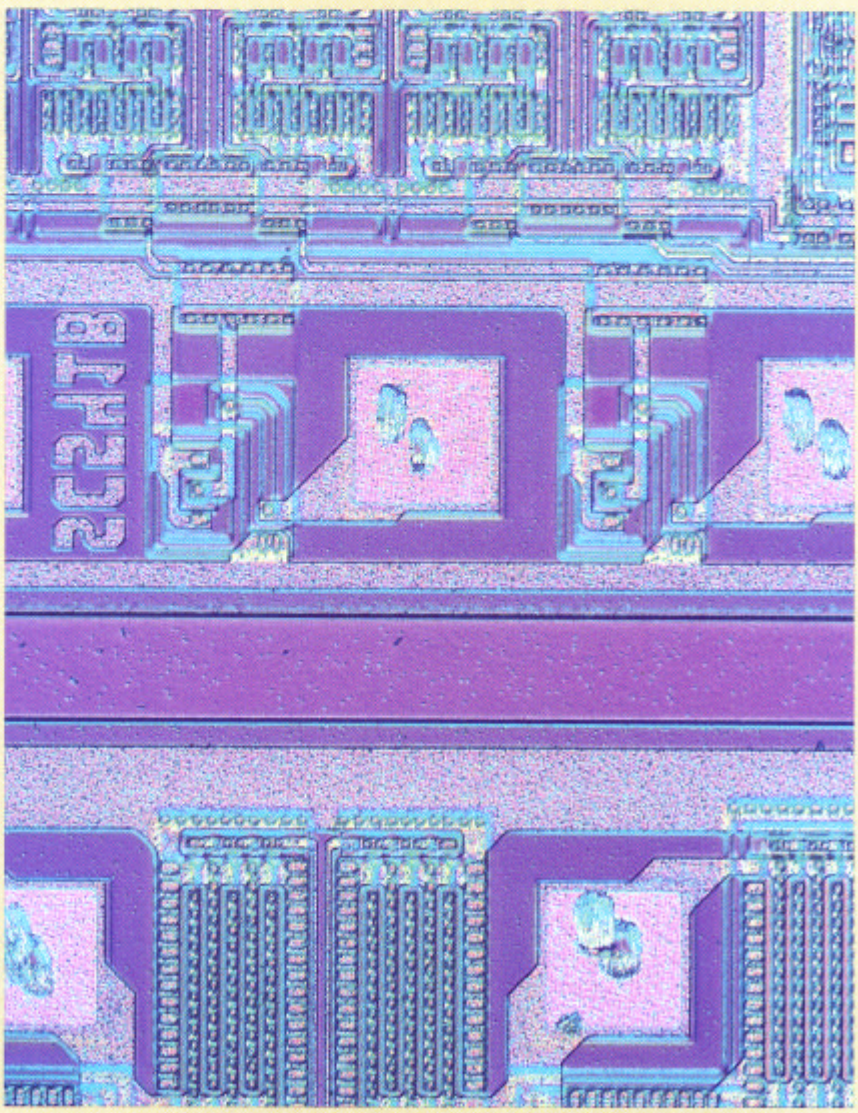
Reflected Light Microscopy

An Overview

by Mortimer Abramowitz
Fellow, New York Microscopical Society

For Olympus Corporation

Volume 3



Reflected Light Microscopy
An Overview

By Mortimer Abramowitz
Fellow, New York Microscopical Society
Consultant, Technical Information, Olympus Corporation

For Olympus Corporation
Volume 3, Basics and Beyond Series

Dedicated to the late
Margaret Cubberly
who, despite serious
physical handicaps,
devoted her life to the
advancement of microscopy
and to the New York
Microscopical Society.

All photomicrography by the author

Front Cover: Computer Chip; Nomarski Illumination
Back Cover: Titanium Heat Etch; Nomarski Illumination

Author acknowledges with thanks permission to use diagrams C, I, J, K from H. Wolfgang Zieler, The Optical Performance of the Light Microscope, Microscope Publications Ltd. 1972.

Table of Contents

Introduction

The world of microscopy may be divided into two domains: transmitted light microscopy and reflected light microscopy. The previous two volumes in this series dealt with transmitted light microscopy. This volume will examine reflected light microscopy,* the principles and the instrumentation.

Reflected light microscopy—sometimes called incident light or metallurgical microscopy—is utilized for the study of specimens that remain opaque even when ground to a thickness of 30 microns or, even if translucent at such thicknesses, are opaque when studied at greater thicknesses. The range of specimens falling into these categories is considerable—metals, ores, ceramics, polymers, semiconductor wafers, slag, coal, plastics, paint, paper, wood, leather, glass inclusions etc. For study of such objects, light, unable to pass through the specimen, must be directed onto the surface of the specimen and eventually returned to the microscope objective by reflectance, specular or diffused. (Fig. A) Such illumination is sometimes referred to as episcopic illumination or epi-illumination or vertical illumination (i.e., from above) as contrasted to diascopic illumination (i.e., transmitted light). There are some microscope set-ups which permit the user to alternate or simultaneously do vertical and transmitted illumination. The world of epi-illumination is frequently the domain of industrial microscopy and thus represents a most important segment of microscopical studies.

* Not including stereomicroscopes or biological fluorescence.

Fig. A
Types of Reflection

Fig. A

THE UPRIGHT METALLURGICAL MICROSCOPE

The typical upright compound reflected light microscope has two eyepiece viewing tubes (in a binocular or trinocular tube head) and a pair of viewing eyepieces, usually of 10X magnification; a nosepiece capable of holding four or more objectives; a stage moveable up or down by means of coarse and fine adjustment focusing knobs; a built-in light source, usually a 50 or 100 watt low voltage-high intensity tungsten-halogen lamp in a lamp house; and a vertical illuminator interposed above the nosepiece but below the underside of the viewing tube head.(Fig. B) The specimen's top surface is upright on the stage facing the objective which has been rotated into the microscope's optical axis. The vertical illuminator is horizontally oriented, 90 degrees to the optical axis of the microscope and parallel to the tabletop, with the lamp housing attached to the back of the illuminator. The coarse and fine adjustment knobs raise or lower the stage in large or small increments respectively to bring the specimen into sharp focus.

(There is another type of reflected light microscope, built upon the Le Chatelier design, which is an inverted microscope. More of this later under the discussion of metallography.)

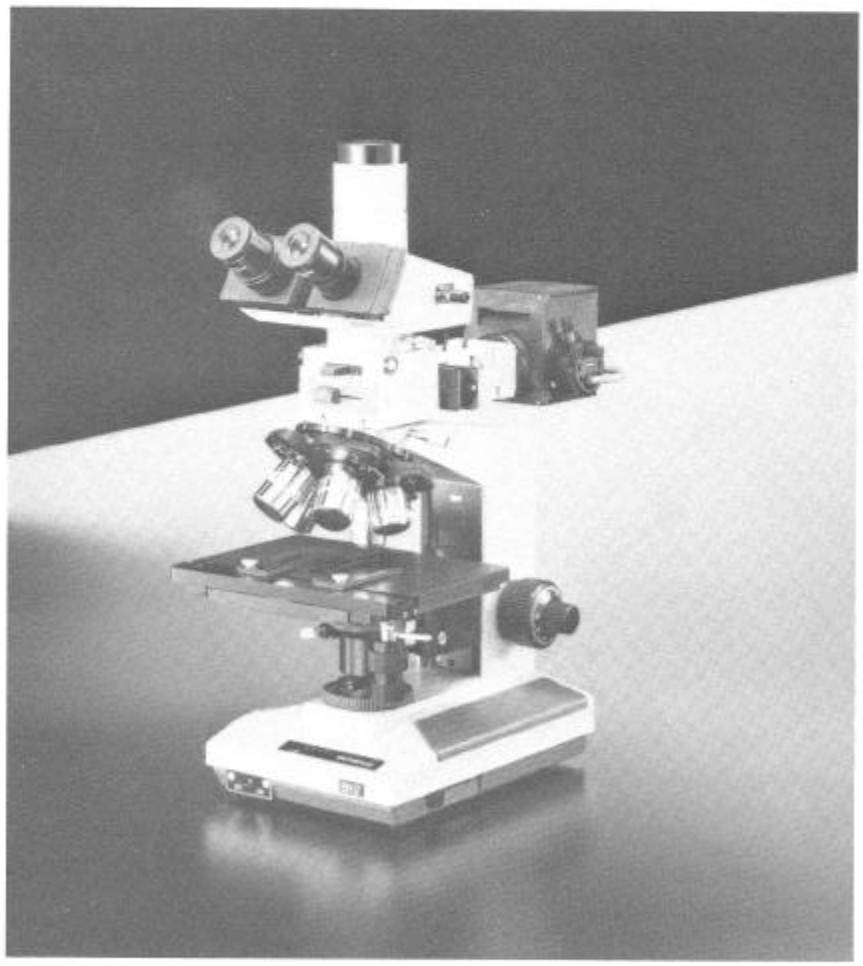


Fig. B
An Incident Light Microscope with Vertical Illuminator in Place

The image of the focused specimen is projected by the objective toward the fixed diaphragm inside the eyepiece, approximately 10 millimeters below the top of the eyepiece tube. This image is a real, inverted image and magnified in accordance with the magnification power inscribed on the objective. This intermediate image is then "looked at" by the eye lens of the eyepiece and further magnified in accordance with the magnification of the eyepiece.(Fig. C) The secondarily magnified, virtual image is seen as if it were about 10 inches from your eyes. The total visual magnification of the microscope is the product of the magnification of the objective and eyepiece. For example, a 20X objective and a 10X eyepiece will yield a total visual magnification of 200X.

