal experiments involving cataract extraction and lens implantation. Leber (1888) inserted small glass tubes into the anterior chamber of the eye in which traces of pus had collected even if no signs of inflammation were visible in the other eye.

It is important to remember that the granulomatous reactions seen in tuberculosis or syphilis, are in principle, also "foreign body reactions." Our tissues cannot

immediately overcome the tubercle bacilli in cells infected with tuberculosis, for example. Thus, the tissues treat these cells as "foreign bodies." In a so-called "granulomatous reaction" macrophages are mobilized and the infected cells are isolated by encapsulation. In this process, the macrophages change into epitheloid and giant cells. This granulomatous reaction typically develops after lens implantation in iris, ciliary body, and pars plana. Histologically, it can be recognized there by the presence of chronic inflammation and epitheloid as well as giant cells on the surface of the reacting parts. This reaction cannot originate "in the fluids of the eye." It is really the result of "a low-grade anterior endophthalmitis" and it not only always involves, but always originates in vascular portions of the eye. All granulomatous inflammations are feared equally by experienced clinicians and pathologists.

Any lens implant, no matter how well it has been manufactured, will elicit a foreign-body reaction. How the eye reacts, depends on individual factors specific to the patient. Characteristic of a reaction against an implant are inflammatory changes which are more serious on the implant than in the adjacent tissue (Wolter, 1983e). The foreign-body reaction to a lens implant in the eye differs from all other foreign-body reactions in the body in that there is no primary direct contact between the implant and any blood vessels. The only exceptions are the parts of the haptic which come into contact with the choroid (Hunold et al., 1989). Inflammatory vascular proliferation on a lens does not occur; there have been reports, however, of ischemic proliferations in cases of diabetic rubeosis iridis (Eifrig et al., 1990). Cystoid macula edema can also occur independently of any inflammatory reaction (Wolter, 1980).

Mechanical irritation due to rough haptics used to be regarded as a cause of post-operative inflammation such as in the "uveitis-glaucoma-hyphema" syndrome, especially when poorly manufactured an-



terior chamber lenses were used (Ellingson, 1977; Apple et al., 1984d). On the other hand some inflammation is welcome, since it may help to stabilize the lens at the level of the haptics (Ridley, 1951; Wolter, 1991).

### *Toxic or Immune Reactions*

Early inflammatory reactions can originate in toxic irritations from the implant. The proportion of such complications has rapidly decreased recently, although lenses unsuitable for implantation do occasionally still find their way onto the market (Schrage et al., 1991). In addition, there has also been some suggestion that an immune reaction to the lens material might be involved in the postoperative inflammation.

The toxic effect of lens sterilization was described by Rintelen and Saubermann (1956) and later by Stark et al. (1980). In 1982 Tuberville concluded that serious inflammatory reactions after lens implantation were due to complement activation by haptics made of polypropylene or nylon. No conclusive proof for the dissolution of significant postoperative iritis following immune reactions to implants made of PMMA has yet been offered.

Galin et al. (1981, 1982) stated that the concentration of C3 and C5 in eyes with bullous keratopathy is increased after lens implantation. Measuring the speed of granulocytes led them to assume that there was a chemotactical activation by lenses made out of PMMA and nylon. Mondino et al. (1985) incubated human serum with lenses made out of PMMA and polypropylene and in so doing found increased titres of C3a and C5a. Kochounian et al. (1991) incubated lenses in human serum and found no difference in the complement activation of regular and surface-passivated lenses. It was possible to reduce surface impurities caused by siloxans by means of such passivating of the surface (Koch et al., 1991).

Moreover Drews (1983), Apple et al. (1984c), Altman et al. (1986), Sievers et al. (1985), Yamanaka et al. (1983), as well as Hofmann (1985) have described the biode-

gradation of haptics made of polypropylene or nylon in the human eye. They emphasized that the assumption that such findings have clinical consequences is pure speculation.

Possible injury to the eye caused by polymethyl methacrylate (PMMA) is apparently not of any clinical importance either. Galin et al. (1977) and Scheiffarth and Kretz (1983) found residual monomeric compounds in the lenses at a concentration of up to 0.5%. They found that at these concentrations monomers were not toxic. Holyk and Eifrig (1979) found that rabbit eyes were not able to tolerate intraocular irrigation solutions upwards of a concentration of 0.5% of monomers. However, such concentrations are not attained in the aqueous humor of human eyes after the implantation of PMMA lenses. Nor could any specific reaction to PMMA be found in animal experiments (Jenette et al. 1992; Redbrake and Biermann, 1988). Rochels and Stofft (1987) even described a marked biodegradation in a PMMA lens, which had been tolerated without reaction for 30 years, but this individual finding has not been confirmed by anyone else since.

HEMA or silicon are regarded as equally compatible (Kilp and Stilla, 1977; Yalon et al., 1984; Allarakhia et al., 1987; Wolter 1988, 1989b). Numerous animal experiments (cf. pp. 20f) as well as examinations in vivo using specular microscopy (cf. pp. 82f) have demonstrated this. On an explanted silicon lens Newman et al. (1986) found no inflammatory cells. On HEMA lenses, too, inflammatory cells have been found, but the cytological behavior seems to be different. There have been several reports of crystalline deposits (Menapace et al., 1990). Supramid loops can be entirely degraded in the eye without any toxic reaction (Langmann et al., 1988).

### **Individual Risk Factors**

Little is known about how individual risk factors affect the extent of postoperative

inflammation. Diabetes mellitus does not necessarily bring an increase in early postoperative inflammations, but rather, tends to cause posterior synechias of the iris and foreign body reactions in the late stages (Sebestyen and Werfai, 1985; Biedner et al., 1989; Oldendoerp, 1989; cf. Chap. 4). Rheumatic illness in adults often does not constitute a high risk either, unless it had already caused an iritis before the operation (Jaffe, 1990).

More serious postoperative iritis can occur in patients who have pseudoexfoliation or heterochromia. The clinical significance of individual risk factors remains a matter of controversy (Hiles and Watson, 1979; Olivius et al., 1989; Jaffe, 1990; Daus et al., 1991a; Gloor and Stegmeier, 1991; cf. Chap. 4).

### **The Cytology of Inflammatory Reactions on Lens Implants**

In order to interpret the *in vivo* cytological findings that can be seen using specular microscopy, some basic knowledge of lens implants is necessary. Living cells are the most frequent and the most interesting findings on implanted lenses. Since we shall be discussing specular microscopy findings in a separate chapter (Chap. 4), what follows is a summary of findings ascertained from explanted lenses.

A survey of the literature (Table 2) reveals just how few posterior chamber lenses have been examined. Most of the lenses explanted were iris-supported, which had resulted in bullous endothelial degeneration making a keratoplasty necessary to restore sight (see below). Cellular deposits were present in most cases, even when no iritis was known to have been present.

### **The Cytological Examination of Explanted Lenses**

When an enucleated eye with an artificial lens is to be examined microscopically, the

lens is usually chemically dissolved, so as not to damage the surrounding tissue when incisions are made (Theobald, 1953; Kincaid et al., 1982; Sievers and von Domarus, 1985; Champion et al. 1985; Garner, 1989). Pathologists were interested mainly in the tissue reactions induced by the intraocular lens (cf. the survey in Apple et al., 1989). However chemical dissolution of the lens, usually means the loss of all information related to the cellular deposits on the lens surface. Only when membranes were connected to the iris or other tissues would lens deposits be examined in cross section. Examining a flat membrane in cross section is more difficult. A more suitable method of examining lens deposits is to examine them in flat preparation. This is possible when the body of the lens has been preserved (Wolter, 1985b). If the artificial lens is removed from the eye before the histological examination of the globe is prepared, there is some danger that gross mechanical damage will be caused to the uveal structures, particularly where the lenses have become firmly embedded. Comparison of the cytological growth of an artificial lens with the histological changes to the choroid may show that the inflammatory reaction on the implant exists independently of any inflammatory reactions in the adjacent tissue (Wolter, 1983e, 1983f).

It is difficult to examine an explanted lens using classical histological methods, because thick lenses are hard to embed between a specimen slide and a cover glass (Wolter, 1989b). Electron microscopy is used as well as optical light microscopy when explanted lenses are to be examined. Scanning electron microscopy provides an easier method, since, like light microscopy, it is suitable for use with large series of explanted lenses (see Table 2). Obtaining findings from cells by means of transmission electron microscopy is technically much more complicated. Up to now there have been only a few case reports (Kraff et al., 1980; Galin et al., 1979; Yeo et al. 1983., Champion and Green, 1985; Ishibashi et al., 1988, 1989a, 1989b; Uenoy-



Table 2 Surveys of the cytology of explanted lenses

Author	Year	explanted IOLs	posterior chamber IOLs	IOLs with cellular deposits	Eyes with iritis	Eyes with endophthalmitis
Sugar et al.	1978	2	—	2	—	—
Wolter	1982c	3	—	3	—	—
Wolter	1982d	6	—	6	2	—
Wolter	1983a	3	—	3	—	—
Wolter	1983c	4	—	4	—	—
Wolter	1983d	3	1	3	1	—
Wolter and Felt	1983	2	—	2	2	—
Siepsner and Kline	1983	27	—	?	9	1
Colin et al.	1984	31	1	?	?	?
Daicker	1984	20	1	19	—	1
Sasaki et al.	1984	17	—	17	?	?
Sievers and v. Domarus	1984	5	—	5	1	—
Wolter	1985a	211	?	?	?	?
Wolter	1985c	4	—	4	1	—
Wolter et al.	1985	2	2	2	1	—
Bryan III et al.	1985	52	7	>31	10	4
Champion and Green	1985	79	4	>39	12	5
Goder and Völker-Dieben	1985	9	—	8	—	—
Kincaid et al.	1985	15	—	11	5	—
Mullaney et al.	1985	4	1	—	—	—
Puck et al.	1985	14	4	14	1	—
Kappelhof et al.	1986b	9	2	8	1	—
Wolter	1987b	2	—	2	—	—
Cameron et al.	1987	396	—	?	?	?
Mertz and Reich	1987	8	?	?	7	1
Wolter and Anderson	1988	2	1	2	2	—
Rochels and Stofft	1988	30	24	?	?	?
Schlote et al.	1988	13	—	13	1?	—
Langmann et al.	1988	2	—	2	—	—
Schmack	1989	16	3	?	2	—
Wolter	1989b	7	2	5	2	—
Wolter	1989c	2	—	2	—	—
Dilly and Sellors	1989	2	2	2	2	2
Ishibashi et al.	1990a	4	4	4	—	—
Cusumano et al.	1991	11	6	5	5	5
Daus et al.	1991c	10	—	5	—	—
Wolter	1991	3	0	3	—	—
Apple (Solomon, 1991 Legler, 1991)	1989	2500	502	?	963	?

ama et al., 1988; Kulnig et al., 1989; Sawusch et al., 1989).

Until the 1970s only a few case reports gave any cytological findings on the surfaces of explanted lenses (Theobald, 1953; Smith, 1956; Krey and Jacobi, 1978; Sugar, 1978). It was not until J. R. Wolter began publishing his work from 1982 onward that interest was aroused in the cytology of lens implantation. Wolter presented a simple method of

examining explanted artificial lenses cytologically. They are stained as in freeze sections (H-E). Aiming for a better surface for easier photography the preparations are always embedded under a cover glass (Wolter, 1985e). Using "Baxter: SIP IM ACCU Mount Mounting Medium", the specimen remains stable much longer (Wolter, 1992, personal com.).

Although it has thus been shown that the presence of cells and of a membrane with deposits can be demonstrated, the actual classification of the cells remains somewhat doubtful (Wolter and Meyer, 1984; Sievers and von Domarus, 1984).

Even more difficult than the evaluation is the interpretation of findings from cells on implanted lenses. The clinical correlation between the histological findings and the severity of the inflammation is difficult. Strong cellular reaction is certainly an undesirable complication (Wolter, 1985b; Bryan III et al., 1985). Chapter 3 of this volume will suggest an approach to reproducible quantification of inflammation (cf. Wolter and Till, 1988).

It is surprising that several authors, who have examined explanted lenses, do not make any mention of inflammatory precipitates (Drews and Smith, 1978; Colin et al., 1984; Apple, 1984c, 1984d, 1985a; Apple and Kincaid, 1985; Pham-Duy and Hoder, 1989). This can be explained on the one hand by the fact that many lenses are in fact free of cells, as can be demonstrated in vivo by examination with specular microscopy. On the other hand, it is also possible that the lenses had not been fixed well enough after explantation. This can lead to loss of cells (Wolter et al., 1982c, 1983b; Kappelhof et al., 1986b).

In comparison to a complete histological examination of a whole eye there are of course limitations to what cytological examination of a lens can tell us. This difference is decreasing, however, as implantation surgery further develops from anterior chamber lenses via sulcus-fixated posterior chamber lenses to capsular fixated lenses. Chronic inflammatory reactions of the uveal tissue are particularly prominent with iris-supported lenses or anterior chamber lenses (Ishibashi et al., 1990a). There is less reaction to posterior chamber lenses. The trend is toward implanting in the capsular sac (Apple et al., 1985a, 1986a, 1986b). This eliminates contact between the lens and the vascular tissue. When lenses are capsule-

supported, the haptic no longer participates in the inflammatory process (Mullaney and Condon, 1985; Wolter, 1987b; Champion, 1988). In modern cataract surgery, inflammation related to lens implantation excess of the actual trauma of cataract extraction is limited to the optics which are not covered by the capsule sac.

## Differentiation of Individual Cells

### *Macrophages (Histiocytes)*

#### *Macrophages on Lens Implants*

The class of cell most frequently found on explanted lenses is the histiocyte in its various manifestations as macrophages, spindle-shaped cells, epitheloid- and giant cells (Wolter and Kunkel, 1983c, 1985a, 1985b; Sievers and v. Domarus, 1984; Goder and Völker-Dieben, 1985; Champion and Green, 1985; Bryan III et al., 1985; Wolter and Kleberger, 1985; Schlote et al., 1988; Kanagawa et al., 1989). The term "histiocyte" is used to describe all cells which originate from monocytes (mononuclear inflammatory cells) of the blood.

Macrophages are difficult to identify using morphological criteria, since these cells may be scattered all over and can elongate and flatten and lack readily identifiable characteristics. Hence, for a clear-cut definition of such cells, monoclonal antibodies are increasingly employed. A host of monoclonal antibodies has been generated against cell surface molecules of macrophages. Additional frequently employed antibodies recognize structures that are shared by other hematopoietic cells (Molty and Osdoby, 1985; Ziegler-Heitbrock, 1989).

