Table 7. Comparison of correct and calculated values for the objects measured.

Object	Correct value	Calculated value	Calculated value less 5.0
A	75.0	79.4	74.4
B	85.0	90.4	85.4
C	65.0	70.6	65.6
D	55.0	59.0	54.0
E	45.0	50.6	45.6

The arithmetic for evaluating the objects is less involved than that used for the drift. To calculate an average for *A*, form the following differences:

Using part *a*, $A - B = -14$
Using part *g*, $A - C = 13$
Using part *j*, $A - D = 18$
Using part *d*, $A - E = 30$
Sum, $4A - (B + C + D + E) = 47$;
Equivalently, $5A - (A + B + C + D + E) = 47$;
And $A -$ average of all $= 9.4$;
Average of all 20 readings $= 70.0$; $A = 70.0 + 9.4 = 79.4$.

Table 7 shows the calculated averages for the objects alongside the correct values. There is evidently a marked discrepancy between the correct and calculated values. The fourth column shows the calculated values all diminished by 5.0, and now the two sets show good agreement. The correction, 5.0, cannot be evaluated in any actual case. It is, in fact, the average value of the drift introduced by the instrument. There is no way, short of the good fortune in having one of the objects a known standard, to separate out the average drift from the average of all the objects.

In much experimental work the *difference* between test items is all that is important to establish. Where absolute values are required, a standard object is indispensable. If the absolute value of one object is known, all other objects can then be determined.

Many choices are available in the construction of the sequence used. The parts or blocks may be of any size. For example, seven objects can be arranged in seven triads, or 10 objects in 10 triads.

ABD | BCE | CDF | DEG | EFA | FGB | GAC
ABE | HIJ | BHC | GEI | IDB | EFH | CJD | JGF | DAG | FCA

The first of these sequences is an example of a class of designs called balanced incomplete blocks. The second sequence is a partially balanced incomplete block design. Various discussions of these designs are available (*4, 5, 7*).

There is a final important comment to make. Comparisons of objects can be made even with a drifting instrument. Even when the instrument has been operating satisfactorily, the experimenter perforce usually has had to *assume* that this state was maintained while making the critical measurements. Statistical design makes it possible to show that the instrument did stay in adjustment and, if not, to introduce appropriate adjustments.

References and Notes

* Based on a talk given at the Gordon Research Conference on Instrumentation in 1954.
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Phase Microscopy 1950–1954

Oscar W. Richards

American Optical Co., Research Department, Southbridge, Massachusetts

THIS paper is an analytic summary of some 200 publications that have appeared for the most part since the publication of *Phase Microscopy* (*1*). Phase microscopy is now so generally used that it often does not appear in the titles or abstracts of papers. This makes a complete listing of papers nearly impossible, and omissions are the result of failure to find the publications. Phase microscopy is useful for the study of colorless transparent or nearly colorless transparent materials containing detail composed of small differences in optical path (refractive index × thickness).

The Phase Microscope

The principles on which the phase microscope is based are illustrated in Figs. 1, 2, and 3. On the left in Fig. 1 is shown light wave A' passing through a transparent object C and slowed down with respect to light wave A, which did not pass through the transparent object. Accordingly, light wave A' is out of phase with light wave A. However, both the human eye and photographic plates are insensitive to phase differences, and as a result the image can scarcely be seen or photographed. Light wave A'' passing through an absorbing medium E is reduced in amplitude (dis-

tance from crest to trough of the wave). In contrast to phase differences, amplitude differences are visible. When light waves of the same phase and amplitude R are combined in the image they add up to produce brighter contrast (Fig. 1, right). Dark contrast can be achieved by producing light waves S which are out of phase or amplitude with each other. Other combinations of amplitude and phase produce lighter or darker grays.

Figure 2 shows how light waves are superimposed. Section 1 shows that the wave P resulting from a slightly retarding particle may be broken up into two waves S and D. These waves, the central wave S and the diffracted wave D, are shown again more clearly in section 2. Section 3 shows the result of using a bright contrast diffraction plate. Wave S has been partially absorbed and retarded so that S and D are in phase and combine to produce a brighter image. Section 4 shows wave S partially absorbed and wave D retarded, so that S and D are out of phase and produce a darker image.

To convert a brightfield microscope into a phase microscope, one replaces the condenser with a phase condenser containing an annulus (Fig. 3, K, L) and the objective with a phase objective containing a diffraction plate (Fig. 3). No change is required in the rest of the equipment.

When the annulus is illuminated, its image is formed at the back focal plane of the objective. A specimen placed on the microscope deviates some of the light throughout the objective. The diffraction plate is made to fit the image of the annulus in the objective by evaporating onto the diffraction plate a metal layer of proper shape and thick enough to absorb as much of the nondeviated light as desired, and by using a dielectric material (MgF_2) to retard the light, or both. These materials can cover the conjugate area of the annular image, the rest of the area (complementary area) or part of each area. The contrast depends on the nature of the diffraction plate, the width of the annulus and conjugate area, and the position of the conjugate area. A retardation by a quarter wavelength is satisfactory for many specimens. Metal layers transmitting 7 to 20 percent give high to low contrast.