Fig. C
Light Path (Diagrammatic) of Two Characteristic Rays Through The Microscope. Finite Tube Length System

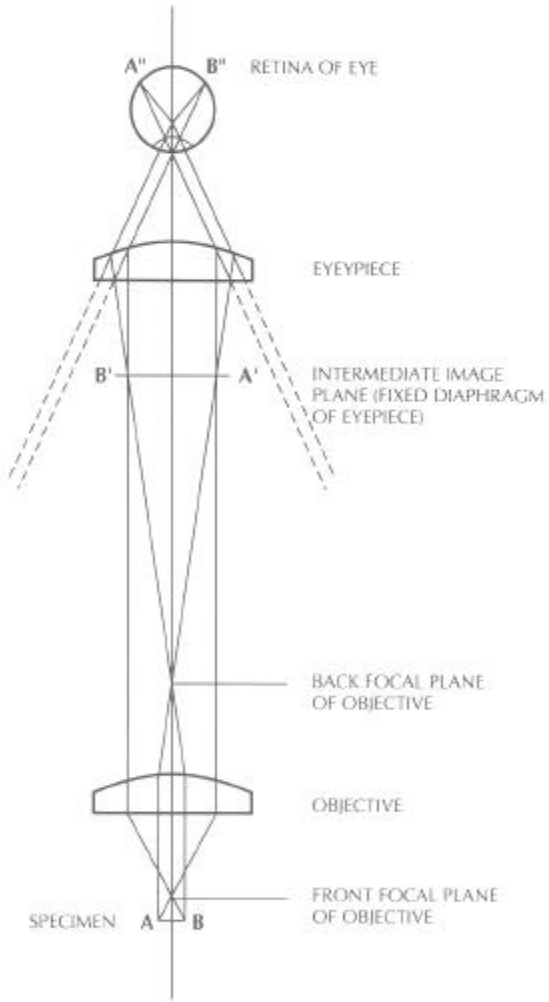
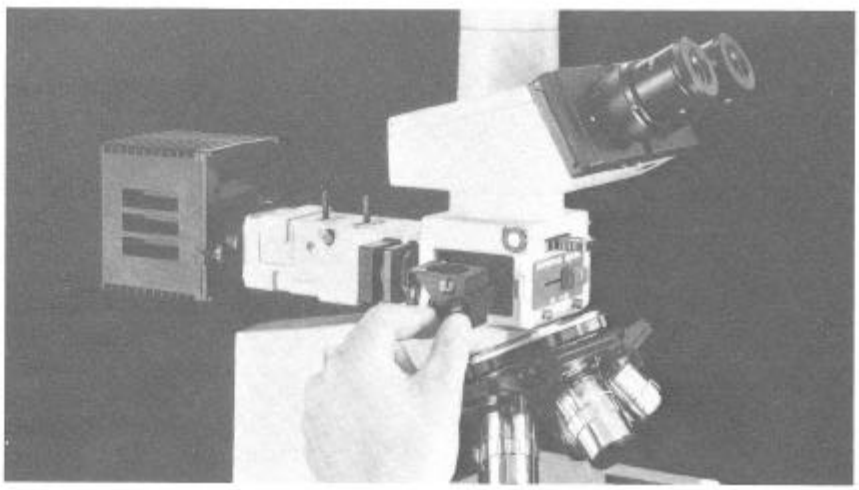


Fig. C

THE VERTICAL ILLUMINATOR

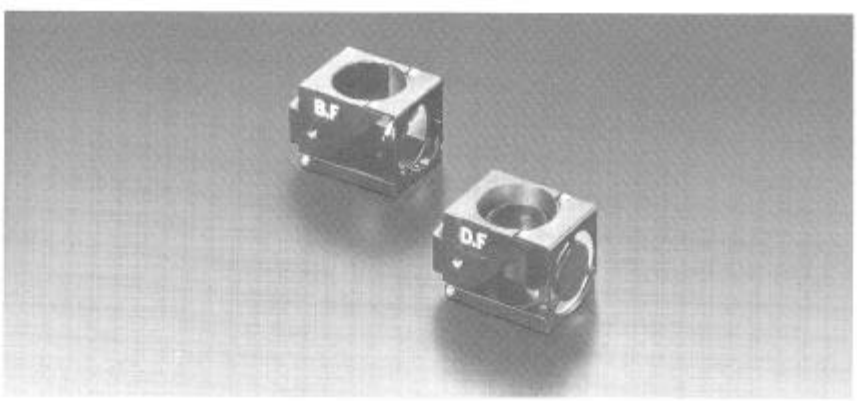
Just as in transmitted light work, reflected light microscopy too can be conducted in any one or more of several modes: brightfield, darkfield, Nomarski differential interference contrast, polarized light and fluorescence. This booklet will deal with each of these types of microscopy, explaining the principles and describing the equipment required as well as the techniques for using such equipment.

Fig. D
Vertical Illuminator



In addition to the basic stand detailed above, the special piece of equipment needed is a reflected light vertical illuminator. (Fig. D) Its function is to direct light (more on light sources later) from the lamp down through the microscope objective and onto the surface of the specimen, then allowing the light from the surface of the specimen, passing through the objective once more on the way up, to reach the viewing tubes for your observation or photomicrography. The generic name for such illumination is coaxial or on-axis illumination.

Fig. E
Darkfield and Brightfield Cubes For Insertion in the Vertical Illuminator



Some of today's reflected light illuminators are described as universal illuminators because, with several additional accessories and little or no dismantling, you can easily switch from one mode of reflected light microscopy to another, or even slide the reflectors out of the path altogether to do transmitted light observation. Such universal illuminators may include a partially reflecting plane glass surface (sometimes referred to as a half-mirror) for brightfield, and/or a fully silvered reflecting surface with an elliptical, centrally located clear opening for darkfield observation. (Fig. E)

Each of these reflecting devices is tilted at a 45 degree angle facing the light traveling along the vertical illuminator and, simultaneously, at a 45 degree angle to the optical axis of the microscope. Each of the respective mirrors directs the light downward at 90 degrees toward the specimen and also permits the upward-traveling reflected light to pass through to the viewing tubes for observation. The best-designed vertical illuminators include condensing lenses to gather and control the light, an aperture iris diaphragm and a pre-focused, centerable field iris diaphragm to permit the desirable Koehler illumination. The vertical illuminator should also make provision for the insertion of filters for contrast and photomicrography, polarizer, compensator plates for polarized light and Nomarski illumination, and an analyzer. In vertical illuminators to be used with infinity-corrected objectives (see below for an explanation of these), the illuminator may also include a telan or body tube lens. Affixed to the back end of the vertical illuminator is a lamphouse containing the light bulb, usually a tungsten-halogen lamp. For fluorescence work, the lamphouse can be replaced with one containing a mercury burner. The lamp may be powered by the electronics built into the microscope stand, or, in simpler models, by means of an external transformer.

INFINITY-CORRECTED OBJECTIVES

Most of the reflected light microscopes currently being manufactured utilize infinity-corrected objectives. Such objectives project an image of the specimen to infinity (the common description is not quite accurately stated as emerging parallel rays). To make the viewing of the image possible, the vertical illuminator itself, or in some instruments the body tube of the microscope, must contain a telan or body tube lens. This lens has as its main function the formation of the image at the plane of the eyepiece diaphragm, the so-called intermediate image plane. The eyelens of the eyepiece "looks at" this real, inverted, magnified image and magnifies that image in the usual second stage magnification of the compound microscope. Infinity-corrected systems are especially valuable because they eliminate "ghost images" (caused by converging light passing through inclined plane glass surfaces) that often accompanied the older forms of instrumentation. Such systems have the advantage of being easier to design and also make possible the insertion of less costly accessories in the "parallel" light path.