Both the giant cells and the spindle-shaped cells, which morphologically resemble fibroblasts, have been defined by immunocytochemistry as macrophages (Hofman et al., 1986; Uenoyama et al., 1990; Becker et al., 1991). In terms of transmission electron microscopy the cytoplasm may be rich in phagolysosomes (Ishibashi et al., 1990a). The cells grow in single-layered fashion, and



only in exceptional circumstances do they overlap (Wolter, 1985a, 1985d; Wenzel et al., 1987a). The application of Healon as a viscoelastic substance does not alter the adhesion of the cells to the surface of the lens (Wolter and Kunkel, 1984). These cells often contain phagocytized pigment, which can be seen in vivo with a slit lamp in focal illumination (Wolter, 1982a; Wenzel and Hahn, 1988; Ishibashi et al., 1990b). The tasks of histiocytes in the body are many and various, which makes evaluation of the causes of such an inflammatory reaction difficult (Scheiffarth, 1989).

#### *Function of the Macrophage in Inflammation*

In 1891, Paul Ehrlich described macrophages as a separate class of leukocytes (Leder, 1967). They are formed in the bone marrow and after their final differentiation, they lose their mitotic activity (Ziegler-Heitbrock, 1989). The young monocyte remains in the circulating blood for about three days (Whitelaw, 1972), where it constitutes only about 5% of the leukocyte population. Their action is outside the blood stream. There they can survive for several months (Werb, 1983). As soon as a monocyte has

left the intravascular space it is called a macrophage (Leder and Nicolas, 1963). Macrophages may give rise to the smaller epitheloid cells or through fusion to larger poly- and multinucleated giant cells. Another typical form of development are fibroblast-like cells or even genuine fibroblasts (Goldstein, 1954; Allgöwer and Hülliger, 1960; Leder, 1967; Goder and Völker-Dieben, 1986; Ziegler-Heitbrock, 1989). Figure 2 demonstrates the surface of an anterior-chamber-IOL, with multiple macrophages, that had to be explanted 14 months after implantation because of a chronic inflammation and endothelial decompensation.

The macrophage has been extensively studied in the last few years. The name itself points to its well-known phagocytic activity (Werb, 1983). Papadimitriou et al. (1975) found that in mice about one-third of the macrophages do not phagocytize. Epitheloid cells in particular belong to this group.

Recent research has focussed more on macrophage secretion than on phagocytosis. Their responses include the secretion of substances. While up to 1984 some 50 such substances had been described (Takemura

Fig. 2 Young macrophages on a lens implant can differentiate into spindle-shaped cells or epitheloid cells. Epitheloid cells can also develop and then retract spindle-like branches. Scanning electron microscope.



and Werb, 1984), by 1987 there were already 100 of them (Nathan, 1987). The molecular mass ranges from 32 ( $O_2$ -) to 440000 (fibronectin), and in biological activity from growth stimulation through to necrosis factors. They can be subdivided into enzymes and their inhibitors, plasma proteins, proteins for the regulation of cell functions and low-molecular substances (Werb, 1983; Takemura and Werb, 1984). Macrophages are important in the regulation of most inflammatory processes (Nathan, 1987).

Functionally macrophages can be subdivided into two groups. *Inflammatory macrophages* have a high secretory activity which affects host tissues and activates inflammation without primarily affecting bacteria or tumor cells. *Activated macrophages* are activated by lymphocytes. They secrete reactive metabolites, which kill off bacteria and tumor cells (Takemura and Werb, 1984). Macrophages of the peritoneal cavity display a biochemical profile different to that from subcutaneous macrophages (Papadimitriou and Wyche, 1976).

Up to now there have been few methods available to examine human macrophages in vivo and help us to gain an idea of the temporal course of inflammation. Using the ear-chamber method, Ebert and Florey (1936) succeeded in demonstrating the emigration of blood monocytes marked with india ink from blood vessels and watched their transformation into macrophages with an optical microscope. This transformation was accompanied by an increase in size from about 11  $\mu\text{m}$  to up to 30  $\mu\text{m}$ . Using the skin-window method, small superficial wounds are created and each is then covered with a small glass plate. On the assumption that the inflammatory reaction takes place under all the glass plates in the same way, one glass plate was removed each day and the cells adhering to it examined. In this way Leder and Nicolas (1965) discovered that within 4 days of the onset of an experiment giant cells with over 200 nuclei can appear.

After the implantation of artificial lenses in the peritoneal cavity of mice, giant cells with up to 80 nuclei were found after only 5 days (Wolter and Kunkel, 1983b). In mice the first giant cells on an implanted plastic foreign body appear after only 2 days, the greatest density of giant cells occurs after 4 weeks, 25% of the adhering cells are then multinucleated (Papadimitriou et al., 1973a). In the eye, on the other hand, the formation of giant cells on implanted lenses proceeds much more slowly (cf. Chapter 4.1.). The mobility of macrophages is discussed in Chapter 4.1., too.

#### *Giant Cells and Epitheloid cells*

Epitheloid and multinucleated giant cells are histiocytic cells which derived from the monocytes of the peripheral blood. They can develop from the transformation as well as from the fusion of macrophages (Goldstein, 1954; Leder, 1967; Wolter, 1983a; Uenoyama et al., 1988c; Kodama et al., 1991). Differentiation of a macrophage into an epitheloid cell is said to take place if a macrophage does not find sufficient material to phagocytise on a foreign body, or if it has digested phagocytized material, or has secreted indigestible material (Papadimitriou and Spector, 1971). Epitheloid and giant cells are typical of foreign body reactions to implants. They develop less phagocytic activity but more secreting functions (Ziegler-Heitbrock, 1989). The ability to phagocytize decreases the more nuclei the cells have. While trinucleate cells remain capable of phagocytosis to a low degree, in studies based on animal experiments cells with five nuclei no longer proved able to phagocytize latex globules with a diameter of 0.5  $\mu\text{m}$  (Saika et al., 1991). Giant cells attempt to grow around a foreign body so as to decompose it chemically. If this does not succeed, many giant cells can surround the foreign body and seal it off from its environment.

Giant cells were described by Johannes Müller as early as 1838; they were further investigated by Virchow (1858) and Langhans (1868). In 1912 Lambert explained



their origin in terms of the fusion of separate macrophages. There were some suggestions that they arose from multiple, amitotic nuclear divisions of a single macrophage (Leder and Nicolas, 1963, 1965). A fusion of macrophages even seemed improbable, since they tend to inhibit contact and mutually reject one another (Forkner, 1930). Until today, prevailing opinion has been that giant cells arise from the fusion of monocytes or macrophages, with amitotic division playing at best a subordinate role (Maximow, 1924; Goldstein, 1954; Oldfield, 1963; Papadimitriou, 1973). Cell fusions by macrophages can also be shown by *in vivo* examinations with specular microscopy. (Fig. 46).

The number of the young cells surrounding giant cells gives some indication of the severity of the inflammation (Wenzel and Reim, 1987).

Where there are no small cells in the immediate vicinity, giant cells slowly disappear from the surface of the lens (Wolter 1985a; Wenzel and Reim, 1987). Wolter (1985a) as well as Uenoyama et al. (1990) subdivide giant cells on lens implants into three groups according to morphological criteria: foreign body giant cells are large, multinucleated, irregularly defined cells which may be found on irregularities of the lens. Several hundred nuclei may lie scattered about in the protoplasm. In the case of Touton giant cells the nuclei are clustered in the center in a disk-shaped area or in a circular, ring-shaped zone. The outer cytoplasm is vesicular. Langhans' giant cells are rarely encountered on implants. Characteristically, the nuclei are arranged in a ring at the (outer) border of the cytoplasm.

#### *Small and Spindle-shaped Macrophages*

Macrophages can also differentiate into spindle-shaped dendritic cells (Figs. 3 and 5). The dendritic macrophages are no more a functionally homogeneous group than are the giant cells. There are already smaller numbers of dendritic monocytes in the circulating blood, where they represent less

than 1% of the monocytes. These cells are not positive for all antibodies against monocytes (Mofty and Osdoby, 1985). Some dendritic cells may be potent stimulators of mixed leukocyte reactions and accessory cells for several immune responses. But those cells lack phagocytic capacity (v. Voothis et al., 1982).

The spindle-shaped macrophages on IOLs, however, usually contain phagocytic material (Wolter, 1985a, 1985b). The smaller the cell, the greater appears to be its ability to phagocytize (Saika et al., 1991). The name "fibroblast-like cells" can be misleading, since we are mostly dealing with macrophages, and not with fibroblasts. Spindle-shaped cells seem to have greater activity in the phagocytosis of erythrocytes than do giant cells or epitheloid cells (Wolter and Lichter, 1983; Wolter and Kunkel, 1985). In the case of the small cells it is difficult to see whether they will develop into an epitheloid cell or a spindle-shaped cell (Wolter 1983b; Wenzel and Machata, 1988).

#### *Other Inflammatory Cells*

Granulocytes are rarely found on well-tolerated lenses, and lymphocytes practically never occur. Both may be present more often in the tissue adjacent to the choroid (Naumann and Ortbauer, 1969; Goder and Völker-Dieben, 1985; Bryan III et al., 1985; Champion and Green, 1985; Champion et al., 1985; Mullaney and Condon, 1985; Bleckmann and Lohotti, 1985; Uenoyama et al., 1990). Inflammatory cells cannot always be differentiated with certainty using the scanning electron microscope. For this reason such deposits are described by some authors as "inflammatory deposits" (Apple et al., 1984d; Kincaid et al., 1985; Champion and Green, 1985; Kappelhof et al., 1986b; Rochels and Stofft, 1988).

#### *Other Cells*

In the case of iris-supported lenses, melanocytes and other cells originating in the iris have been reported in the vicinity of the



haptic. Some authors also reported lens epithelium and endothelium cells on the lens implant, which judging from the illustrations could also have been histiocytes. They are said to have remained adherent to the lens after direct contact with the endothelium or to be derived from the regenerative effect secondary cataract of the anterior lens capsule (Sugar et al., 1978; Siepser and Kline, 1983; Puck et al., 1985; Champion and Green, 1985, cf. Fig. 30). Wolter (1984b) found in a deceased female patient endothelial cells adhering to both lenses which still had their typical hexagonal pattern; but in many cells the nucleus was missing. He assumed that they had only adhered to the IOL following the collapse of the anterior chamber after death. Implanted epithelium of the cornea was rarely found on the surface of explanted IOLs (Wolter, 1985b; Goder and Völker-Dieben, 1985; Champion and Green, 1985; Puck et al., 1985; Kappelhof et al., 1986a).

### Membranes on Lens Implants

In addition to cellular growth, Wolter has from his earliest publications pointed to the presence of a thin eosinophilic membrane covering practically all lenses (1982b, 1983c). His first findings led him to assume that these had been formed by macrophages present on the lens surface (Wolter and Kunkel, 1983a, 1983c, 1983d, 1983e). Yet it soon became clear that the membranes can occur independently of cells and even prior to them (Wolter 1985c; Wolter and Kleberger, 1985). In contrast to cellular growth on an implanted lens, which can be tolerated or is to be regarded as pathological, the presence of such a membrane is desirable (Wolter, 1984b). Schmack, (1989) too, drew a distinction between well-tolerated lenses, where only the protein membrane was found, and less well-tolerated lenses where in addition masses of macrophages were found. Where there are many macrophages on the membrane it seems to adhere more firmly to the lens than

where there are no cells at all (Wolter et al., 1985; Wolter and Kleberger, 1985). Since this membrane must be functionally distinguished from other lens deposits, it is referred to as a "membrane (Wolter)" in this volume.

Sievers and v. Domarus (1984) as well as Ishibashi et al. (1990a) confirmed by means of electron microscopy that the cells adhere to and are not within the membrane.

Most recently this membrane has gained a new significance with the development of surface-modified lenses. It appears that surface modification does not directly affect cellular growth, but it modifies the nature of membranes on which the deposits occur. A membrane altered in this way might bring about better tolerance of the lens (Kilp and Stiller, 1977; Ratner, 1990; Versura and Caramazza, 1990).

Similar membranes also form when a lens is implanted in an artificial open chamber in the peritoneal cavity of a mouse or is incubated in blood with 10 IE heparin/ml (Wolter and Kunkel, 1983d; Wolter and Till, 1988). Other authors maintained that the membrane was not present in all cases, which is difficult to prove, especially using a scanning electron microscope (Dilly, 1989). Goder and Völker-Dieben (1985) as well as Kappelhof (1986a, 1986b) assumed that it sometimes covers only parts, and not all of the lens.

Apple et al. (1987) made no special mention of such a membrane; however the printed scanning electron micrograph does lead one to suppose that such a membrane exists. On transmission electron microscopy it consists of a fine-grained material. In the vicinity of the haptic collagenous deposits may also be present in this membrane (Ishibashi et al., 1990a; Saika et al., 1992). Boy et al. (1992) described fibronectin as a component of pseudophakic acellular membranes. Langmann et al. (1988) examined a membrane on a lens, which has been implanted for 30 years, using electron microscopy. They described it as being porous. However, this may be fixation artifacts of



the homogeneous membrane, since no porous membrane has yet been observed in vivo.

Fibrinous deposits occur in different degrees of severity as separate, transitory threads or as dense membranes in less than 10% of lens implantations (Böke, 1987; Walinder, 1989; Nishi, 1988, 1989; Norris, 1990; Wenzel et al., 1992). They may appear either after infections or other inflammatory complications or after intraocular hemorrhages or other primarily noninflammatory complications. They are usually accompanied by cellular growth on the lens, including collagen-producing fibroblasts (Theobald, 1953; Smith, 1956; Jaffe, 1978; Yeo et al., 1983; Wolter and Felt, 1983; Apple et al., 1984a; Champion and Green, 1985; Humalda et al., 1986; Wolter, 1989c). Hiles and Johnson (1980) believed that "postpseudophakos" membranes may be caused by lens epithelial cells. In creeping infections due to staphylococcus epidermis the pathogens may form their own membranes, which exercises a retarding influence on the body's defenses (Peters, 1988).

### Varieties of Cytological Findings

In describing cytological reactions on explanted lenses the different types of cells are often described without any quantitative evaluation of the findings being given. Data from the various authors are not comparable with one another. Independently of the varying symptoms it appears that in almost all of them, except for infection, macrophages, epitheloid- and giant cells are the prevailing types of cell.