Infinity-corrected objectives come in a wide range of magnifications, from 1.5X to 200X, and in various qualities of chromatic and spherical correction—from simple achromats to planachromats and planapochromats. Most, but not all, are designed to be used dry, that is with air in the space between the objective and the specimen. The brightfield series have the customary microscope thread for screwing into the nosepiece. (Fig. F) The objectives which are used for brightfield/darkfield observation usually have wider diameter threads and require a nosepiece with wider openings for attaching such objectives (these objectives are called Neo or BF/DF or B/D objectives). Some of the reflected light objectives are designed to focus at a longer working distance from the specimen than is usual; such objectives are labeled on the barrel of the objective as LWD (long working distance) or ULWD (ultra-long working distance). The manufacturer usually designates the objective series to be used for reflected light Nomarski differential interference contrast studies; e.g., in the case of Olympus, the appropriate series is the MS Plan series for brightfield objectives and the Neo S Plan in the brightfield/darkfield series. Such objectives are sometimes labeled NIC on the objective barrel or designated as strain-reduced.

Fig. F¹ (top) & Fig. F² (bottom) A Sampling: 1) Brightfield and 2) Brightfield/ Darkfield (B/D or Neo) Objectives

OBJECTIVES: RESOLUTION AND MAGNIFICATION; CORRECTIONS

Although it may seem that one could go on indefinitely enlarging the image with more powerful eyepieces and objectives, useful total magnification, i.e. magnification retaining resolution, sharpness and clarity, is limited by the numerical aperture of the objective. This term, devised by Ernst Abbe in the 19th century, is a measure of the light-gathering capability of an objective. Numerical aperture is determined by the angle of the cone of light rays reflected (or transmitted) by the specimen and "captured" by the objective. Half that angle is called μ; numerical aperture or N.A. is equal to the mathematical sine of the angle μ for "dry" objectives (objectives which function with air between the front lens of the objective and the specimen). For "dry" objectives, the highest N.A. practically obtainable is 0.95. (Fig. G)

Useful total magnification is sometimes estimated by the "rule of thumb" which suggests that you multiply the N.A. by no more than 1000 or no less than 500. For example, with a 100X objective having an N.A. of 0.95, this would suggest an upper total limit of useful magnification of 950X. Some microscopists more conservatively would recommend an upper limit of 750X. There are some instances where you might go to an upper limit of 2000X, where enlargement is more important than absolute clarity for some specimens. In any event, the figure may vary according to the visual acuity of the particular observer.

Most reflected light microscopy is done with "dry" objectives. Occasionally, when very high magnification and high resolution are required—and the specimen can withstand such treatment—oil immersion or water immersion objectives may be used (objectives designed for immersion are always inscribed as such). For oil immersion objectives, the N.A. may be as high as 1.4; the front lens of the immersion objective must be in contact with the immersion oil that is placed on the top surface of the specimen. For such objectives, the N.A. equals the product of the sine μ multiplied by the refractive index of the oil (1.5+). The practical upper limit of N.A. for oil immersion objectives is 1.4. For objectives designed for water immersion (refractive index 1.33), the upper practical limit of the N.A. is 1.25.

Fig. G
Numerical Aperture and Light Gathering Ability
N.A. = Numerical Aperture
n = Refractive Index of Medium between Front Lens and Specimen
μ = 1/2 the Angle of Cone of Light "Captured" by the Objective

Almost all reflected light objectives are designed to be used with uncovered objects, i.e. without a cover glass. If you examine the inscriptions on such objectives, you will note that, in addition to magnification and N.A. information, the objective also carries the inscription ∞/-. This inscription means that the objective is infinity-corrected and used with uncovered objects. On some manufacturers' objectives there will also be an inscription, e.g. f=180, which tells you the focal length in millimeters of the body tube or telan lens of that system. Objectives come in various degrees of correction. The most common type are achromats, which are corrected for two colors longitudinally, the red and blue light of the spectrum being brought to a common focus. Achromats are also corrected spherically (that is, to bring light passing through the center and periphery of the lens to common focus) for the apple-green wave length. Fluorites or semi-apochromats are also corrected for blue and red chromatically; for red, green and blue spherically. Apochromats, the highest kind of correction in objectives, are corrected for three or more colors chromatically—blue, red, and green—and every attempt is made to correct for spherical aberration for all colors to eliminate sphero-chromatism.

Any of the three major types described above are available with an additional correction, called plan (and so labeled) to deliver an image which is flat from edge to edge in the field of view.

There is an additional chromatic correction that is usually required, the correction for chromatic difference of magnification or lateral color correction. This is needed to ensure that the images for each of the wave lengths of red, green, and blue in white light are all the same size. This correction is often effected by using compensating eyepieces (marked K or C) which complete the final color correction. Some manufacturers effect this correction in the body tube lens or in glass in the observation tube head. These different approaches underscore the importance of using eyepieces and objectives that are made by the same manufacturer.

The importance of the quality of the objective cannot be overemphasized. The objective, assuming proper illumination technique, has the critical function of replicating the specimen in the form of an image which is eventually formed at the plane of the fixed diaphragm of the eyepiece, ready for the second stage magnification. Unless that image is well-resolved and sufficiently contrasty, all the eyepiece would do is to magnify an inadequate image. Therefore, you should choose the best objectives consistent with your needs and your budget, preferably objectives with high numerical aperture for their respective magnification.

OPTIONAL OBSERVATION TUBE HEADS

In addition to the usual observation tubes, there are several optional tubes that are worthy of mention because of their usefulness especially in the examination of semiconductor wafers. These observation tubes have several significantly different features; they come in either a binocular or trinocular version. Each of these tubes contains an erecting prism (similar in function to such components in a low power stereomicroscope). The image is erect and unreversed. When you move the specimen toward you, the image moves toward you; when you move the specimen away, the image moves away; similarly, for movements in left/right directions. Also, the binocular observation tubes and the viewing tubes of the trinocular are easily tilted at any angle from 0 degrees to 45 degrees, thus making the observation tube head easily adjustable for users of different height. The eyepieces of each of these tiltable observation tubes comes so far forward of the stage that there is little likelihood of "debris" from the user's face or hair landing on the specimen.

STAGES AND OPTIONAL NOSEPIECE ON SOME STANDS

Stages on the upright metallurgical stand come in a variety of shapes and sizes from the standard rectangular or circular stage to a 4"X4" or a 6"X6" stage. The large stage can be outfitted with fixtures to handle wafers of various sizes, or masks for transmitted light inspection. There also are available stages with motorized devices to move by electronic joystick controls in x and y directions; even some with z controls for focusing.

Some of the microscope stands come equipped with a motorized nosepiece thereby making it unnecessary for the observer to reach over the stage to rotate an objective into place.

LIGHT SOURCES

There are numerous light sources available for illumination for observation or photomicrography. A most common light source, because of its low cost and long life, is the 50 or 100 watt tungsten halogen lamp. These lamps are relatively bright but require color conversion filters to raise their color temperature to daylight equivalence. Another popular source is the 75 or 150 watt xenon lamp because of its very high brightness and long life, its relatively even output across the visual spectrum, and its color temperature which approximates that required by daylight film. Where very high intensity is required, tin halide lamps are used. For fluorescence work, 100 watt or 200 watt mercury burners are employed. In former years, carbon arc lights or zirconium bulbs might have been used.

LIGHT PATH IN VERTICAL ILLUMINATION

In the vertical illuminator, the light travels from the light source, usually a 12 volt 50 or 100 watt tungsten halogen lamp, passes through collector lenses, through the variable aperture iris diaphragm opening and through the opening of a variable and centerable pre-focused field iris diaphragm. The light then strikes the partially silvered plane glass reflector, partially silvered on the glass side facing the light source and anti-reflection coated on the glass side facing the observation tube in brightfield reflected illumination; or strikes the fully silvered periphery of a mirror with elliptical opening for darkfield illumination. The light is thus deflected downward into the objective. The mirrors are tilted at an angle of 45 degrees to the path of the light travelling along the vertical illuminator. (Fig. H)

The light reaches the specimen which may absorb some of the light and reflect some of the light specularly (mirror-like) or diffusely. The light which is returned upward can be "captured" by the objective in accordance with the objective's numerical

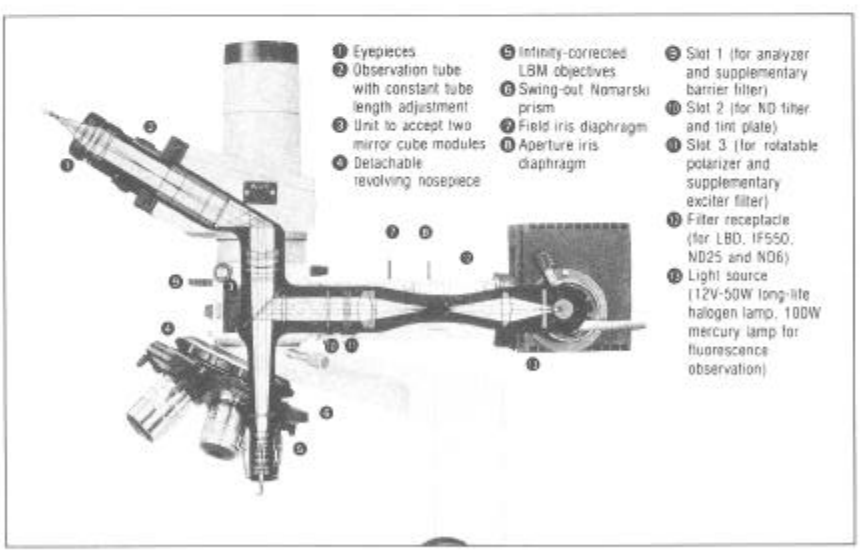


Fig. H
Light Path in the Vertical Illuminator, Brightfield Koehler Illumination (Diagrammatic)

aperture. In the case of infinity-corrected objectives, the light emerges from the objective in so-called "parallel" (parallel from every azimuth) rays projecting an image of the specimen to infinity. The "parallel" rays enter the body tube lens which forms the image at the plane of the fixed diaphragm opening in the eyepiece. (Fig. I)

It is important to note, that in these reflected light systems, the objective serves a dual function: on the way down as a matching well-corrected condenser properly aligned; on the way up as an image-forming objective in the customary role of an objective projecting the image-carrying rays toward the eyepiece.

Fig. I
Light Paths, Finite Tube Length and Infinite Tube Length Systems

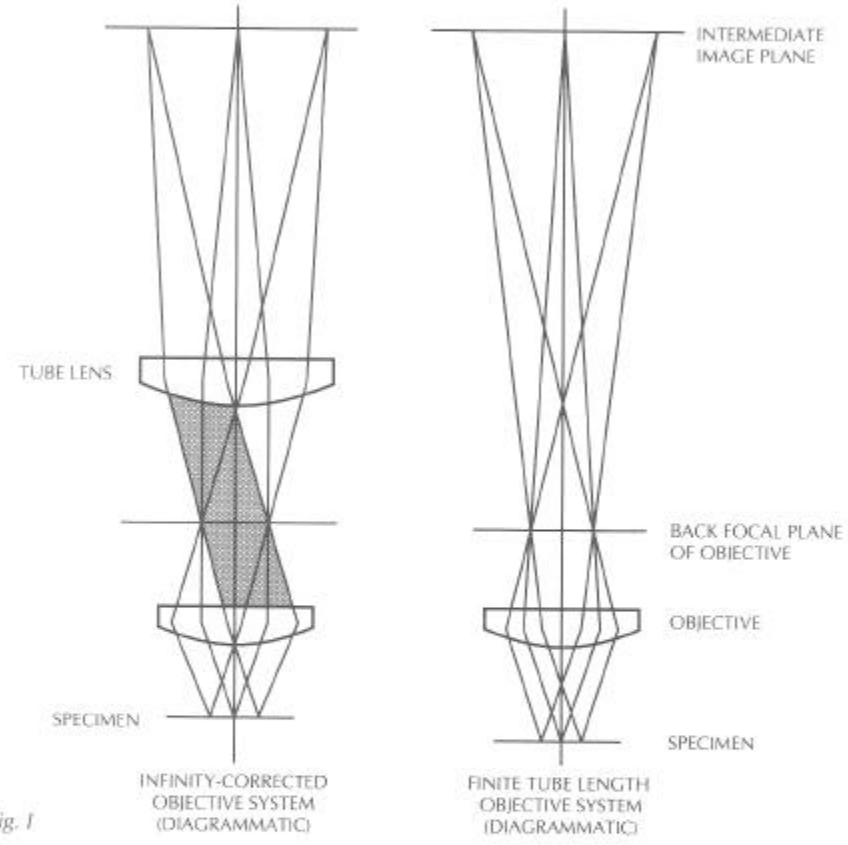


Fig. I

KOEHLER ILLUMINATION

In brightfield reflected light microscopy, the proper use of the two variable diaphragms, the aperture iris diaphragm closer to the light source and the field iris diaphragm closer to the specimen (the opposite of their respective positions in transmitted light) enables you to use the highly desirable Koehler illumination. Such illumination provides bright light evenly dispersed across the plane of the field of view of the focused specimen. Koehler illumination provides glare-free light utilizing the maximum share of the objective's numerical aperture consistent with good contrast.

In practice, you focus the specimen with the objective, e.g. 10X, and close down the pre-focused field iris diaphragm. You will see this field iris diaphragm narrowing the field of view of the focused specimen. If the field iris diaphragm is not centered, you center it by using the centering screws on the vertical illuminator. You then open the field iris diaphragm until it just disappears from view. The field iris diaphragm in this setting prevents stray light from entering the field of view and it also controls the size of the diameter of the field of view which you choose to observe. You then lift out an eyepiece and observe the back of the objective. If you open and close the aperture iris diaphragm, you will see the aperture iris diaphragm opening and closing at the back focal plane of the objective. (A so-called phase or centering telescope inserted in place of the regular eyepiece will enlarge the image of the aperture iris diaphragm for easier viewing of the back focal plane of the objective). The aperture iris diaphragm should not be left wide open because such a setting would result in severe loss of contrast. Instead, the aperture iris diaphragm should generally be opened so that $^2/_3$ to $^4/_5$ of the circular area of the back focal plane of the objective is illuminated. This setting represents the best compromise between maximum usable aperture and good contrast. Every time you change the objective, the settings of the field diaphragm and the aperture diaphragm must again be made in conformance with the above- described procedure. (Fig. J)

A function of Koehler illumination (aside from providing evenly dispersed illumination) is to ensure that the objective will be able to deliver excellent resolution and good contrast even if the source of light is a coil filament lamp. The aperture iris

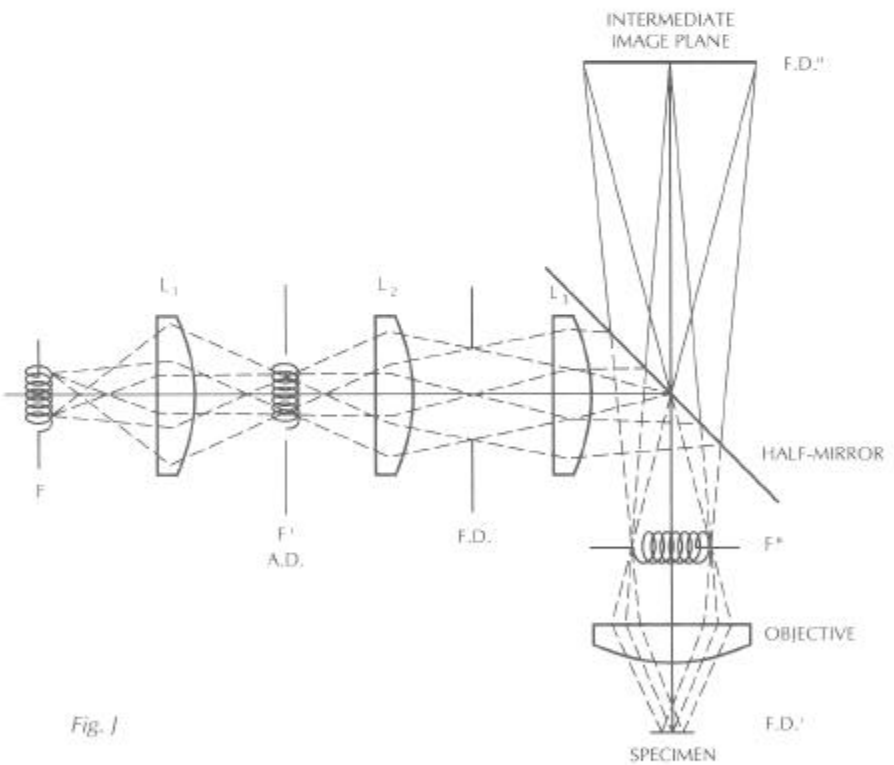


Fig. J

Fig. J
Koehler Illumination for Brightfield Reflected Light (Diagrammatic - for Finite Tube Length Objective System)

F = Light Source

F' = Image of Light Source at Aperture Diaphragm

F'' = Image of Light Source at Back Focal Plane of Objective

A.D. = Aperture Diaphragm

F.D. = Field Diaphragm (Conjugate with Specimen Plane)

L_1,L_2,L_3 = Lenses of Vertical Illuminator

F.D.', F.D. = Conjugate Plane to Field Diaphragm*

diaphragm controls the angle of light striking the specimen from every azimuth in a full cone in brightfield reflected light. The objective's numerical aperture determines the angle of light which can be "captured" as it is reflected from the specimen. Other factors being equal, the higher the numerical aperture, the better the resolution of the objective, i.e. the better the objective is able to clearly separate small details lying close together. The system's vertical illuminator contains the aperture iris diaphragm so that the back of the objective itself does not have to be occluded. In order to get excellent resolution in brightfield reflected light, the objective must "capture" the zero order of light and one or more of the diffracted orders of light coming from the specimen. As the zero order and diffracted orders proceed to the intermediate image plane (plane of the eyepiece diaphragm), the zero order light and the diffracted orders interfere with one another; the image of the specimen appears darker than the background, and the resulting contrast renders the image visible to the observer. The more diffracted orders that enter the objective from the specimen, the more accurately the image replicates the specimen. (Fig.K) The greater the numerical aperture of the objective, the better its capacity to "capture" more of the diffracted orders of light.