#### *The Cytology of Clinically Well-Tolerated Implants*

Up to now the methodology used in describing the cytology of clinically well tolerated implants was poorly established, for where could one obtain sufficient material for examination? An explanted lens cannot be described as "clinically well-tolerated." Lenses which have been explanted during

keratoplasty because of endothelium decompensation display a somewhat different pattern of cellular growth than do those of eyes from autopsies. An autopsy eye with an intraocular lens where enough is known about the ophthalmic history is very rare. But even lenses where no incompatibility was known to have been present may have caused a chronic (sub?)clinical foreign-body reaction.

Wolter reported for the first time (1982a, 1982d, 1983c, 1984a; Wolter and Kleberg, 1985; Wolter and Anderson, 1988) on several clinically well-tolerated lenses which had been examined after the death of the patients. The lenses were covered with inflammatory cells. In 1984 Daicker described 20 lenses, 17 of which had been worn without any clinical complications. He had mainly obtained them from autopsies. Even in those 19 cases when there was no clinical evidence of iritis, there was still a mixed cell population with foreign body giant cells on 18 of them. The cells mainly grew from synechias between the iris and the lens. In two autopsy eyes with posterior chamber lenses Bleckmann and Lohoff (1985) found an inflammatory reaction in the vicinity of the haptic and the choroid. Mullaney et al. (1985) found minimal signs of inflammation in four autopsy eyes with clinically well tolerated lenses; cellular lens deposits were not mentioned. Nor were deposits noted by Apple et al. (1987a).

#### *The Cytology of Implants after Keratoplasty*

Most lenses which have been examined cytologically have up to now been iris-supported or anterior chamber lenses which were removed after decompensation of the corneal endothelium (Table 2). These data cannot simply be transposed to cytological findings for posterior chamber lenses. In these cases, complications other than a bulbous endothelial decompensation were rare. However in eyes that were clinically free of irritation, cellular deposits with histiocytes were found in well over half the lenses (Sievers and v. Domarus, 1984; Goder and Völ-



ker-Dieben, 1985; Champion et al., 1985; Puck et al., 1985; Kappelhof et al., 1986b; Cameron et al., 1987; Schmack, 1989). Apple et al., (1989) suggested that corneal decompensation was often due to this inflammatory reaction (p. 227). The corneal edema made it difficult to see into the anterior chamber of the eye, so that even histologically demonstrable fibrin deposits could not be seen in vivo with the slit-lamp (Wolter, 1989c).

In iris-supported lenses the most intensive inflammatory reaction had been in the vicinity of the lens haptics and the pupillary margin (Wolter, 1983c; Sievers and v. Domarus, 1984; Goder and Völker-Dieben, 1985; Champion et al., 1985). While interconnected inflammatory cells were usually found on the haptics, an increased number of isolated cells were found on the optics (Sievers and v. Domarus, 1984; Champion and Green, 1985). Macrophages were found in the vitreous of patients undergoing penetrating keratoplasty for pseudophakic endothelial bullous decompensation (Tuberville et al., 1984). Patients with lenses had significantly more cells in the vitreous than did patients without them. Patients who had undergone an intracapsular operation had just as many vitreous cells as did those who had undergone an extracapsular operation. Giant cell proliferation on the lens surface can extend to the cornea and cause decompensation there (Wolter, 1987a, 1987c, 1989a). Macrophages can cause the vitreous body to fuse with the implant (Wolter, 1983d).

### *The Cytology of Chronic Inflammatory Reactions*

After reviewing more than 2500 explanted lenses, Apple et al. (1989) conclude that inflammation and its consequences are apparently the most frequent cause for the removal of a lens implant or for an enucleation after implantation of a lens. Other authors have reached the same conclusions (Garner, 1989; Nover and Rochels, 1990). Chronic inflammation can be recognized in

vivo early by using specular microscopy, allowing early treatment of this inflammation.

In cases of severe inflammation fibrin exudation may be predominant (Yeo et al., 1983; Wolter, 1989c). The formation of fibrin or the emigration of histiocytes is non-specific. The cytological findings do not allow one to draw any conclusions as to the cause of the inflammation. A lens which had been removed on account of severe inflammation was covered with fibrin, leukocytes, and erythrocytes (Krey and Jacobi, 1978). Kincaid et al. (1982) reported on a patient who from a clinical standpoint had a mild chronic inflammatory reaction with vision of 20/40. The eye was examined at autopsy after the patient died of a heart attack. The inflammatory cells on the lens, which had been seen using a slit lamp, could be confirmed histologically; there were also some signs of a granulomatous inflammation of the iris and cystoid macula edema. Half of the 79 lenses examined by Champion and Green (1985) had a granulomatous inflammation, which was rarely suspected clinically. The prevailing cell types were fibrocyte-like cells, macrophages, multinucleated giant cells, and cells of the iris. In 58% of the cases only the optical portion was affected, in another 25% the haptic was also involved. Only in 17% was the haptic alone involved in the granulomatous process. Cystoid macula edema often occurs in connection with chronic granulomatous inflammation, the edema is the result of the inflammatory reaction (Obstbaum and Galin, 1979; Champion and Green, 1985; Bryan III et al., 1985; Wolter, 1989a). Mullaney and Condon (1985a) described a case of severe postoperative inflammation after postoperative trauma in which the eye had to be enucleated because of sympathetic ophthalmia. The lens itself was covered with histiocytes and giant cells; in addition, lymphocytes were also seen in other parts of the eye. In all 14 lenses examined by Puck et al. (1985), cells were seen which they felt were derived from the iris. From their morphol-

Fig. 3 Cocci-shaped structures on a lens are phagocytized by a spindle-shaped macrophage. Incidental findings after explantation 3 days following the implantation. Scanning electron microscopy.



ogy, however, they were very similar to histiocytes, which have been described by many authors. In culture these cells developed into giant cells, which is an indication of their histiocytic origin. In cases of UGH-syndrome (uveitis, glaucoma, hyphema) erythrocytes can also be shown to be present, as is to be expected (Apple, 1989).

#### *The Cytology of Implants after Infection*

In contrast to the other complications, infections are a less frequent cause of lens explantation (Garner, 1989; Nover and Rochels, 1990). Hence only case reports are found in the literature (Table 2).

From their cytological features infections can, however, be distinguished from granulomatous inflammation. In infections, granulocytes are the most common type of cell on the lenses. Fibrinous deposits occur as well. Isolated macrophages or even epitheloid cells may also be observed. A number of authors agree on an important observation which is also relevant for in vivo diagnosis by specular microscopy, namely, that giant cells do not occur when there is bacterial inflammation on the surface of the lens (Riedl et al., 1985; Wolter et al., 1984, 1985d; Bryan III et al., 1985d; Schemann, 1987; Dilly and Sellors, 1989; Faschinger,

1990). Both Piest et al. (1987) and Apple et al. (1988) described "nonspecific inflammatory cells," but no giant cells. In a case of bacterial inflammation due to *Propionibacterium acnes* sp. which originated in the capsular sac, Sawusch et al. (1989) reported mainly on macrophages. In animal experiments the greater the number of nuclei the more the histiocytes lose the ability to phagocytize staphylococci. In giant cells with more than seven nuclei, this is almost completely lost (Papadimitriou et al., 1975). In another series of experiments macrophages had phagocytized staphylococci, whereas the epitheloid cells no longer possessed the capability of doing so (Papadimitriou and Spector, 1971). Cusumano et al. (1991) reported on five patients who, it was assumed, were suffering from sterile post-operative inflammation. In the cytological examination bacteria were unexpectedly found on all five explanted lenses. All the lenses were covered with membranes and cells. The cells were flat and had an average diameter of no more than 15  $\mu$ m.

Many lenses explanted after infection are not examined histologically but are submitted in toto for culture (Salvanet-Bouccara et al., 1990; Gloor, 1991; Posenauer and Funk, 1991).



A bacterial infection may be followed by a foreign-body reaction to the implant. Thus, Wolter (1983) reported on a case in which 3 days after the operation a bacterial infection appeared; this was successfully treated with antibiotics. However, the bacterial infection was followed by a sterile inflammation, which, 3 months later, made enucleation of the eye necessary. The histologic examination revealed a granulomatous inflammatory reaction with many giant cells on the implant.

A cataract operation can never be carried out under entirely sterile conditions (Dickey et al., 1991). Transitory infiltration of bacteria is a quite frequent consequence of the operation. A clinical example of this is shown in Figure 3. A lens was implanted in an 85-year-old patient in spite of a defect of the posterior capsule. Since the lens was found to be dislocated in the vitreous 2 days later, it was explanted again on the 3rd day. Although there was no clinical evidence of any infection, a routine examination by scanning electron microscope revealed the presence of two collections of cocci on the lens, one of which had just been phagocytized by a spindle-shaped macrophage. Such occurrences are probably often present, but they are discovered by accident. Accordingly it may be one of the many tasks of spindle-shaped macrophages in cases which go well enough, clinically speaking, to phagocytize accidentally implanted bacteria before they produce an infection which manifests itself clinically.

#### *The Cytology of Implants in Animal Research*

Unfortunately the testing of new lens models took place over several decades directly on human beings. Harold Ridley thought of trying out animal experiments. He did not actually carry them out since enough had become known about the biocompatibility of the PMMA from war wounds, and the reaction of animal eyes may be quite different from that of human eyes (Ridley,

1953). In later years, animal experiments still remained the exception (Simpson, 1956; Schillinger et al., 1958; Galli, 1962). Since the end of the 1970s animal models or cell culture have been used to test the biocompatibility of new devices for implantation surgery. This development was pioneered in D. J. Apple's Center for Intraocular Lens Implantation in Salt Lake City (Utah), later in Charleston (South Carolina) (Apple et al., 1987b). Their efforts extended our knowledge of the cytology of lens implants.

Eifrig (1976, 1977, 1980) did examine the cytological reaction on lens implants in animal experiments. The cells were mainly macrophages, from which epitheloid or giant cells can develop. He also found isolated granulocytes. By administering pilocarpine over a long period or in high dosage, this reaction can be intensified; with adrenocortical steroids it can be slowed down. Chronic iritis or a great deal of cortical residue from the operation can also cause such a reaction. Macrophages were found only on the lens, not on the cornea. Yalon et al. (1984) implanted lenses in seven cat eyes. Scanning electron microscopy of aminopolyamide IOLs revealed "fibroblast growth" on the surface. No fibroblast growth was noted on the surface of HEMA IOLs.

Kappelhof et al. (1986a) examined cytological findings on lenses implanted in seven rabbits which had been enucleated 3 days to 12 weeks after implantation. They found giant cells with lengths of 100  $\mu\text{m}$  as early as the third day after the operation. They were able to confirm the existence of a thin membrane on the lens surface as already described by Wolter (1982b) and assumed that it consisted of fibronectin; it was 1  $\mu\text{m}$  thick. The cells were located on the surface of the membrane. Kanagawa et al. (1990) carried out a similar study on ten rabbits and submitted the implanted lenses for immunocytochemical examination. They found fibronectin only in the cells, not in the surrounding membrane. In the weeks after the operation, the fibronectin production of



the macrophages seemed to decrease constantly.

Ishibashi et al. (1989b, 1990b) implanted lenses in 12 monkeys after extracapsular cataract extraction. The eyes were enucleated 1 day to 2 weeks after the implantation. Using a transmission electron microscope they were also able to confirm the presence of a homogeneous membrane on the lens surface. On this membrane, first granulocytes and macrophages were seen, then a week later macrophages and giant cells were found. The presence of granulocytes could only be demonstrated up to the 4th day; they were fewer in number compared to macrophages. The macrophages adhering to the lens surface turned from round cells into flat ones stretched out across the surface. The macrophages contained finely distributed chromatin, lysosomes, vacuoles, vesicles, rough endoplasmatic reticulum, ribosomes, and mitochondria. The surface had many cytoplasmic branches with numerous lysosomes. Microfilaments helped them adhere to the surface. The membrane lying across the lens contained finely granulated material.

Tamura et al. (1990) compared the cytological findings of IOLs which had been implanted in the anterior chamber without the lens previously having been extracted to others that had been implanted in the posterior chamber after extracapsular cataract extraction. In these cases the cellular inflammation was less on the "anterior chamber lenses." Tamura assumed that lens residues were responsible for the difference. From our own experience, however, it would seem more probable that the additional operative trauma of cataract extraction was the cause of the more intense inflammation. Porter et al. (1991) also found cellular reactions on most anterior chamber lenses which had been implanted in phakic eyes of monkeys. The dominant cell types were histiocytic giant cells, epitheloid cells, spindle-shaped, and other inflammatory cells, and were located mainly in the area of the lens haptic. In rabbits, polymorphonuclear

leukocytes are frequently observed in the aqueous humor immediately after surgery and then rapidly disappear. Initially after implantation neutrophils predominate for the first day and then gradually decrease in number while the macrophage becomes the predominant cell type until the first month postoperatively (Lundgren et al., 1992).

In a study of 30 rabbits hydrophilization of silicon lenses did not bring about any reduction in the giant cells on the surface of the lens (Hettich et al. 1991). Klatt et al. (1988) found that inflammatory changes were intensified in the vicinity of irregularities in the material from which the lenses were made.

The implantation of a lens in the eye of a rabbit produces similar but weaker reactions than does implantation in the peritoneal space of mice (Wolter, 1983b; Tamura et al., 1988). In so doing, Uenoyama et al. (1988a) found that isolated lymphocytes were present as well as histiocytes. Giant cells arise through the fusion of macrophages (Uenoyama et al., 1988b, 1987). In a comparison of PMMA and silicon lenses they did not note any difference between the cell reactions on both materials (Uenoyama, 1990). In animal experiments Bucher and Faggioni (1988; Bucher et al., 1989) found fewer inflammatory cells on silicon lenses than on lenses made of PMMA. The findings of Kulnig and colleagues (1989) were similar. Kamps and Wenzel (1991) implanted lenses of the same design made from PMMA and from silicon into rabbits. The cell density was noticeably higher than would have been expected in human beings. After 13 weeks there was a maximum of 215 small and spindle-shaped cells/mm<sup>2</sup> on silicon lenses, and 220 on PMMA lenses, this difference not being statistically significant. What was significant, on the other hand, was the difference in the case of giant cells, where silicon lenses had a maximum density of 4/mm<sup>2</sup> and PMMA lenses a density of 6/mm<sup>2</sup>.

## Comparison of Specular Microscopy with Other Kinds of Microscopic Examination—Why Specular Microscopy?

### Specular Microscopy—Slit-Lamp Microscopy (Focal Illumination)

Examining the lens in the specular area provides clinically relevant information which corresponds in importance to that of other findings using slit lamps. If it is deemed necessary to examine a patient with the slit-lamp after implantation of a lens, then the lens should be examined not only in focal, but also in specular illumination.