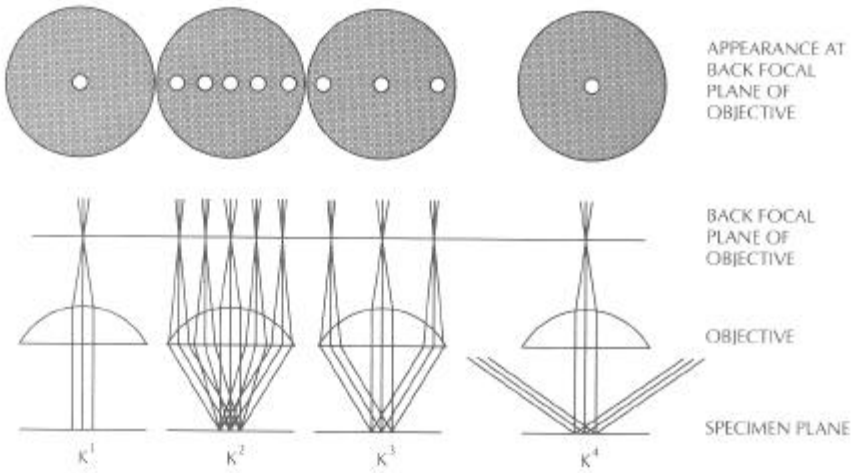


Fig. K

Fig. K Numerical Aperture and Resolution (Diagrammatic) Specimen is a Set of Closely-Spaced Vertical Parallel Lines

K^1 = No Specimen on Stage 0 Order Only

K^2 = High N.A. Objective 0 Order, 1st 2nd Orders "Captured" - Very Good Resolution

K^3 = Lower N.A. Objective 0 Order, 1st Orders "Captured" - Satisfactory Resolution

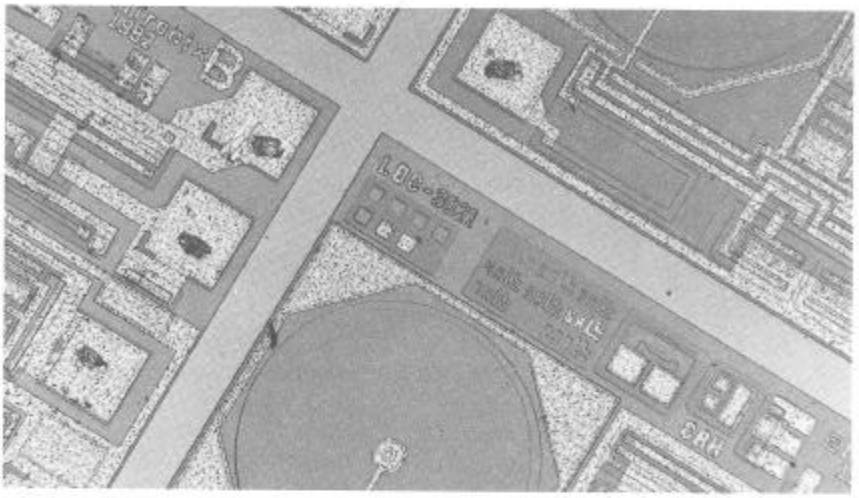
K^4 = Very Low N.A. Objective 0 Order Only - No Resolution

In Koehler illumination, the system is arranged so that the image of the coil filament of the lamp is brought to focus at the plane of the aperture iris diaphragm; it is also in focus at the back focal plane of the objective. Assuming there is no frosted filter* in the light path of the illuminator, if you take out an eyepiece, you will be able to see the image of the lamp filament at the back of the objective. In most systems, the lamphouse exterior has a set of centering screws which enable you to center the lamp filament by moving the filament in a north-south or east-west direction. Also the closing or opening of the aperture iris diaphragm is observable at the back focal plane of the objective, as described above.

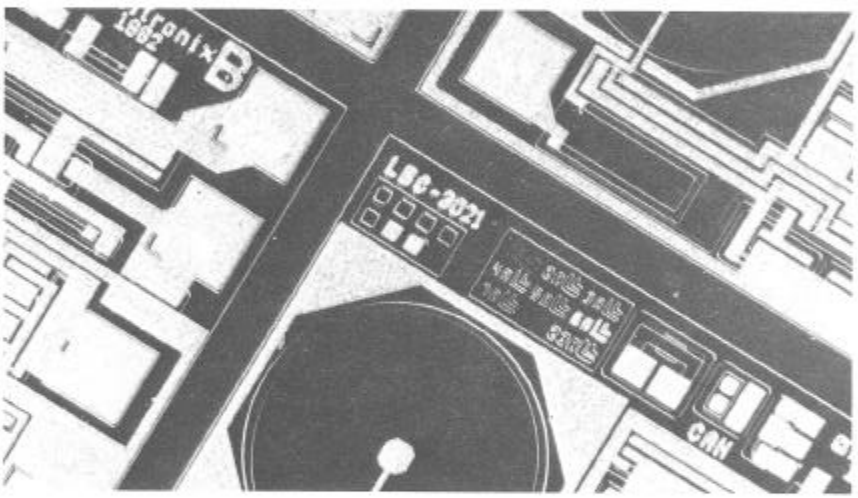
The field iris diaphragm is conjugate, i.e. in focus, with the focused specimen, the intermediate image plane at the plane of the fixed diaphragm of the eyepiece and the retina of the eye.

*If a frosted filter is present, you will see an even circle of light instead of a filament.

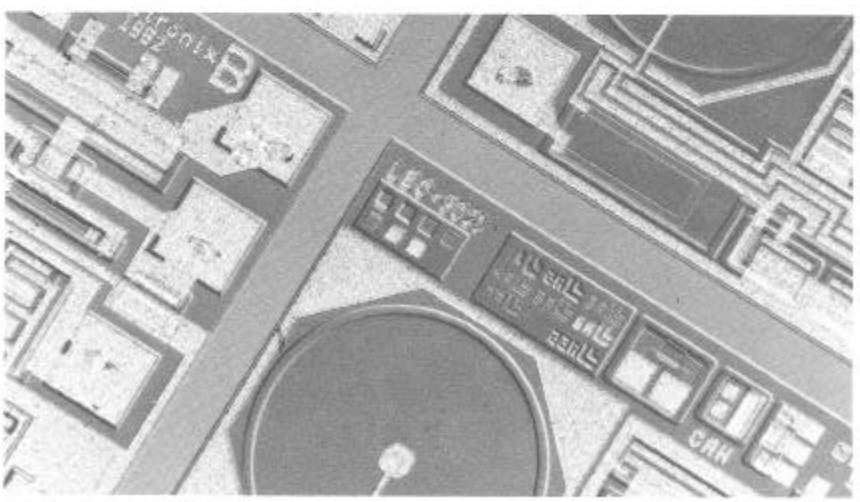
Computer Chip
Brightfield
Illumination



Same View
Darkfield
Illumination



Same View
Nomarski
Interference
Contrast
Illumination



SPECIMENS: AMPLITUDE AND "PHASE-TYPE"

For many specimens, the absorption and diffraction of the incident light rays by the specimen lead to readily discernible variations in the image, from black through various shades of gray, or color if the specimen is colored. Such specimens are known as amplitude specimens and may not require special contrast methods or treatment to make their details visible.

Other kinds of specimens show so little difference in intensity or color that their feature details are extremely difficult to discern and distinguish in brightfield reflected light microscopy. Such specimens behave much like the phase specimens so familiar in transmitted light work. Such objects require special treatment or contrast methods, e.g. darkfield, Nomarski differential interference, polarized light, etching, staining, etc., to produce sufficient contrast (amplitude difference or color) to make the details visible. It must be borne in mind that our eyes are sensitive only to variations in light intensity or to the colors of the visible spectrum.

DARKFIELD REFLECTED LIGHT ILLUMINATION

One of the most important ways to improve contrast in the reflected light microscope is to utilize darkfield illumination. In darkfield work, an opaque occluding disk is placed in the path of the light traveling through the vertical illuminator so that only the peripheral rays of light reach the deflecting mirror.(Fig. L) This mirror has an elliptically shaped opening surrounded by a fully silvered front surface mirror. The field diaphragm is opened all the way. The peripheral rays are deflected downward through specially constructed objectives, known as Neo or BF/DF objectives. The light travels down the 360 degree hollow chamber surrounding the centrally located

Fig. L
Brightfield and Darkfield Reflected Light Paths

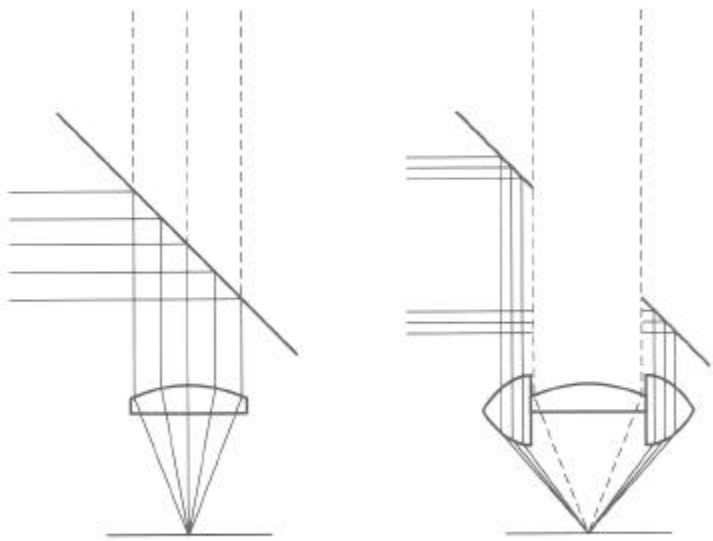


Fig. L

LIGHT PATH
HALF-MIRROR
BRIGHTFIELD
REFLECTED LIGHT
(DIAGRAMMATIC)

LIGHT PATH
ELLIPTICAL OPENING
MIRROR DARKFIELD
REFLECTED LIGHT
(DIAGRAMMATIC)

lens elements of the objective. The light, by means of circular mirrors or prisms located at the bottom of the hollow chamber, is directed at the specimen from every azimuth in oblique rays. Without a specimen on the stage, the field of view appears black because the oblique rays miss re-entering the objective. When a specimen is placed on the stage, the features of the specimen, be they details or ridges or scratches or depressions or particles, etc., now shine brightly against a black background. The contrast is enormously increased with the result that features of the sample, otherwise almost invisible in brightfield, are readily discernible. The light scattered by the specimen details has been able to enter the objective and pass through the central lens elements of the objective eventually to reach the eye or camera.

In many modern microscope stands, the Neo type objectives, with appropriate modules or accessories, can be used for darkfield, brightfield, polarized light, Nomarski differential interference, and reflected light fluorescence observations.

POLARIZED REFLECTED LIGHT ILLUMINATION

For some specimens, the use of polarized light enhances contrast and makes visible details that are otherwise difficult to see. This technique is especially valuable for

Fig. M
Polarized Reflected Light (Diagrammatic)
1. Light Source
2. Polarizer
3. First Order Red Compensator
4. Half-Mirror
5. Objective
6. Specimen
7. Tube Lens
8. Analyzer

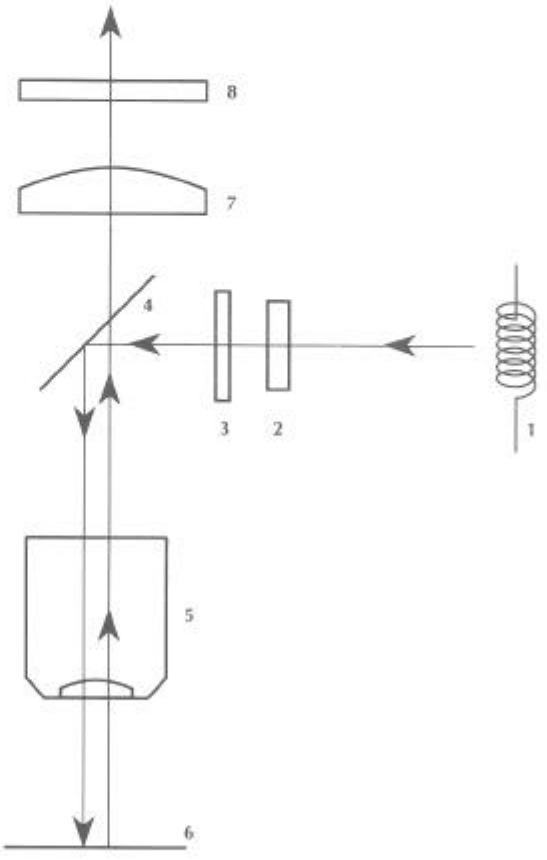


Fig. M

specimens that are anisotropic (having more than one refractive index, depending on the direction of the impinging light) or are rendered so by deep etching or deposition of thin surface films. Polarized light examination requires the placement of a polarizer, oriented in east-west position, in the light path of the vertical illuminator on the "lampside" of the deflecting plane glass brightfield half-mirror and an analyzer above the mirror in the light path going toward the observation tubes. The analyzer is oriented in north-south position, thus effecting "crossed polarizers". (Fig.M) Anisotropic details of the specimen will affect the impinging polarized light according to their thickness and refractive index by causing elliptical polarization or rotating the plane of polarization. The light returning to the objective will pass through the half-mirror as usual; the light beams which are slightly out of phase with one another but vibrating at 90 degrees to each other are brought into the same plane by means of the analyzer. As a result, some portion of the spectrum of the white light is subtracted because of destructive interference; contrast or color results. The insertion of a full wave plate (also called lambda plate, sensitive tint or first order red compensator) somewhere in the light path between the "crossed polarizers", usually in a slot on the vertical illuminator, will often produce a dazzling array of colors making particles or boundaries of the specimen details readily visible. For polarized light work, it is often helpful to be able to rotate the stage or specimen for best effect. There also should be provision for rotating either the polarizer or the analyzer to ensure the "crossing" of the polarizing filters. Some manufactures use prism reflectors (these do reduce resolution) or mirror devices to direct the polarized beam before it enters the plane glass deflector in the customary brightfield reflected mode.

NOMARSKI DIFFERENTIAL INTERFERENCE CONTRAST REFLECTED LIGHT ILLUMINATION

Increasingly in recent years, microscopists have employed a contrast enhancement technique known as Nomarski differential interference contrast (also known as DIC or NIC). For this optical contrast method, most manufacturers provide an individual modified Wollaston prism specified for each objective you use. Manufacturers recommend the particular objective series in their offerings that are suitable for Nomarski use. A polarizer and an analyzer, in "crossed position", are placed in the respective positions as described above for polarized light. The modified Wollaston prisms are fixed to the nosepiece for each objective to be used and then the objectives are screwed into the threaded receptacle of each prism. The polarizer is oriented to pass east-west vibrations of light; the analyzer is oriented to pass north-south vibrations. A first order red compensator is placed in front of the polarizer on the "specimen-side" of the polarizer on the illuminator. (Fig. N) For a range of color effects, the polarizer is rotatable 45 or 90 or 360 degrees. The polarizer should be on a slider to make it possible to move it out of the light path altogether; likewise for the first order red compensator. When Neo objectives are used with the brightfield plane glass half-mirror reflector, it is important, to avoid dismantling as you may wish to convert to ordinary brightfield or darkfield reflected light, to be able to swing the Wollaston prism out of the light path altogether. In some of the designs, the color effects or alteration of the image appearance of the specimen from dark to gray to color is achieved, not by use of a compensator, but by a rotating screw which moves the Wollaston prism in a horizontal direction left or right. In either case, DIC examination is done with the brightfield plane glass reflector in the light path, not the darkfield module mirror.

Fig. N
Nomarski Differential Interference Reflected Light (Diagrammatic)

1. *Light Source*
2. *Polarizer*
3. *First Order Red Compensator*
4. *Half-Mirror*
5. *Modified Wollaston prism*
6. *Objective*
7. *Specimen*
8. *Tube Lens*
9. *Analyzer*

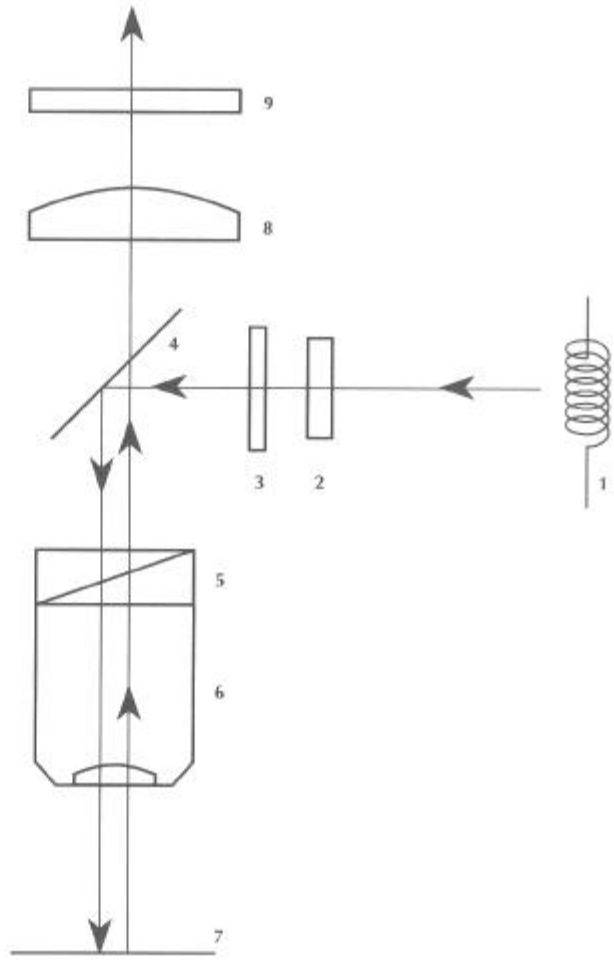


Fig. N

Light, coming through the polarizer vibrating east-west only, is deflected by the brightfield half-mirror and enters the modified Wollaston prism mounted behind the objective. The prism serves a beam splitter and divides the rays into pairs vibrating at 90 degrees to each other with one set of rays traveling at a slower speed than the other. The prism is mounted so that the axis of vibration of the upper half of the prism is at a 45 degree angle to the vibration plane of the polarizers. The respective pairs of rays are separated by a distance (shear) which is less than the resolving power of the objective, thus eliminating the possibility of double images. The pairs of beams reach the specimen and the varying heights of the specimen as well as the slopes of the specimen's features and the varying refractive indices of these features alter the optical path differences of the impinging beam pairs. The light is reflected back into the objective and once more passes into the Nomarski prism. The net result is differences in optical path, depending on the original path difference, the specimen's effect and the effect of the prism serving as a beam recombiner in the upward path. The beams emerging from the prism cannot interfere with one another until they are brought into the same plane of vibration by means of the analyzer which is in place above the deflecting mirror as well as above the prism. The net result of all of this is