### Disadvantages of Specular Microscopy

The greatest disadvantage with examining in the specular area is the fact that the technique is difficult to learn and is therefore not used by many ophthalmologists. It is a major aim of this volume to contribute to eliminating this disadvantage. Under the usual examination conditions a slit-lamp examination will be lengthened by only a few seconds if a lens implant is examined in the specular area as well as under focal illumination.

In most cases, examining the posterior surface of the lens in the specular area does not reveal as much as does examination of the anterior surface. Only the extent of the giant cells on the back surface of the lens can be seen and up till now it has not been possible to determine the limits of the central raised regions. Differentiating giant cells from other, acellular membrane-like deposits on the back of the lens is not possible with any certainty. Additional examination in focal illumination using a slit lamp may be of assistance here, since this also makes it possible clearly to distinguish the central region of cells on the posterior surface of the lens as gray shadows.

Beyond this it is difficult in specular microscopy to determine the focusing plane without concomitant examination in focal illumination. Cell deposits on the front surface of the lens can be mistaken for those on the back surface or even on the cornea. The usually high initial magnification of about 100 $\times$  in commercially available specular microscopes makes an overall picture difficult to obtain. This disadvantage does not apply to examination in the specular area using the slit lamp (Fig. 4).



Fig. 4 Dense growth of giant cells on a lens 3 months after the operation. The cells in the specular areas can be seen more clearly than can those in the areas of focal illumination. (15 $\times$ )



### *Advantages of Specular Microscopy*

The disadvantages of specular microscopy pointed out above are not of great importance, since specular microscopy is not so much an alternative to slit-lamp microscopy as an often essential component of slit-lamp microscopy itself. In principle, there is no difference between a specular microscopic examination and a slit-lamp examination, as long as the slit lamp is also used for examination in the specular area (cf. Chap. 2). Usually, the slit lamp is used with lower magnifications and with focal illumination. The picture obtained with specular microscopy is, in principle, quite a different one from that obtained with focal illumination, and it can thus provide additional information.

All cellular deposits on a lens can be seen in much greater detail and contrast using a specular microscope than they can with the slit lamp under focal illumination. Most cells cannot be seen at all unless specular microscopy is used! (Fig. 47).

In focal illumination, small and spindle-shaped cells are only visible as faint, reflecting marks if they are examined close to the specular area (Oak and Laing, 1983). Outside the specular area phagocytic pigments in the cells do of course remain visible in focal illumination, but differentiating them from free pigment is barely possible (Wolter, 1982a; Wenzel and Hahn, 1988; Ishibashi et al., 1990a). Differentiation between erythrocytes and melanin-containing cells remains uncertain in focal illumination.

Giant cells with a large cytoplasm are likewise noticeable in focal illumination only because of their small central clusters of pigment. The experienced observer can sometimes also make out the outline of the cell as a fine margin of pigment. Fast and sure recognition in the case of these cells is only possible through examination in the specular area.

In focal illumination with the slit lamp, it is only large giant cells with many nuclei that can be seen with any certainty as gray stains

(Wolter 1985a, 1985b; Wenzel and Reim, 1987). This type of cell is also known in the form of "mutton-fat deposits" on the back surface of the cornea. Figure 4 shows an example. Three months after an uncomplicated cataract operation the lens was thickly covered with giant cells, and vision is reduced to 0.3. Whereas in focal illumination the cells can only be made out as gray-brown stains, in the specular area the cell structure can clearly be seen. The specular microscopic picture may be very similar to that obtained by retro illumination, but the contrast is worse than with examination in the specular area. If examination by means of specular microscopy happens not to be possible, one can have recourse to retro illumination. This is sometimes necessary, particularly in the case of experiments on animals. With the aid of laser-scan microscopy or with ultrasonic microscope examination, it is possible to describe cellular growth without examinations in the specular area (Rochels et al., 1989; Rochels and Ziegler, 1989). This will, however, technically speaking, always remain a more complex procedure than examination in the specular area.

### **Specular Microscopy In Vivo— Microscopy of Explant Material**

If it is accepted that explanted lenses should be examined microscopically, it is all the more important that this should be done in vivo. It would be nonsensical to devote one's attention to the cytological and pathological findings of explants without at the same time being interested in the cytological and pathological findings of the patients one is caring for.

### *Disadvantages of Specular Microscopy*

Specular microscopy requires a clear view. Many serious complications which may render the explantation of a lens necessary accompany clouding of the refracting media, and this may mean that specular microscopy is either not possible at all or only to a



limited extent. For this reason it is not possible, particularly in severe clinical cases, to fully exhaust the diagnostic possibilities *in vivo*. This disadvantage is not so serious, however, since by means of regular examinations with specular microscopy pathological developments can be recognized and treated early before irreversible damage is done to the corneal endothelium. Also, a fibrinous reaction in the anterior chamber of the eye makes specular microscopy evaluation of the lens surface much more difficult or even impossible.

Not all sections of the lens are accessible to examination by specular microscopy. The optics elude an examination of this type only when badly tilted. With synechiae of the iris only the areas of the lens left free by the pupil are visible. But it is precisely in the vicinity of the synechias that the maximum cell densities are to be expected. Depending on their positions, the haptics of anterior chamber lenses and iris-supported lenses can be examined with specular microscopy only to a limited extent. But it is the haptics of these lenses in particular where inflammatory reactions most frequently tend to occur. In the case of sulcus-fixated posterior chamber lenses, the haptics cannot be examined with specular microscopy. With modern operating techniques, however, this limitation has lost in importance. With capsular fixation the lens optic of an implant is the primary location of inflammatory reactions. The haptic loops adhering to the capsular sac are no longer affected by the inflammation (Eifrig and Doughman, 1976; Champion, 1988). It is for this reason that in cases of capsular fixation the inflammatory reaction on a lens implant can be adequately understood by means of an examination using specular microscopy. The concomitant inflammatory reaction of the uvea is less with capsular support than with all other implantation methods of lens implantation (Apple et al., 1985a).

Since in specular microscopy the cells can only be observed when not stained, the only details that can be seen are those which

attract attention through some optical difference from their environment. In microscopic examination of stained preparations the number and structure of the cell nuclei is important. Even spindle-shaped cells can have two nuclei or can overlap (Fig. 5). In many cells the nucleus cannot be precisely defined by specular microscopy. Thus it is often the case that differentiation between lymphocytes, granulocytes, and macrophages is unfortunately not possible *in vivo*. Nevertheless, criteria for determining the dominant cell type in a cell population can be found. In specular microscopy, macrophages attract attention because of their longevity and their differentiation potential (cf. Table 10). In specular microscopy the maximum resolution is about 100 $\times$ . Beyond that, it is now possible to carry out immunofluorescent examinations *in vivo*, which in principle can also be applied to cells on a lens implant (Scheiffarth, 1989).

### *Advantages of Specular Microscopy*

All the inadequacies which examinations using specular microscopy entail and which we have listed above are rendered less serious by the fact that specular microscopy does not hinder examination by other means. Cytological techniques otherwise change the

Table 3 Particularities of specular microscopic examination

#### **Disadvantages**

- Depends on clear anterior ocular media
- No view behind posterior synechias
- Lens haptic cannot be evaluated
- No recognition of details, which only become visible on staining
- Low resolution

#### **Advantages**

- Combination possible with all other techniques of cytological examination
- Artifact-free examination
- Noninvasive technique of examination
- Observation of course of disease possible
- Basic research also possible on humans



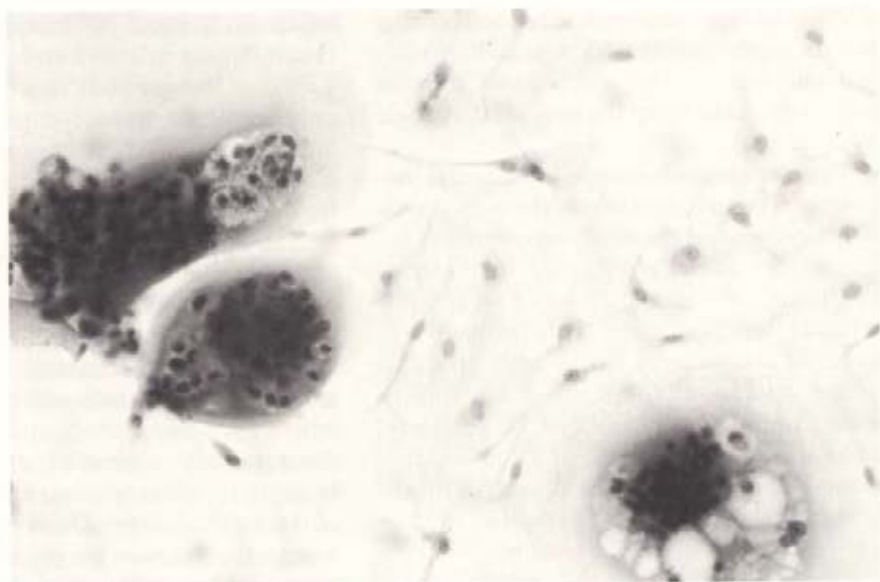


Fig. 5 Spindle-shaped macrophages and multinucleated giant cells on a lens 3 years after implantation. Papanicolaou. (300 $\times$ )

cells to be examined so much that no further staining is possible. Examination with a scanning electron microscope remains possible only after an examination with light microscopy (Stacholy et al., 1989). But after scanning or transmission electron microscopy, light microscopy is no longer possible. After H-E staining of the cells there is no point in any other staining. After an immunocytochemical examination, examination with other antibodies is much more difficult. Only specular microscopy may be repeated at will and may be combined with all other techniques of cytological examination.

It is also true that only specular microscopy guarantees artifact-free examination. The ability of all other techniques of cytological examination to provide meaningful information is limited, because the manipulations involved in explantation and processing of the lens causes severe changes. Just because a pathologist finds no cells on a lens does not mean he can assume that there were none in vivo. The explantation of in-

traocular lenses may be difficult to perform and the necessary manipulations may alter the cytological findings on the lens surface. Cells may be wiped off or they may adhere to the lens due to changes caused by death or by the removal process itself (Wolter et al., 1982c, 1984a, 1985; Wolter et al., 1985; Kappelhof et al., 1986a). Difficulties of explantation occur with some anterior chamber lenses, but in particular with posterior chamber lenses after capsular fixation. Since the loops usually adhere firmly to the capsular sac, cytological examination of a capsular fixated lens succeeds either when the loops are cut off on explantation or when the capsular sac is also removed (Apple and Kincaid, 1985; Isenberg et al., 1986; Dilly, 1989). But cells may also be lost during the preparation of a cytological examination (Rochels and Stofft, 1987; Wolter, 1989b).

In spite of the relatively low resolution in specular microscopy, it has been possible to use the process to discover irregularities in the manufacture of diffractive lens implants

which had not attracted attention during routine examination with a scanning electron microscope, but which could only be found after examining the lens at a flat angle (Wenzel et al., 1992a).

The decisive advantage of specular microscopy is that it constitutes the only noninvasive cytological examination technique.

Some examples may serve to illustrate this. If after the implantation of an anterior chamber lens a patient with incipient endothelial insufficiency has to undergo a corrective operation, there are three choices open to him or her: isolated keratoplasty, isolated lens explantation, or a combination of the two operations. The operative procedure can be planned after a specular microscopy examination. If there clearly is an inflammation reaction on the implant and if there are sufficient endothelial cells, the explantation of the lens or long-term cortisone therapy will suffice. Where a posterior chamber lens has been firmly inserted and there is no inflammation, but the number of endothelial cells is too low, keratoplasty of the implant will suffice. Only where the endothelium has been irreversibly destroyed and there is an inflammatory reaction on the implant keratoplasty combined with lens explantation must be carried out.

In chronically recurrent inflammations after lens implantation it is possible to use

specular microscopy to differentiate between benign infective endophthalmitis and a chronic foreign-body reaction. If there are only small or spindle-shaped cells on an implant, an infection is probable. If many giant cells appear on the lens, one is confronted with a foreign-body reaction.

The advantages of a noninvasive examination technique offer, in addition, an opportunity unique in anatomy and pathology—that of carrying on cytological observations of the course of a disease in individual human cells without any manipulation. Previously, every attempt to describe the temporal course of an inflammatory reaction to a lens implant failed for reasons of the methodology. Thus, we are unable to accept the courses suggested by some authors (Schlote et al., 1988; Bryan III et al., 1985). The way the course of a disease differs from patient to patient is too great.

Furthermore, for the purposes of anatomical and pathological research there is the advantage of being able to examine clinically well-tolerated human material to an almost unlimited extent. It was always a weak point in the evaluation of explanted lenses that too little was known about the normal, uncomplicated processes of healing after the implantation of lenses.



## 2. Techniques of Examination

### Physical Preconditions

When light strikes a medium which has a different index of refraction, one part is refracted and allowed to pass through, another part is absorbed, and a third is reflected or scattered. The respective proportions vary with the density of the medium and the quality of its surface. The reflected light may either be returned diffusely or reflected specularly, the specularly reflected light may be polarized. Diffuse reflection, diffraction and absorption are also possible within any medium; the process may be limited to individual pencils of rays. A specular reflection, on the other hand, is a phenomenon produced by a large pencil of rays which strike a surface in parallel and it occurs only on even interfaces, whose irregularities are significantly smaller than the wavelength of the light.

The refractive index of a medium reflects the reduction in the speed of light within a given material. Thus, light is 1.333 times slower in water than it is in air. Table 4 shows the refractive index ( $n$ ) for some of the media important in specular microscopy (Duke Elder, 1970; Zeiss, 1977; Hecht, 1987; Tighe, 1989).

The greater the difference between the refractive indices of two media, the more light is reflected and the less light is allowed to pass through. If  $n_1$  and  $n_2$  are the refractive indices of two adjacent non light absorbing media, in case of vertical incidence the reflectance ( $R$ ) is calculated as

$$R = \left( \frac{n_1 - n_2}{n_1 + n_2} \right)^2$$

Whether the ray of light comes from the medium of higher or lower density does not

affect the reflectance. In the case of an implanted lens, the reflection on its front surface (aqueous/PMMA) is, in principle, just as large as that on the back surface (PMMA/aqueous). To facilitate orientation, the reflectance on various ocular interfaces calculated according to the above figures have been listed in Table 5.