to produce a so-called Nomarski-type image which appears three-dimensional with one side of individual features appearing less bright than the other. This pseudo-3-dimensionality results from differences in light intensity caused by the optical path differences described above. In some instruments a series of Newtonian colors can be produced by placing a first order red compensator on the "specimen-side" of the polarizer and rotating the polarizer to change optical path differences; in other prisms, the path differences can be altered by a screw device attached to the prism itself which moves the prism back and forth in a horizontal plane. The appearance of the image can sometimes be enhanced by rotation of the stage. Best contrast is achieved when the specimen details to be enhanced are positioned at 90 degrees to the direction of the shear.

The Nomarski system has the advantage of enabling you to use high numerical aperture (Koehler illumination) with accompanying shallow depth of field to yield excellent resolution and less confusion in the image from features below or above the exact plane of focus. This is known as optical sectioning. The color resulting from the manipulation of the light is known as optical staining. The distance of the prism from the back focal plane of the objective is critical to ensure that a so-called interference fringe of color or gray fills the entire field of view. The greatest 3-dimensional effect is achieved when the prism or the polarizer/compensator is set to the position which gives a gray background.

MODES POSSIBLE WITH UNIVERSAL ILLUMINATORS

In today's universal vertical illumination systems, you may wish to use only Neo type objectives. With the brightfield half-mirror in place, these objectives can do brightfield reflected light; with the darkfield module in place, you can do darkfield illumination; with the brightfield module, polarizer, analyzer and a compensator, you can do polarized light work. Add modified Wollaston prisms to the latter polarizing accessories and you can do Nomarski differential interference contrast.

FLUORESCENCE REFLECTED LIGHT ILLUMINATION

It is also possible, with these universal vertical illuminators, to do vertical fluorescence illumination for the detection of contaminants or residual photo-resist left on semiconductor wafers. The standard halogen lamphouse is replaced by a lamphouse with a 100 watt mercury burner powered by an external power supply; a replacement collector lens is easily screwed into the back of the illuminator in front of the lamphouse. The brightfield or darkfield module is slid out and a fluorescence cube is inserted in its place. This cube is self-contained in that it includes an exciter filter, dichroic mirror and emission-barrier filter. The light (Fig. O) from the mercury burner travels through the exciter filter (which rejects longer wave lengths) and reaches the dichroic mirror which reflects the lower wavelengths down upon the sample. The sample's contaminants or photo-resist absorb the lower wavelength exciting light and emit longer wave length light which is now able to pass through the dichroic mirror and to pass through the barrier filter and then to the viewing tubes of the microscope. Positive residual photo-resist, for example, will fluoresce in red color when excited by green light. Negative photo-resist will fluoresce green when excited by blue or violet light. Cubes are available for violet, blue, or green excitation.

Fig. O
Fluorescence Reflected Light (Diagrammatic)

1. *Mercury Light Source*
2. *Exciter Filter*
3. *Dichroic Mirror*
4. *Objective*
5. *Specimen*
6. *Barrier Filter*

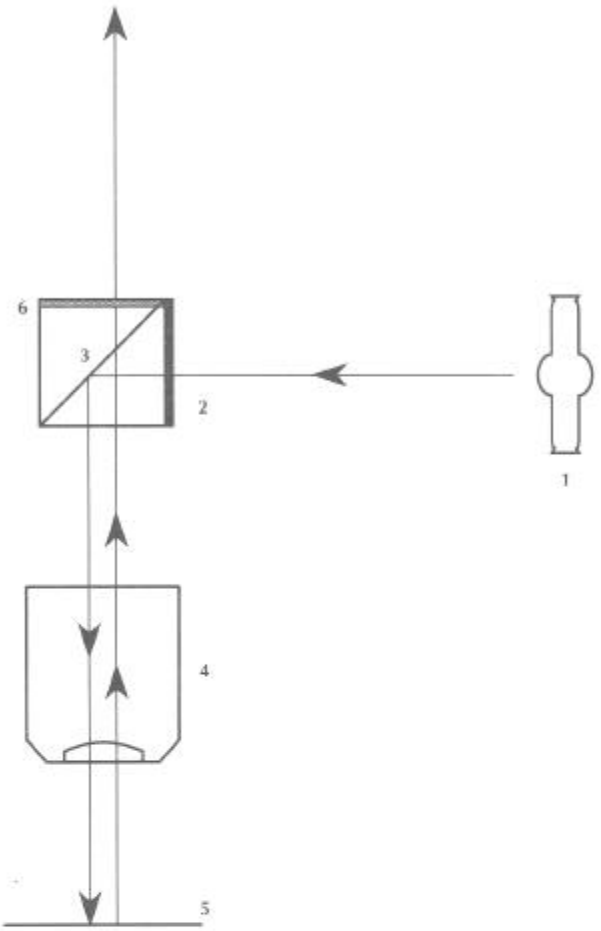


Fig. O

MEASUREMENT

Often, the microscopist may wish to measure details of the specimen being observed. The simplest form of measurement is done with an eyepiece containing a metric scale (inch fractions are also available) and a stage micrometer which is usually a one millimeter scale divided into a hundred equal divisions, each division thus spanning ten microns. The eyepiece micrometer scale, placed at the eyepiece diaphragm plane or a plane conjugate to it, is calibrated with the stage micrometer for each objective so that its scale value is known for each objective magnification. Thereafter, only the eyepiece scale is needed for measurement. There are also eyepiece scales available which compare grain size or place concentric circles, a net or a bar in the image plane of the eyepiece. These scales are known generically as reticles or graticules. A finer measurement is possible with a filar micrometer eyepiece which also has to be calibrated with a stage micrometer for each objective. There are more expensive versions of a filar micrometer which yield a digital readout of measurements.

Much more sophisticated measurement equipment is available which does very fine line width measurement using a video camera, video monitor, line width generator

as well as control panel display. The most versatile and sophisticated measuring instruments involve image analysis, using chip-type video cameras, high resolution video monitors, desk computers and software designed to do automatic measurements of many parameters, in addition to enhancing images.

PHOTOMICROGRAPHY; VIDEO

For photomicrography on upright metallurgical microscopes, the straight tube of the trinocular observation tube head is used. A photographic eyepiece is dropped into the straight tube and any one of the standard photomicrographic cameras can be attached. All formats of standard film size, from 35 mm. to 3 1/4" X 4 1/4", 4" X 5" and 8" X 10" (on large research stands) are available. The film may be either standard camera film or Polaroid instant-type film. Cameras are offered which range from manual to the most advanced automatic systems with auto exposure, auto wind, digital readout, reciprocity adjustment, and spot as well as averaging metering. For further information, consult other Olympus publications or the Kodak manual cited in the bibliography.

A film back of special interest is the Polaroid CB33 back which takes a packet of ten exposures, approximately 3" X 4", of positive prints requiring no coating. The film is ejected from the holder by pressing a button which utilizes the accompanying transformer to power the film ejection. For clean room applications, an attachable plastic cone receives all the prints as they come forth. The film is high speed Polaroid color (339) at 640 ASA or black/white (331) at 400 ASA.

For video observation and recording, a photoeyepiece is dropped into the straight tube of the trinocular and a parfocalizing adapter connects the video camera to the microscope so that the image on the video screen is in focus when the image is in focus in the observation tube. If parfocality is not required, a simple C-mount adapter is connected to the video camera and then inserted into the vertical phototube.

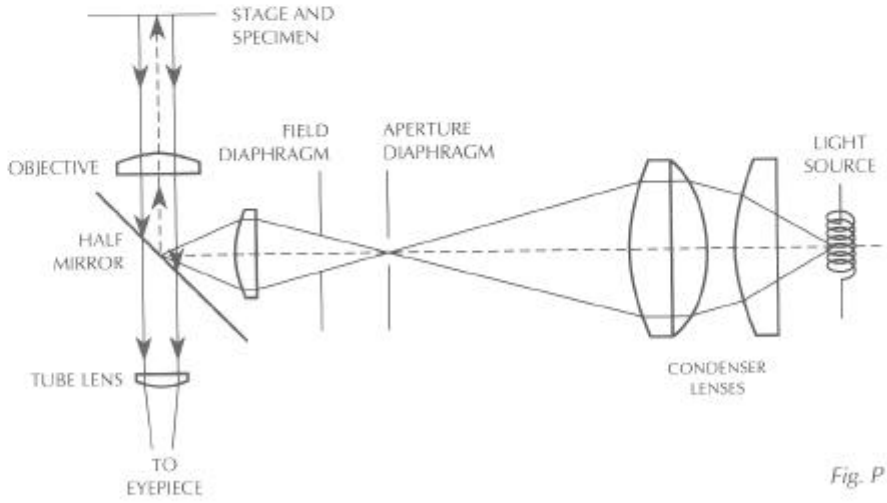


Fig. P

Fig. P Vertical Illuminating System for Inverted Metallurgical Microscope (Diagrammatic-Infinity Corrected Objective)

THE INVERTED METALLURGICAL MICROSCOPE

In addition to the upright metallurgical microscopes, there are inverted microscopes—of the Le Chatelier design. On the inverted stand, the specimen is placed on the stage with its surface facing downward. The objective is mounted on a nosepiece, with its front lens facing upward, located under the stage. Focusing is accomplished on some instruments by having the focusing knobs move the entire nosepiece up or down toward the specimen; on other inverted microscopes, the nosepiece is in a fixed position and the focusing knobs move the stage closer or further from the objective.(Fig. P) The advantage of an inverted microscope is that only one side of the mounted specimen under inspection need be perfectly flat, the side facing the objective. (On the upright stand, a leveling stage may be needed or the lower side of the specimen mount is pressed into plasticene to ensure its horizontality, in addition to having the polished specimen perpendicular to the optical axis). Another advantage is having an unobstructed stage so that relatively thick or heavy objects can be viewed since the illumination through the objective is all below the stage. The inverted stands incorporate the vertical illuminator into the body of the microscope. The same kinds of objectives are used as those on the upright stands. Here too, all modes of reflected light illumination may be possible: brightfield, darkfield, polarized light, Nomarski, and fluorescence. Many of the inverted microscopes have built-in 35 mm. and/or large format cameras or are modular to allow such accessories to be attached. Some of the instruments include a built-in magnification changer for zooming the image; contrast filters; and a variety of reticles, e.g. for grain size comparison.

The inverted microscope is a favorite instrument for metallographers; such stands are sometimes called metallographs.

PREPARATION OF METALLURGICAL SPECIMENS

The preparation of the specimen in metallography is most important. Although in the past few decades, there has been increasing automation of the grinding, polishing and finishing of the sample, there still is considerable skill and experience needed in selecting the procedures that are best suited for particular specimen types.

Each step in preparing the sample builds upon previous steps. The sample should be typical of the specimen being studied. The sample is selected by fracturing or by sectioning with special saws using diamond or silicon carbide, or by microtomy or other suitable means. The sample is embedded in phenolic or acrylic resin which hardens at room temperature (or in a special press under pressure at elevated temperature) into a block 1"-1$^1/_2$" in diameter and $^1/_2$"-$^3/_4$" high. The sample is cleaned before the grinding and polishing process is begun.

The grinding, a wet process to minimize deformation and keep the heat of friction down, is usually done on automatic grinding and lapping machines going from 120 grit down to a fineness of 600 grit. Then the coarse polishing is undertaken, using such material as diamond paste or SiO_2 slurry down from 30 micron surface unevenness down to a 3 micron unevenness to fine polishing down to 1 micron; usually the final polishing will be done by hand down to an unevenness of a fraction of a micron, 100 nanometers or less of unevenness horizontally.

Some metal (or ceramic) samples may then be examined as is. However, many types of specimens will require treatment of the surface to make the micro-grain structure

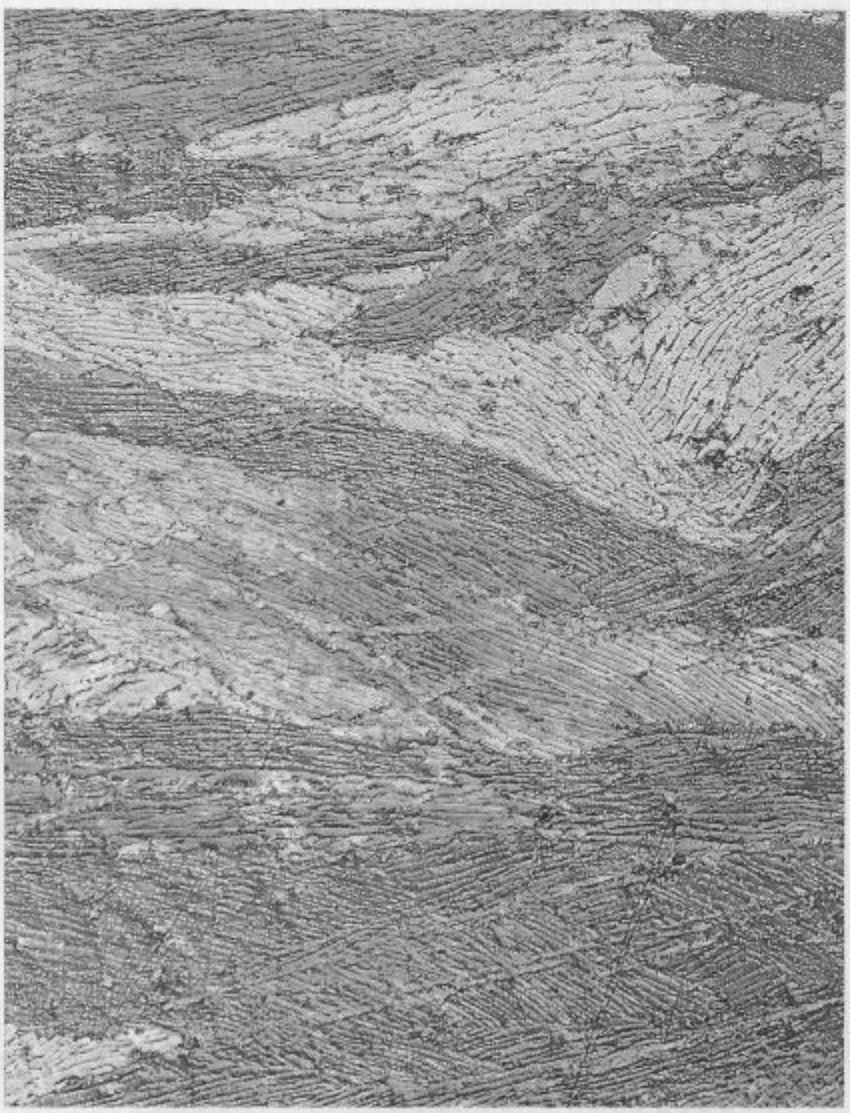
visible in the microscope. The most common treatment is chemical etching, essentially a controlled erosion process utilized to delineate clearly the boundaries of the various crystals, grains, or phases. There are other processes employed, e.g. tint etching, thermal etching, vapor deposition, electrolytic etching, anodizing, vacuum cathodic etching, etc.

Since many of the metallurgical samples are subject to deterioration from oxidation, it is generally good practice to keep samples in closeable plastic containers with desiccation packets; even to cover these samples with non-linting cotton wads to prevent scratching.

Brief Selected Bibliography

1. Abramowitz, M. Microscope Basics and Beyond, Vol. 1 Olympus Corp. Publication
2. Abramowitz, M. Contrast Methods in Microscopy, Transmitted Light, Vol. 2 Olympus Corp. Publication
3. American Society for Metals Vol. 9, Metallography and Microstructures 1985
4. Conn, G.K. and Bradshaw F.J., Polarized Light in Metallography, Butterworth and Co. 1952
5. Crouse, R.S., Gray, R.J., Leslie, B.C., Interpretive Techniques for Microstructural Analysis, Plenum Press 1977
6. Various editors and volumes Microstructural Science, Elsevier 1975
7. Photography Through The Microscope Publ. P2, Kodak 1988
8. Photomicrography of Metals, Kodak Publication 1971
9. Richardson, J.H., Optical Microscopy for the Materials Sciences, Marcel Dekker 1971
10. Vander Voort, G.F., Metallography, Principles and Practices, McGraw Hill 1984
11. H. Wolfgang Zieler, The Optical Performance of the Light Microscope Part One and Part Two, Microscope Publications Ltd. 1972
12. Handbook of Incident Light Microscopy, Carl Zeiss Corp.

Price $5.00



OC8-10907.5 RLM