These figures are only rough approximations, since the reflectance also depends on the wavelength and the angle of incidence of the light. Blue light is more strongly reflected than red. However, in case of biological fluids and tissues, the dispersion of  $n$  is very low. The figures below are valid for light

Table 4 Refractive indices ( $n$ ) of optical media

Air	1.000
Water	1.333
Crown glass	1.518
Flint glass	1.613
Tear film	1.336
Cornea	1.376
Aqueous	1.336
Lens (cortex)	1.375–1.387
Lens (nucleus)	1.403–1.409
PMMA	1.48–1.495
Silicon	1.409–1.44
HEMA	1.39–1.44
Vitreous	1.336

Table 5 Reflectance of various ocular interfaces

Air to crown glass of spectacles	4.23%
Air to tear film of the cornea	2.07%
Endothelium-aqueous	0.02%
Aqueous-human lens	0.03%
Aqueous-PMMA IOL	0.28%
Aqueous-silicon IOL	0.13%
Aqueous-HEMA IOL	0.13%

which enters vertically. When the angle of incidence is oblique, the light may be more strongly reflected if it strikes a medium which has a lower index of refraction. Since the interfaces listed above are mainly optically curved, the reflection may be more influenced by the curvature than by the refractive index of the media. And finally, a thin coating can change the reflectance—a fact which is put to use in coating spectacle lenses.

In the case of specular microscopy, what this basically means is that the brightest ocular reflection occurs at the interface of air and the eye. The reflection of an implanted PMMA lens is more than  $10\times$  less; the reflection of the human lens or of the endothelium is more than  $100\times$  less. Of the different materials used in implantation, the reflection of PMMA lenses is about twice as intense as that of silicon or HEMA lenses.

## The Specular Areas of a Lens

### Definition of Terms

In some respects a *specular area* is similar to a *mirror image*. A simple, everyday example will suffice to illustrate the difference: if we look into a mirror from about half a meter away, we see our own face—but it appears to be a meter away. Small impurities on the mirror will not be noticed at all. If, however, we study the surface of the mirror very closely, our own reflection goes blurred, but then we can make out small specks of dust or dried drops of water on the mirror, in the “specular area.”

If a ray of light is shone onto a lens in the eye from an arbitrary source, two *specular images* are visible to the naked eye in the form of Purkinje images 3 and 4. One of them is reflected off the front surface of the lens, the other off the back. These specular images reveal the source of light. In former times this would have been a candle; nowadays it tends to be the filament of an incandescent lamp. Specular images may be virtu-

al images that appear to hover in space behind or in front of the lens.

The *specular area*, on the other hand, is not the reflection itself but the area of the lens where the light is specularly reflected. Specular reflection and specular area are derived from the same rays of light, only the plane of focus is different. If the specular reflection is brought into focus, the specular area on the surface of the lens will seem out of focus. If the specular area on the surface of the lens is focused on, then the specular reflection will seem completely out of focus. This implies that the specular area is largely independent of the form of the light source. As its unsharp reflection, it is not sharply defined, but the light is attenuated toward the edge. *Specular microscopy* is the microscopic examination of an object in the specular area.

## Three Varieties of Specular Microscopy

### The Significance of the Plane of Focus

Both the anterior and the posterior specular reflections of a lens (Purkinje images 3 and 4) are suitable for specular microscopy. The planes of focus are, in the former case, the anterior specular area, in the latter, the posterior. Since in specular microscopy the optical depth of field is low, different variants of illumination may arise when the focus is changed. This allows for a third possibility of carrying out specular microscopic examinations, which is to examine the anterior surface of the lens in the light reflected off the posterior surface. In this case the plane of the specular reflection does not coincide with the plane of focus.

The optical path is shown in Figure 6. The light entering from the slit lamp is reflected on both surfaces of the lens. The anterior specular area (aS) arises out of the 3rd Purkinje image, while out of the 4th Purkinje image arises the posterior specular area. If the front surface of the lens (Focus 1) is brought into focus, the direct anterior specular area (aS) can be examined with the microscope. The front surface of the lens



can also be illuminated by the light reflected off the posterior surface (pS). In this way the anterior lens surface (Focus 1) can be examined both directly in the incident light and in the second case, indirectly in retroillumination. In the case of the posterior lens surface, only reflected light microscopy is possible (Focus 2); the specular area of the anterior lens surface remains completely out of focus and tends to be something of a nuisance.

The two ways of illuminating the anterior surface of the lens may overlap during examination (Figs. 7 and 9), but mostly, depending on the angle between axes of illuminating and imaging beam, they are separate and adjacent to one another. (Figs. 9 and 17).

#### Reflected Light and Transmitted Light Microscopy

(Reflected direct) light microscopy is used primarily to examine surface structure. It creates an image which appears to be in relief. Irregularities form dark shadows in the specular area. Elevations may be mistaken for depressions if the direction of the illumination is not known. (Transmitted indirect) light specular microscopy is used to examine transparency. The image does not appear in relief. It ensures sensitive recording of differences in transparency and color, even if these are not associated with any prominence. The resolving power of both varieties of illumination is higher than with focal illumination using the slit lamp (Figs. 4 and 47).

The effects of different methods of illumination are demonstrated in Figures 8 and 9. Both photographs in Figure 8 show the cytological findings on the front surface of an anterior chamber lens implanted 2 years earlier. In Figure 8a a large giant cell can be seen lying at the edge of the specular area. On the right and on the left edges of the cell, extensive raised vesicular structures can be seen. The surface appears to be in

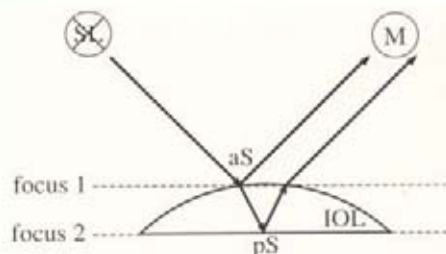


Fig. 6 The light from the slit lamp is reflected on the front side of a lens (IOL) in the anterior specular area (aS) and on the back side in the posterior specular area (pS). The posterior specular area (pS) can be brought into focus by the microscope on two planes (Focus 1 and Focus 2).

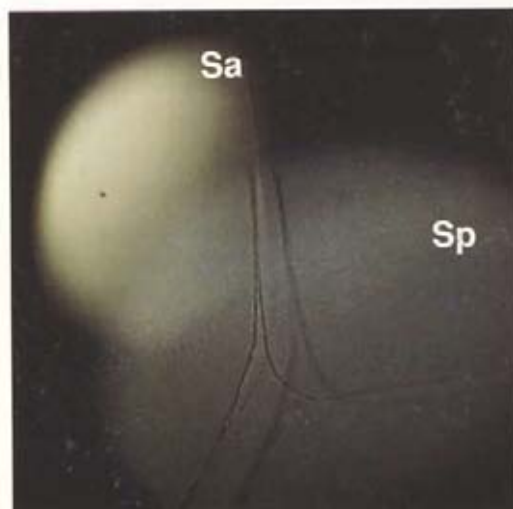


Fig. 7 Superposition of two specular areas of a planoconvex lens with a thin fibrinous strand with the front surface in focus. The specular area of the anterior surface (aS) is smaller and bright; the posterior specular area (pS) is larger and darker. The shadow of the strand can be seen in the posterior specular area. (70 $\times$ )

relief without any details of the cell content being visible. An adjacent cell has a drop-shaped branch. The same cells were transilluminated in Figure 8b. The reflection is more regularly illuminated. The wavy surface of the cell can no longer be made out; in its place are embedded brown pigmentations. The giant cells in Figure 9 have been

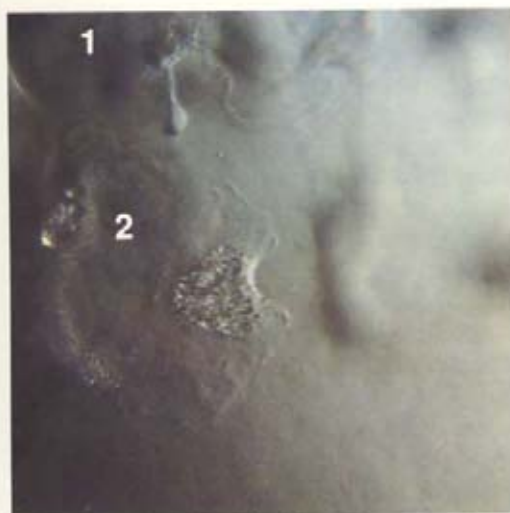


Fig. 8a Giant cells (1, 2) on an anterior chamber lens in reflected light microscopy; the surface of the cell appears in relief. (70 $\times$ )

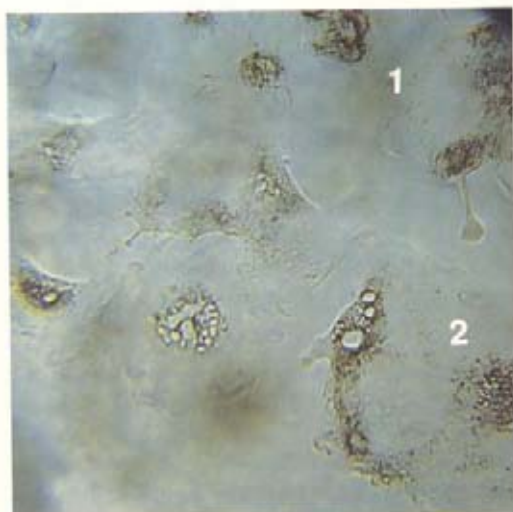


Fig. 8b In transmitted light microscopy; the brown pigment of the cell content of the two giant cells (1, 2) can be clearly seen; the cell outlines are less well contrasted. (70 $\times$ )



Fig. 9 Giant cells and spindle-shaped cells in direct reflected light microscopy and indirect transmitted light microscopy. The cytoplasm of the giant cells (gc) appears gray when reflected, but transparent when trans-illuminated (upper part of the cell). (70 $\times$ )

illuminated using both methods. While the lower portion appears gray under direct illumination, the outline of the cell of the

upper portion under indirect, more attenuated light cannot be seen so easily.

Examination under retroillumination could also be called indirect lateral examination, according to Vogt's definition.

### *An Example for Practice*

Examination in the specular area creates conditions which are not observed with other examination techniques. The theory outlined above can be simply demonstrated by marking a non sterile IOL with india ink. In this way, inexperienced operators can develop a certain skill in the techniques of specular microscopy.

With the naked eye the mark can best be seen against a bright background. Where the contrast with the background is high, it can also be easily recognized using the slit-lamp in focal illumination. This is why a piece of paper was held behind the lens in Figure 10a.

Examination of the anterior surface in reflected specular light, makes the same mark bright and shiny against a somewhat





Fig. 10a A black India ink mark on a PMMA lens in focal illumination. White paper behind the lens serves to improve contact (50 $\times$ )



Fig. 10b In reflected light specular microscopy the black mark appears bright and shining. Wiping marks from the felt pen look like scratches. Dust particles deposited appear black. (50 $\times$ )

darker background (Fig. 10b). The color can no longer be determined. This reversal of brightness occurs because in the area that is optically dense more light is reflected than in the transparent areas. Minute irregularities in the mark made by the felt of the pen make it look as though it is in relief.

The situation changes if the mark is transilluminated using the light reflected off the posterior surface of the lens (Fig. 10c). The mark appears as a strong, transparent brown image. Fine irregularities in the thickness of the line can cause an interference pattern forming Newton's rings. This phenomenon also occurs with vivo examinations (Figs. 12b, 25, 26, 32). If the optical properties of all the media involved are known, the thickness of the layer may be calculated (Okada, 1991). The thickness lies between 0.2  $\mu\text{m}$  and about 4  $\mu\text{m}$ .

#### *Particular Features of Clinical Specular Microscopy*

During clinical examinations, determining the plane of focus may be difficult. Yet this is the most important step to be able to correctly interpret the findings obtained using specular microscopy.

If one has a clearly focused picture of a biconvex lens, it may be difficult to tell



Fig. 10c In transmitted light microscopy the mark appears to be brown and transparent. Differences of thickness in the nanometer range give rise to Newton's rings. Wiping marks left by the felt pen look like scratches. (50 $\times$ )

whether the front or the back surface of the lens was in focus unless the plane of focus is already known. This has consequences for the interpretations of the results, since cellular deposits only rarely occur on the back surfaces of posterior chamber lenses. In specular microscopy a technically perfect picture which offers no evidence of any cellular deposits can be obtained, even though the front surface of the lens is thickly co-



Fig. 11a Small spindle-shaped cells and a fibrinous filament on a posterior chamber lens. Transmitted light microscopy of the anterior surface. On the periphery the remains of the anterior capsule are slightly out of focus. The shadow of the fibrinous filament on the posterior surface has not been brought into focus. (70 $\times$ )



Fig. 11b The same lens. Reflected light microscopy of the posterior surface. The shadows of the cells and of the filament are in focus, the deposits on the anterior surface are out of focus. (70 $\times$ )

vered with cells, when the posterior surface is focused on.

Certain limitations apply to the foregoing discussion. In normal circumstances it is assumed that the incoming beam of light runs exactly parallel. In such an ideal case all irregularities in the optical path would be sharply projected onto lower-lying surfaces. With specular microscopy *in vivo*, cellular deposits on the front surface of the lens can only rarely be seen as sharply contoured shadows on the back surface of the lens. Such an examination is shown in Figure 11. In Figure 11a the anterior lens surface is illuminated off the posterior specular area. The remains of the anterior capsule are on the periphery. Toward the center a fibrinous filament may be noticed, surrounded by smaller inflammatory cells. Next to this filament its shadow may be made out indistinctly on the posterior surface of the lens. If this shadow on the posterior surface is now brought into focus (Fig. 11b), it will appear

just as sharply defined as in the anterior plane; all the cells around the filament are also projected as sharply defined shadows onto the back surface. The filament itself and the remains of the anterior capsule are out of focus.

Such shadows on the back surface of the lens are seen only rarely. In many cases the incoming rays of light do not proceed exactly parallel from the source of illumination. For this reason, anterior deposits appear blurred when projected backwards. In Figure 12a, for instance, there are many collections of cells on the anterior surface of the lens. The deposits on the back side appear out of focus. In focusing on the same area of the posterior surface of the lens (Fig. 12b) the cells of the front side can be perceived only as blurred shadows. Gray marks, which look rather like giant cells, are predominant on the specular microscopy picture of the posterior surface.



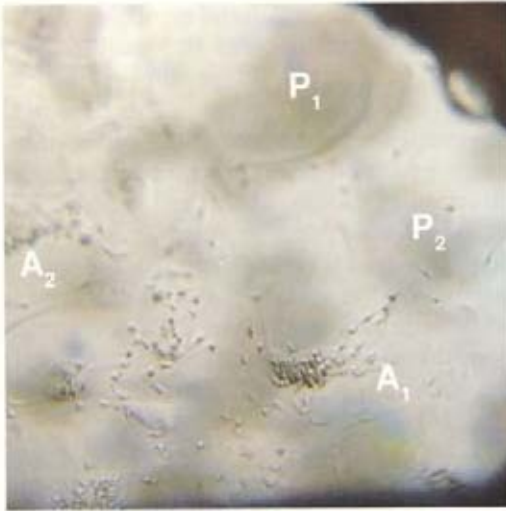


Fig. 12a Collections of cells (A1, A2) on the anterior surface of a lens in transmitted light microscopy. The deposits on the posterior surface are not focused on here (P1, P2). (70 $\times$ )

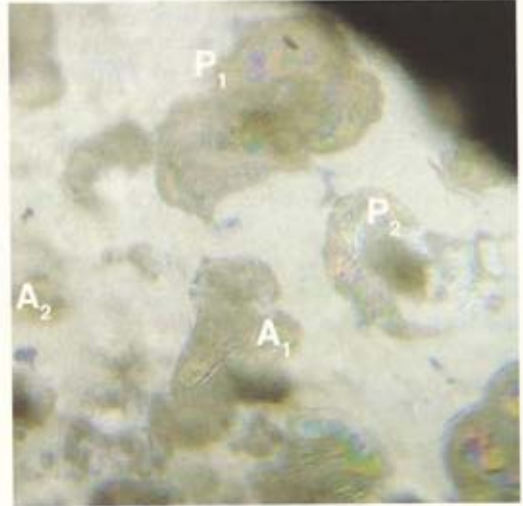


Fig. 12b The same lens. Reflected light microscopy of the posterior surface. Its deposits (P1, P2) are in focus, the cells of the anterior surface (A1, A2) can be seen as blurred shadows. (70 $\times$ )

### Influence of Optical Curvature on Specular Images

Specular microscopy of lenses is complicated by the fact that the reflecting surface is rarely flat and it is mostly curved. Only when light is reflected off a flat surface are the angle of incidence and the angle of re-

flection of all rays the same. If the light from a slit lamp (Figs. 13 and 14) strikes curved surfaces, it will be reflected in a concentrated or in a diffuse manner. Since different areas of a lens are at different angles to the entering light, it is not possible to examine the whole surface of a curved lens in the

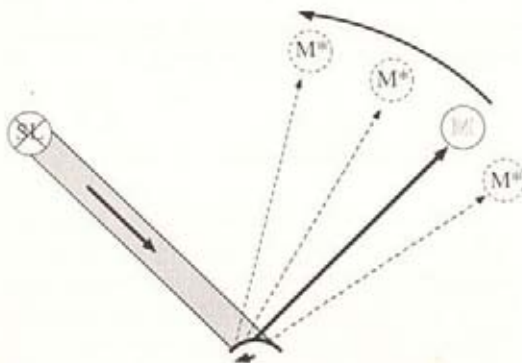


Fig. 13 Light entering parallel is divergently reflected on the anterior surface of a convex lens. Using the microscope (M), only the specular area of one part of the anterior surface of the lens can be examined. If the microscope (M\*) is moved, other specular areas of the lens may be seen.

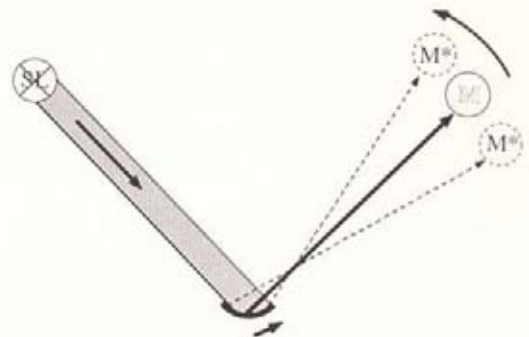


Fig. 14 Light entering parallel is first reflected convergently on a concave surface, then collects at a focal point before diverging with the image in reverse. With the microscope (M), only the specular area of part of the anterior surface of the lens can be examined. If the microscope (M\*) is moved, other specular areas of the lens may be seen.

specular area at one time. If the whole surface of the lens is evenly illuminated, the specular area will be limited to a small area of the lens surface. Adjacent areas will reflect the light in a different direction. By turning the incident light, one can get other sections of the lens to light up in the specular area.

Deposits on the surface of the lens will appear the same size in the specular area, independently of whether the lens is flat, concave, or convex. The optical refractive power of the lens only exercises an influence on the size of the specular area.

In the case of a biconvex lens the anterior surface of the lens functions as a convex mirror. The optical effect of a convex mirror corresponds to that of a concave, or diverging lens. The posterior surface of a biconvex lens functions as a concave mirror. The optical effect of a concave mirror corresponds to that of a convex, or collective lens. While the focal length of a lens depends on the optical density ( $n$ ) of the limiting optical media, the focal length of specularly reflecting surfaces do not.

### *Distance of Examination*

There are significant differences between the specular areas of convex and concave mirrors. The reflected image and therefore also the specular area of a convex mirror (Fig. 13) are reduced in size, but remain upright. The more the front side of a convex lens is curved, the smaller the specular area becomes and the further the microscope must be turned to allow for the examination of all its specular areas.

In concave mirrors the position of the focal point affects the specular areas (Fig. 14). The posterior surface of biconvex lenses functions as a concave mirror. In the area between the focal point and the surface the reflected image appears magnified and remains upright. The closer it comes to the focal point, the larger the reflected image, and therefore also the specular area appears. At the focal point itself all parallel

entering rays meet and the whole lens lights up in the specular area. Behind the focal point the reflected image appears upside down and laterally reversed. This does not, however, alter the specular area itself. The image inversion does, however, change the direction of motion of the reflected image. If, when observing in front of the focal point, the lens is rotated upward, the specular area on the surface will shift downward. If the lens is rotated upward when observation takes place behind the focal point, the specular area will also shift upward (Fig. 14).

Directly behind the focal point the specular area still appears magnified. The further from the focal point examination takes place, the smaller both the reflected image and the specular area become. Beyond the radius of curvature of the mirror, the reflected image of the source of illumination becomes smaller than it would on a flat mirror.

To enable further calculations to be made, the focal length ( $f$ ) of lens surfaces acting as curved mirrors must be known. A working approximation is half the radius of curvature. If the radius of curvature ( $r$ ) is 10 mm, then the focal length ( $f$ ) will be 5 mm. The radius of curvature of one side of a lens can be calculated if the refractive power (measured in diopters) is known and if  $n_1$  and  $n_2$  are the indices of refraction for the materials on question (Table 4):

$$r = \frac{n_1 - n_2}{\text{lens refraction (dpt.)}}$$

From this, one may calculate approximately whether the focal point of the posterior specular area of biconvex intraocular lenses is situated in front of the cornea (Table 6). Often it is situated in the eye.

To calculate the focal point of specularly reflecting surfaces of artificial lenses in vivo, it is necessary to take into account—in addition to the data given in Table 6—the fact that the rays of light will also be bundled by the cornea or, in biconvex lenses, possibly



Table 6 Position of the focal point of specular reflections from the curved posterior surfaces of planoconvex lenses (in front of the lens, in mm)

Refractive power in water	PMMA lens (n = 1.49)	Silicon/HEMA lens (n = 1.42)
5 dpt	16	9
10 dpt	8	5
15 dpt	5	3
20 dpt	4	2
25 dpt	3	2

Table 7 Magnification contribution of structures on the IOL by the cornea depending on the depth of the anterior chamber

Distance of the IOL from the cornea	magnification contribution
2 mm	11%
3 mm	14%
4 mm	20%
5 mm	26%
6 mm	33%

even by the anterior surface of the lens, so that the focal point in the eye will be even closer to the lens (see below).

This means that in specular microscopy even where the working distance of a microscope is short and the posterior surfaces of lenses are barely curved, the specular area almost always has to be examined behind the focal point.

#### *The Effect of Corneal Curvature on the Specular Areas*

In the above calculations the curvature of the cornea was not taken into account. It does, however, have an effect on the focal point of specular reflections of lenses, and in examinations of lens surfaces it functions like a magnifying glass.

The deeper the anterior chamber, the greater the magnification of lens deposits. The magnifying contribution by the cornea ( $V'$ ) to the overall magnification of the microscope can be approximately calculated according to the formula

$$V' = \frac{f'}{f' - s},$$

where " $f$ " is the focal length of the cornea. If the refractive power is 42 dpt, it is about 2.4 cm. " $s$ " is the distance of the lens behind the cornea. The magnification of intraocular structures by the cornea can be seen in Table 7.

Vogt (1930, p. 9) had arrived at very similar values when measuring the size of intraocular needles in autopsy eyes.

Curvature of the cornea also means that the rays are bundled. This magnifies the specular area on the anterior surface of biconvex lenses, whereas the specular area of the posterior surface of a biconvex lens, which functions as a concave mirror, is reduced in size. In the case of the anterior surface of a biconvex lens with a refractive power of about 4 dpt in water, rays striking the cornea in parallel would be reflected back, off the lens, in such a way that they are almost in parallel when they leave the eye. Thus this type of lens has similar reflecting properties to a flat surface in space. In specular microscopy, the whole anterior surface of this lens is lit up (given that the illuminating slit is wide enough). Implanted artificial biconvex lenses whose front surfaces are curved by less than 4 dpt even function as weak concave mirrors due to the bundling of the incident rays of light by the cornea. In the case of the anterior surfaces of convex lenses in vivo, at least another 4 dpt must be added to the values quoted in Table 6 in order to allow for the data on the location of the focal point of the lens: the focal point of an *implanted* PMMA lens whose back surface has a refractive power of 11 dpt is about 5 mm away. The precise value depends among other things on the depth of the anterior chamber, the radii of the cornea and, in some cases, on further bundling if the focal point is in front of the cornea.

### *The Size of the Specular Areas*

Any convex or concave surface reflects a round specular area which seems brighter at the center than at the periphery (Figs. 4, 7, and 18). The size of the specular areas depends on the slit aperture or on the size of the illumination diaphragm. A large aperture yields a large specular area. Looking at photographs taken with specular microscopy, it is possible to distinguish the anterior specular area from the posterior area by its shape, provided one knows whether the lens used was planoconvex or biconvex, since the shape and brightness of both specular areas of a lens differ.

In *planoconvex lenses*, where the flat surface is at the back, the anterior specular area is bright and smaller, *the posterior is darker and larger* (Figs. 7, 9, and 18). In *biconvex lenses* with similar radii of curvature *the anterior specular area is larger but darker* than that of the posterior surface.

A strong curvature of the lens does have the advantage for the examination of making it easier to catch a specular area (see pp. 42). If the lens is only minimally tilted, the specular area of flat surfaces will not light up in the initial position. With optically curved surfaces, however, tilting to a slight degree will only cause the specular area to light up in the peripheral portions of the lens rather than in the center. Thus, in locating a specular area, a lens with a pronounced curvature is relatively insensitive to tilting. If a lens with a strong curvature were to be tilted upwards by about  $10^\circ$ , the specular area would light up in the lower peripheral area circumscribed, where the curvature is just  $10^\circ$  to the optical center of the lens. The specular area is very small in lenses with a strong curvature, however, and it would not permit a survey of the lens surface. More difficult to locate but more revealing in what they have to tell us are the large specular areas. With biconvex lenses this is mostly the anterior specular area, with planoconvex lenses (where the flat surface is at the back) the posterior.

### **Summary of the Clinical Consequences**

For specular microscopy the most important conclusions to be drawn from the above may be summarized as follows:

1) The anterior surface of a lens can be examined using direct specular reflection and by means of the specular reflection from the posterior surface of the lens.

2) In lenses with strong optical curvature *the specular area is smaller* than with lenses where the curvature is less. In biconvex lenses both lens surfaces are less curved than is the curved surface of a planoconvex lens. The diameter of the specular area of the curved surface of a planoconvex lens is thus smaller than the two specular areas of a biconvex lens. Lenses made out of material with a high refractive power (PMMA) have a weaker curvature than do lenses made of soft material (silicon, HEMA). Thus the diameter of specular areas of soft lenses are smaller than those of hard ones.

3) A photograph of the specular area of a curved surface cannot be uniformly illuminated. The center is slightly over-exposed, the degree of exposure then decreases toward the periphery. The less the lens surface is curved, the more evenly it will be illuminated.

4) In planoconvex lenses (where the flat surface is at the back) the anterior specular area is smaller and round, the posterior specular area is larger and not so bright. In biconvex lenses with similar radii of curvature the anterior specular area is larger and darker than the one on the back surface.

### **Slit-Lamp Examination of Patients**

#### **Preparation for the Examination**

Specular microscopy can be carried out on all patients with whom a detailed slit-lamp examination is possible. The patient must be able to hold his or her head and eye steady, which means that this method cannot be applied to small children or to patients suffering from disorders of the central nervous



system, such as untreated Parkinson's disease.

Before the actual examination with specular microscopy, the eye should be examined in *focal illumination* using the slit lamp. The upper lid should not cover the pupil. The motility of the eye must be unrestricted, since the extremely accurate movements of the patient's eye are the prerequisite for finding the specular area and for scoring the lens surface. Only complete *transparency of the cornea* renders perfect specular reflection possible. Any turbidity enlarges the proportion of disturbing diffuse reflected rays of light, which reduce contrast making recognition of smaller structures impossible. Any remaining ointment or mucous deposits should be washed off. Epithelial edema can be reduced by applying hyperosmol to the corneal surface. Prior administration of anesthetics can cause the tear film to break up early, which seriously impairs the quality of the specular reflections. A clinical example of such irregularities of the tear film is shown in Fig. 21.

Examination with *mydriasis* is preferred, since it is easier to perform and it allows examination of the whole lens surface.

While examining the lens in focal illumination, one should watch for findings of relevance to specular microscopy. Posterior synechias of the iris can cause a reaction with foreign body giant cells. If fibrinous deposits are present, the optical irregularity will make it more difficult to carry out specular microscopy in this area. Pigmentation can be an indication of cellular growth. The position of the lens is particularly important. If it lies exactly parallel to the pupillary plane, specular microscopy is easy. Positioning holes or the remains of an anterior capsule can be used as fixation aids and orientation points.

Immediately following focal illumination the lens can be examined in the *specular area* using the slit lamp. For someone with practice this only lengthens the examination by a few seconds, and valuable information will be obtained on inflammatory processes

which cannot be demonstrated in any other way. If no deposits are seen in the specular area using the slit lamp with an enlargement of 20-40 times the natural size, then the technically much more difficult use of specular microscopy with 100-fold enlargement will not be necessary.

### Locating the Specular Areas with the Slit-Lamp

Locating the specular areas with the slit lamp is much simpler than with the specular microscope. To locate the specular areas *enlargement of 20-25 times* the natural size should be selected in such a way as to provide an overview of the whole lens. The slit should be opened to the maximum; the level of illumination can be lowered in order to reduce discomfort to the patient being examined and to enhance his cooperation. Examination in the specular area should be *monocular* in contrast to examination in focal illumination! In a binocular examination the reflections described in what follows would be perceived by the right eye differently than by the left. The specular area can only be seen by one eye, since the angle of observation of the right eye is different from that of the left.

Figure 15 shows the optical path in the examination of an IOL. A small proportion

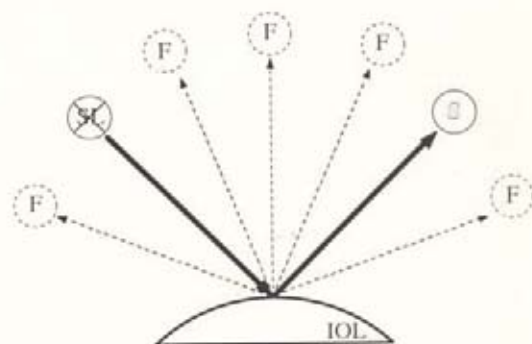


Fig. 15 A lens (IOL) examined using a slit lamp (SL) reflects the observation beam diffusely in all directions and can be seen there in focal illumination (F); specular reflection occurs in only one direction (S).

of the incoming light from the slit lamp (SL) is diffusely reflected off the surface of the lens in all directions. In examinations with the slit lamp this diffuse reflection yields the focal illumination (F). Examination in focal illumination is largely independent of the angle of incidence of either the illuminating or the observing beams. The greater portion of the reflected light is not, however, diffusely reflected, but in a single direction (S). There are many areas of focal illumination but only one specular area.

The sketch in Figure 16 shows how this area can be located with the slit lamp. The direction of view should be upward toward the eye and the refracting unit. Over and above what was shown in Figure 15 some light is scattered diffusely in all directions at the slit itself. In focal illumination of the lens (IOL) a part of this light is reflected back to the microscope. For this reason what is seen in focal illumination is not the slit (S) alone, but also a reflection (R) of it. This reflection is a great irritation to the beginner in slit-lamp microscopy. The more experienced operator will hardly notice it. When the adjustment is poorly done, the reflected image of the light filament can be focused.

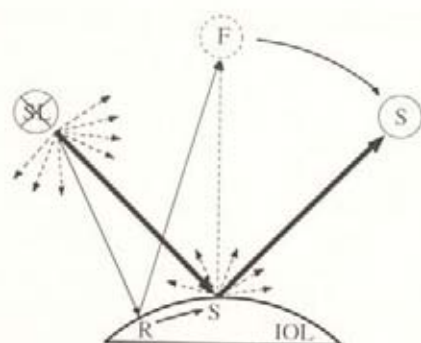


Fig. 16 Some light is scattered diffusely at the slit lamp (SL). It is for this reason that during the examination in focal illumination (F) not only the slit (S) but also a reflection (R) of the illumination source can be seen. If the slit (S) and the reflection (R) are aligned by swinging the observation arm, the specular area (S) lights up.

The specular area is located by swinging the observation arm of the slitlamp sideways. This causes the reflection and the slit to move nearer to one another on the surface of the lens. If the slit (S) is aligned with the reflection (R), the specular area (S) will light up. To do this, the angle of incidence of the illuminating beam must be equal to the angle of reflection of the reflected beam. The best thing is to swing both arms of the slit lamp about 20° outward. A specular microscopic examination with parallel illuminating and observation beams both at 0° respectively would be possible, only then the bright specular areas of the cornea may swamp out the mirror reflection of the lens behind it.

In examining patients the process of locating the specular area can be rendered more difficult by the fact that it has to be sought both in the horizontal and in the vertical planes. Only in the horizontal is it possible to focus on the specular area by turning the illumination or observation arm and without the patient's assistance. In order to align the specular area in the vertical plane the patient has to look straight ahead and the lens has to have been implanted in the eye parallel to the pupillary plane. Where the lens has been fixated in the eye obliquely, for instance in cases where the lower haptic has been implanted in the capsular sac and the upper in the sulcus ciliaris, the upper part of the lens will be tilted forwards. In order to be able to locate the specular area nevertheless, the patient must look up slightly, so that the lens level is exactly parallel to the examination level of the slit lamp.

This procedure may be demonstrated by reference to Figure 17, which shows findings obtained using the slit lamp shortly after the implantation of a posterior chamber lens. If the illumination of the surrounding area is reduced, the slit on the cornea, as well as on the anterior and posterior surfaces of the lens becomes visible. Next to the slit on the cornea (SC) the reflection on the cornea (RC) can be seen; the slit and the corre-



sponding reflection do not coincide. In contrast, however, the slit and the reflection do coincide on both the anterior and the posterior surfaces of the lens. The resultant specular areas (aS and pS) are much brighter than were either the slit or the reflection.

It may very well be that the inexperienced examiner will find it difficult at first to align the reflection of the cornea and the front and back surfaces of the lens with the corresponding slit. In addition there are other, less intense reflections caused by internal reflections between the cornea and the lens which cannot be used for specular microscopy.

After the specular area has been located, maximum magnification may be selected, the intensity of the light further lowered and the width of the slit reduced, without causing a reduction in the specular area. The barely illuminated slit is no longer to be seen on the photograph (Fig. 18). The three specular areas of the eye all light up together. The brightest specular area at the transition from air to the tear film of the cornea (Sc) appears out of focus, since it was the front surface of the lens that was brought into focus. The specular area of the anterior surface of the lens (aS) is in focus and slight-

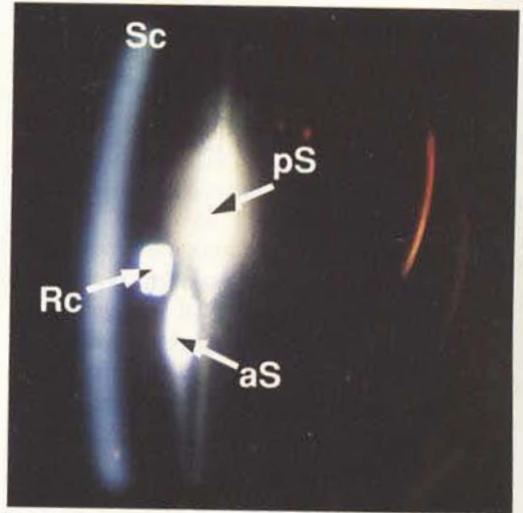


Fig. 17 Slit-lamp findings after lens implantation. On the cornea the slit (Sc) and reflection (Rc) are not aligned. On the anterior and posterior surfaces of the lens, the slit and reflection are aligned, so that the specular areas (aS, pS) are brightly lit. (12 $\times$ )

ly over-exposed, which is why only peripheral cell deposits are to be seen. The specular area of the posterior surface of the lens (pS) is much larger and more uniformly illumi-

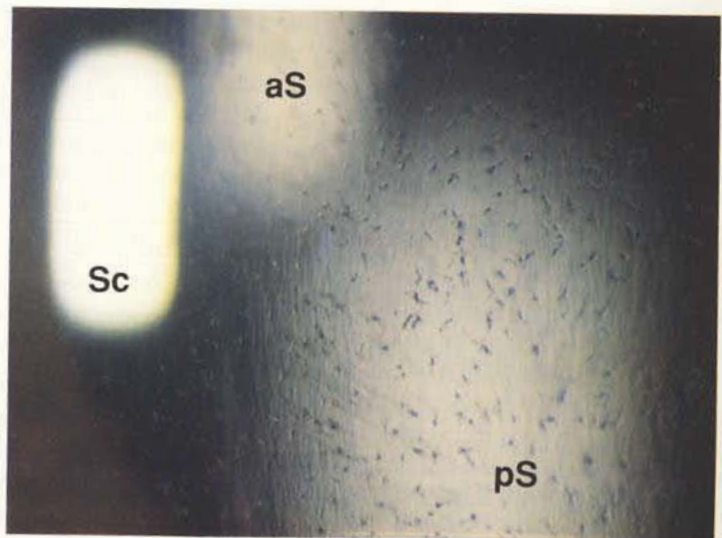


Fig. 18 Specular microscopy with the slit lamp. The cornea specular area (Sc) is out of focus. The anterior surface of the anterior chamber lens is in focus. In the specular areas of the anterior and posterior surfaces (aS, pS), cellular deposits can be seen. (40 $\times$ )

nated. The cells on the anterior surface of the lens show up in transmitted light.

### Searching the Lens in the Specular Area of the Slit-Lamp

Once a specular area has been found, searching the lens there becomes much simpler. To do this, the specular area must be deflected onto other areas of the surface of the lens by changing the angles of the incident and emergent rays of light. This is possible in two ways: either the illuminating or observation apparatus is rotated, or the patient turns his or her eye in such a way that the movement changes the reflective level of the lens. Moving the slit-lamp apparatus is possible on the horizontal plane, which means that the specular area can be made to travel along the horizontal both by moving the apparatus and by movements of the eye. This can be seen in Figures 13 and 14. If, in these illustrations, the microscope (M) is swung to the right or left, it is not the central but the peripheral areas of the lens that will light up in specular illumination. A movement in the vertical plane, on the other hand, can only be produced by the corresponding movements of the patient's eye. The principle here is that the specular area is

turned down by twice the amount that the lens plane turns. A movement of the eye through  $10^\circ$  causes a change in the reflected ray of light of  $20^\circ$ . When examining with a slit lamp in the specular area the examiner usually holds the observation arm by one hand, so as to "scan over" the surface of the lens in a continuous series of small movements and compensate for horizontal movements of the patient, while with the other hand he or she holds the central control unit in order to control the focus level and to follow the changes, desired or undesired, of the specular area on the lens.

This procedure is outlined in Figure 19. A lens (IOL 1) examined with a slit lamp (SL) specularly reflects the illumination beam in only one direction (S1). On tilting the lens (IOL 2 and 3), the specularly reflected beam (S2 and S3) is twice as strongly deflected. In this process the anterior and posterior specular areas travel in opposite directions. The movements noted in Table 8 tell us which manipulations are necessary to direct the specular area specifically to other parts of the surface of the lens. They are valid for both biconvex or planoconvex lenses, where the refraction of the front surface of the lens is less than 4 dpt. Where the anterior surface of the lens is flat or

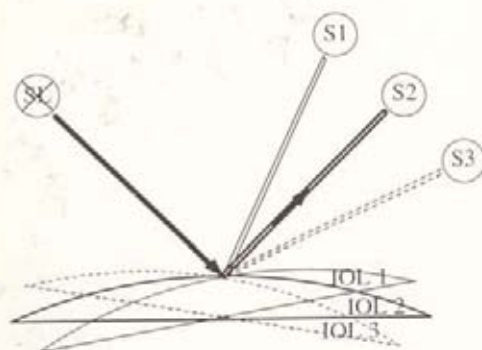


Fig. 19 A lens (IOL 1) examined with a slit lamp (SL) specularly reflects the illumination beam only in one direction (S1). If the lens is tilted (IOL 2 and 3), the beam reflected (S2 and S3) is deflected twice as strongly.

Table 8 Eye movements of the patient or rotations of the observation arm necessary to direct the specular areas to other areas of the lens

Movement	Specular area anterior side (convex)	Specular area posterior side (plane or convex)
Looking up	down	up
Looking down	up	down
Looking to the right	left	right
Looking to the left	right	left
Observation arm to the right	right	left
Observation arm to the left	left	right



concave, the instructions for the posterior surface of the lens will have to be followed; where the posterior surface of the lens is concave, the instructions for the anterior surface apply. If the posterior surface of the lens is flat it reacts optically in the same way as it would if it were convex, since the incident light is slightly bundled by the cornea and the anterior surface of the lens.

At first the patients usually make movements which are far too sweeping, and they then have to be asked to move their eyes only a very few degrees. If a lens is curved peripherally  $10^\circ$  to the center plane, then the maximum permissible movement of the eye is only  $10^\circ$  in all directions, if one still wishes to be able to see the specular area on the lens. Since many patients can only maintain a particular line of sight for a few seconds, the examination has to be performed rather quickly.

### Examination of Patients with the Specular Microscope

If the theoretical, basic ground rules outlined above are observed, an examination of the specular area using the slit lamp is easy. But if the examiner is focusing on a lens with a specular microscope for the first time, then everything usually looks black. He or she has to look for the specular area. There are hardly any structures which can be used as a guide to the specular area. Even when one is quite close to it, there are no more contours on the lens surface than there are when one is searching a considerable distance away (cf. Fig. 18). It is a question of "all or nothing": either the specular area is there, or it is not.

Moreover, it is difficult to provide any general guidelines for locating the specular area, since the operation of commercially available microscopes varies considerably from model to model. What follows, then, is really only valid for microscopes which have fixed illumination and observation arms such as the microscopes manufactured for-

merly by Leitz, distributed by Rodenstock and presently by Hund, Bio-Optics, Keeler-Konan, Pocklington and Pro/Koester. Zeiss, Topcon, and Nikon market noncontact specular microscopes which have been converted from slit-lamps. In some ways, largely because of the free mobility of the illuminating arm and the observation arm, the operation of these devices is similar to that of the slit lamp. The same image is reflected into both the oculars, so that in binocular examination the examiner can see the specular area with both eyes. A further significant way in which specular microscopes differ from slit lamps is the fact that with specular microscopes the examination distance of 1 to 2 cm between the objective and the cornea is much smaller than with slit lamps. Mayer (1984) gives a market survey of microscopes.

In the specular microscopy of lenses a contact element is not necessary and in many cases more of a nuisance. In contrast to the specular microscopy of the endothelium the reflection of the tear film provides no hindrance to the examination of lenses. Depending on the manufacturer, some contact elements are only suitable for examining the endothelium and not the deeper structures.

At this high degree of magnification it is not only arbitrary movements of the eye which can cause a disturbance but also movements synchronous with the patient's pulse or respiration. Such movements may be reduced by asking the patient to lie down, but it is usually not necessary.

### Locating the Specular Areas with the Microscope

The patient's eye should be positioned in such a way that the specular area can be found right away. If the implanted lens has not been tilted, he or she should look straight ahead; any tilting of the lens will be known from the slit-lamp examination and should be compensated by the appropriate changes in the line of vision. If during the slit-lamp examination the specular area lies



in the center of the lens when the line of vision was  $10^\circ$  upward, the patient should also look upwards by about  $10^\circ$  when being examined with a specular microscope. Unfortunately some patients tend to experience convergence movements caused by fusion when the microscope is moved slowly toward their eye. This should be avoided.

The intensity of the light can be set much lower than is necessary when examining the endothelium with a specular microscope. A PMMA lens reflects approximately 14 times more light than the endothelium (Table 5). In certain cases, to locate the specular area one can open the slit of the illumination beam further, if this makes the specular reflection area larger and therefore easier to find.

In specular microscopy of the IOL the first thing to find is the plane of focus and only then the specular area. The examination itself is begun by preliminary focusing on the specular area of the tear film of the cornea, to exclude the possibility of touching the cornea by mistake. This must not be allowed to happen, as it would cause contamination of the objective, and the patient would become anxious. After focusing on the tear film the microscope may be moved towards the eye by no more than a maximum of 5 mm, depending on the depth of the anterior chamber.

The plane of focus in the area of the anterior surface of the lens should be set as precisely as possible. Even if hardly any details are to be seen at this high degree of magnification and low-light focal illumination of the specular microscope, the attempt should nevertheless be made to focus on the pupillary margin of the iris, on deposits on the lens or on positioning holes, even if they do not lie within the specular area. When the posterior lens capsule is recognized, one has to refocus forward again. If no such structures are found, the focus cannot be firmly set.

Ideally, part of the lens will light up in the specular area as soon as the plane of focus is reached. But unfortunately, the specular

area is not often found immediately. If the microscope is focused on the lens, but the specular area has not yet been found, the first thing to do is to look for it by moving the microscope in the horizontal and then in the vertical plane. While this is being done, the patient should maintain his or her line of vision. In specular microscopes the field of view embraces an area of about  $1 \text{ mm}^2$  where magnification is  $100\times$ . A lens whose diameter measures 6 mm has an area of about  $28 \text{ mm}^2$ . Searching in this manner tends to be more successful with dilated pupils than in cases of miosis.

If the specular area is not found in this way, further searching is likely to be difficult. The patient must be asked to move his or her eye very slightly in various directions. This has to be done in a rather aimless fashion since there is no way of knowing in what direction the specular area may lie. Sometimes the patient only has to look up a bit. The position of the eye should be regularly checked, since too large a deviation from the primary position rarely brings success. Searching is a strain for both patient and examiner. If unsuccessful, one should stop after one or two minutes, so that the patient can take a break, and then start over again.

### **Searching the Lens in the Specular Area with the Microscope**

After locating the specular area it is hardly feasible technically to search all sections of the lens with the specular microscope. Which areas of the lens have to be examined is determined from the examination of the lens in the specular area using the slit lamp; these alone should now be located with the specular microscope. A few findings from other areas of the lens might be documented in addition. Once again the patient should go through a series of fine movements of the eye. The specular area, now changed in this way, must again be located by tracking it down with the microscope. Here it has



proved advantageous to alter the reflex by such small movements of the eye that it does not entirely disappear from the field of view, but remains partially visible in the periphery. If the patient mistakenly moves his or her eye too far, the specular area will vanish from the area of observation. The microscope should not then be moved, but rather an attempt should be made to "catch" the specular area again by corresponding movements of the patient's eye. In contrast to examinations using the slit lamp, the guidelines set out in Table 6 cannot be adopted in their entirety. Depending on what make of microscope one is using, the image visible in the ocular may be upside down and laterally reversed due to the optical path within the microscope, to optical reflection or to inversions, so that the movements have to be correspondingly modified.

The examiner should take care to see that the desired plane of focus is maintained throughout, otherwise he or she will switch between the anterior and posterior specular areas by mistake.

## Avoiding Typical Errors

### The Specular Area Has Not Been Found

The inexperienced examiner will encounter initial difficulty even in locating the specular areas of a lens. Thus, in focusing on the posterior capsule of a lens, for instance, a blur may be seen which, out of disappointment, may be taken for a specular area. But after only minimal experience it will no longer happen that such a dark area of focal illumination is mistaken for the bright specular area. In addition to the specular areas described here there are also other insignificant reflections within the eye, which are much darker than the specular areas of the lens.

It can also happen that when the microscope is poorly adjusted, reflections occur within the microscope and particles of dust there may be mistaken for cellular deposits

on a lens. This is particularly true when working with the Pocklington microscope manufactured by Keeler-Konan. In such cases it always helps to check the focus and compare the slit lamp findings in order to determine the extent of the artifacts. Unfortunately the examiner's spatial awareness is considerably reduced due to the very restricted field of vision and the quasi monocular examination procedure.

However, with some experience, artifacts can be differentiated from true findings making misinterpretations unlikely.

### Artifacts of the Cornea

In examinations using specular microscopy there are two pitfalls to avoid, namely the tear film and the endothelium. Both of these boundary layers can give rise to optical phenomena, which even someone with experience may on occasion mistake for the surface of the lens.

*The specular area of the anterior surface of the cornea* is relatively easy to recognize. It is about 7 times as light-intensive as the specular area of a PMMA lens in the eye (Table 5). The movements of the lipid film and other components of the tear film which come from blinking are characteristic. During the examination particular attention should be paid to such movements, since it will not be possible to evaluate mobility when looking at photos of the examination series later on.

It is very difficult to distinguish between *the area of indirect lateral illumination (Vogt) of the tear film or of the corneal endothelium* on the one hand and the lens findings on the other. The endothelium and the tear film can both be examined in the transmitted light specularly reflected off the lens. Which is simply to say that the specular area of a lens can light up at the same time as the cornea is being focused on. Cellular deposits on the endothelium are morphologically very similar to lens deposits, but there is no correlation between the forms both

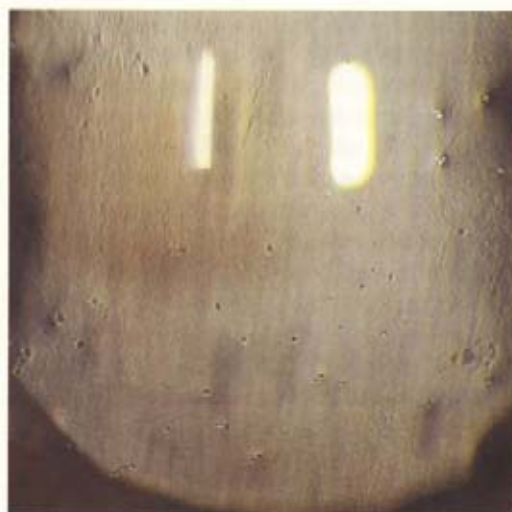


Fig. 20 "Indirect" specular microscopy of the tear film. The cornea is transilluminated by the light reflected on the surface of the lens. Such defocussing entails the danger that corneal findings will be misinterpreted as lens findings. (20 $\times$ )



Fig. 21 "Indirect" specular microscopy of the cornea. Patterns caused by the drying out of the tear film may be of assistance in identifying the plane of focus later on. (20 $\times$ )

reactions take (Vogt, 1930, 1931, 1942; Wenzel and Hahn, 1989).

Since planes of focus are confused fairly frequently and since this can entail serious misinterpretation, a number of examples will be given to show just how similar specular microscopic findings on the cornea are to those on the lens.

In Figures 20 and 21 the *tear film* is in focus. Since these photographs were taken with a slit lamp, the plane of focus may be recognized by the blurred representation of the iris. If only the small cells in the center of the photograph had been photographed at high magnification using the specular microscope, it would hardly be possible to establish the plane of focus retrospectively. It is easier to make out the tear film in Figure 21. In areas where the tear film dries out, net-like patterns are produced, which would not be seen in this way on an implanted lens. Figure 22 shows the *endothelium* findings from a patient with many small, spindle-shaped cell deposits. By contrast, the surface of the lens was practically

free of cells. In determining the plane of focus retrospectively the particles near the bright specular area of the tear film, whose images are relatively sharp, are of assistance. No details can be seen there, since the specular area of the tear film is much brighter than that of the lens surface and is therefore completely over-exposed in this photograph.

Figure 23 may be interpreted simply as the "indirect specular area" of the corneal endothelium, since the image of the pupil is quite blurred. In this case, too, it would be difficult to arrive at an interpretation if the eye had been examined at high magnification with a specular microscope without one having been able to check the plane of focus.

Misinterpretations of specular microscopic findings due to artifacts of the cornea may be avoided if care is taken to maintain the plane of focus. The plane of focus has usually been correctly chosen if a positioning hole of the lens or the remains of the anterior lens capsule or the pupil can be



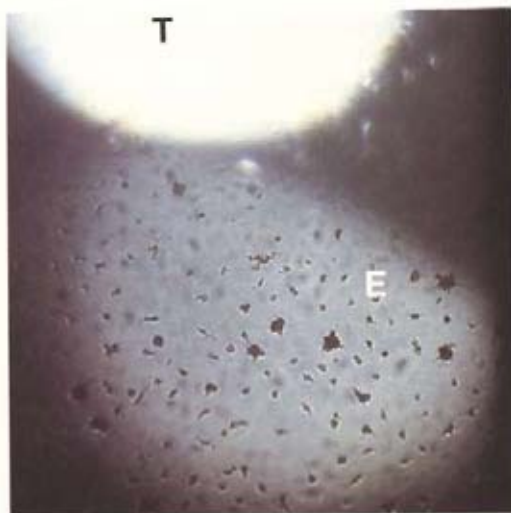


Fig. 22 Dense cellular deposits on the corneal endothelium (E) in a patient after lens implantation. Indirect illumination. The specular area of the tear film of the cornea (T) is only slightly out of focus. These findings should not be confused with those from a lens. (70 $\times$ )

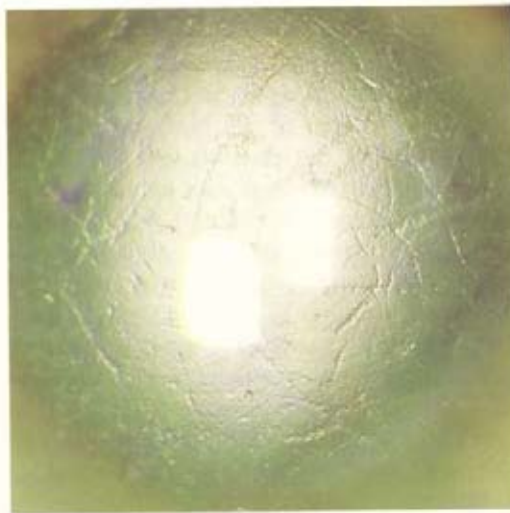


Fig. 23 "Indirect" specular microscopy of Descemet folds 3 months after the operation. (20 $\times$ )

seen in sharp definition. It is often useful to pay careful attention to the findings over the iris, when one assumes that there might be deposits on the lens near the pupil. If the same changes are also found over the iris, the plane of focus is usually wrong, and what one is dealing with are deposits on the cornea. Only when there is a great distance between the iris and the lens, and the pupil can only be seen as a blurred shadow in front of the specular area of the lens, may some lens deposits be formed on the edge of the iris.

If the right plane of focus is maintained, irregularities of the tear film can bring about shadow formation on the lens surface, which the inexperienced may tend to misinterpret as lens deposits. Such irregularities occur due to the absence of blinking when the tear film dries out. They regularly occur when the cornea is anesthetised shortly before examination by specular microscopy, for instance, in order to measure intraocular pressure. One example of such diffuse shadows can be seen in Figure 24. The out-

line of the capsulorhexis can be clearly seen. Irregularities of the lens surface could be focused exactly. In contrast, the black blurs in the area of the lens cannot be focused on.



Fig. 24 Shadows on the lens produced by irregularities of the tear film. The lens is in focus, and the outline of the capsulorhexis is clearly visible. These findings should not be mistaken for lens deposits. (20 $\times$ )

### Inadequate Searching of the Lens

Since the visible specular area of a lens in the microscope is significantly smaller than the whole surface of the lens and since an overall view is not possible using a microscope, relevant findings may be easily overlooked. To correctly evaluate findings on a lens it is not enough to have only *one* specular microscopic photograph, even if it is of superb quality. For this reason the lens must first be closely examined in the specular area using the slit lamp at medium magnification (25 $\times$ ) before each examination with a specular microscope. Only in this way can one be certain one has an overall view. This examination is more important than specular microscopy at high magnification. If the possibility of lens deposits can be excluded using the slit-lamp, there is no need to perform specular microscopy at higher magnification. However, if cells or unclear lens deposits are seen in the specular area using the slit lamp, then these should be sought specifically with the specular microscope at a higher magnification.

In addition, one should guard against mistaking the anterior specular area of the lens for the posterior, as described in detail above.

### Selection of Unsuitable Patients

One reason for difficulties in learning the technique may also be that an unsuitable group of patients has been selected. During the first postoperative week the lens may be covered with lens remnants, erythrocytes or loose pigment, all of which make the recognition of cellular deposits difficult. Even the slightest degree of corneal clouding or folds in Descemet's membrane noticeably reduce the quality of an examination. In uncomplicated cases maximum cell density and the best examination results can be expected about a month after the operation. Later on, high cell densities may appear in the vicinity of posterior synechias of the iris. Often the cell density is so low that a beginner will give

the whole technique up in despair. Really positive results with a larger number of cellular deposits only occur in about 10% of operated cases.

Table 9 summarizes once again the most important steps in carrying out an examination using specular microscopy.

**Table 9 The most important steps in carrying out specular microscopy in vivo**

#### Slit-lamp examination in focal illumination

- Is the patient cooperative? Can he or she open the eyelids wide and perform a series of tiny movements of the eye?
- Have sources of haze in the anterior optical media been eliminated (epithelial edema, ointment, mucus, ruptures of the tear film after local anesthesia, fibrin)?
- Is mydriasis sufficient?
- Is the lens exactly parallel to the level of the pupil or tilted?
- Are there any synechiae or deposits on the lens?
- Are the outlines of the capsulorhexis visible in front of the IOL?

#### Slit-lamp examination in the specular area

- Monocular examination
- Reduction of the intensity of the illumination
- Widening of the slit size
- Basic position: look straight ahead, illumination and observation arms both swung 20° apart
- Locating the specular area
- Magnification to a maximum (>20 $\times$ )
- Searching the lens by means of slow movements of the patient's eye or by moving the illumination arm; if necessary, raising the patient's upper eyelid

#### Examination with the specular microscope

- Magnification to ca. 100 $\times$
- No contact element
- Reduce the intensity of light, widen the slit if possible
- Check that the patient is looking straight ahead
- Focus on the tear film
- Focus on the pupillary level
- Locate any specular area by searching with the microscope or by means of tiny movements of the patient's eye
- Search the lens by means of tiny movements of the patient's eye and tracking with the microscope



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