A diffraction plate with the dielectric retarding material and the metal on the conjugate area weakens and retards the undeviated background light without affecting the light deviated by the specimen; with it

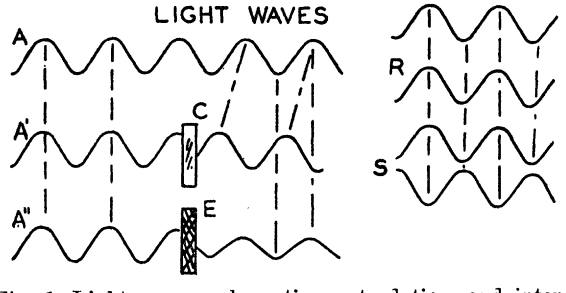

Fig. 1. Light waves: absorption, retardation, and interference.

regions of higher index in the specimen appear brighter than regions of lower index. This gives bright or negative phase contrast.

For dark, or positive, contrast the undeviated background light is absorbed by metal on the conjugate area and the deviated light from the specimen is retarded by the dielectric on the complementary area (A-type); or the undeviated light is weakened or retarded by metal and dielectric on the complementary area of the diffraction plate in the B-type of dark contrast phase microscopy.

These essentials briefly describe the ordinary phase microscope. Instead of fixed contrast diffraction plates, experimental work has been directed toward variable and color contrast. Details of construction, theory, and application are available in (*1*) and the papers mentioned in this review, which should be consulted for further information.

Theory and Instrumentation

General elementary papers have been published by Brice (*2*), Discombe (*3*), Hopkins (*4*), Hughes (*5*), Hughes and Cardew (*6*), Haselmann (*7*), Inagaki (*8*), Keuning (*9*), Lippert (*10*), Martin (*11*), and Richards (*12*). More advanced discussions include those of Berti (*13*), Françon and Šaez (*14*), Royer (*15*), and Kenohane (*16*), Radojičič and Brausil (*16a*). Osterberg (*17*) includes a simplified explanation of phase microscopy and states the conditions for maximum and minimum contrast. The vector theory of phase microscopy has been elaborated by Barer (*18*) and nomenclature for phase microscopy has been proposed by Barer (*19*) and Locquin (*20*). A book has appeared in Japanese by Mizuhira (*21*). Hansen *et al.* (*22*) present briefly the theory of the phase microscope and discuss its application to medicine. Hori and Hori (*23*) describe an approximation system for phase microscopy, and Torikai (*24*) discusses image formation in phase microscopy.

The Reichert vertical illumination phase equipment has been described by Gabler (*25*) and the Reichert phase microscope by Luschin (*26*). Haselmann (*27*) has written a general article on phase microscopy and has described the Winkel-Zeiss phase equipment. Zernike (*28*) has obtained a patent for achromatic and color diffraction plates. Françon and Nomarski's vertical phase illuminator has been patented and has been described for a Nachet microscope (*29*). Other patents include a slide for phase microscopy by Bennett (*30*), a turret by Aitcheson (*31*), and vertical illumination by Osterberg and Jupnik (*32*). Harrison (*33*) has written on homemade equipment for phase microscopy. Gottschewski (*34*) has described the Leitz phase microscope with a simple annulus in the condenser that may be magnified to match the diffraction plate by focusing the condenser and which also gives darkfield illumination (*35*).

Variable phase microscopy has progressed slowly because of technical difficulties in manufacturing. Cooke, Troughton, and Simms have made two variable phase microscopes, similar to the Polanret system of

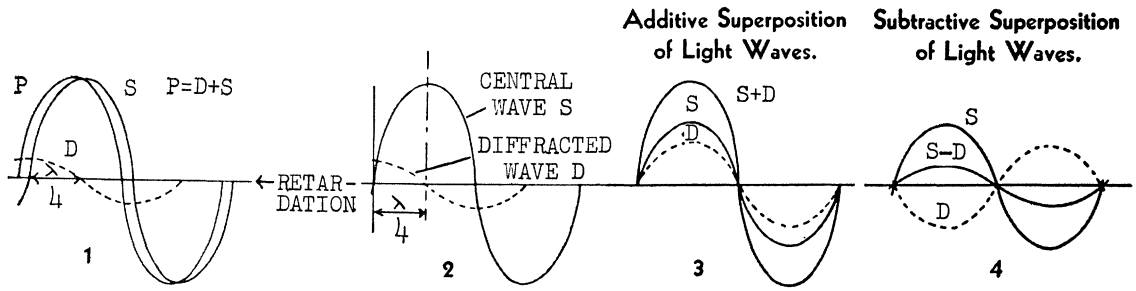

Fig. 2. Contrast control by adding or subtracting light.

Osterberg and Bennett (*36*) except that quartz was used in the diffraction plate instead of Polaroid, but they are marketing a multipupil system with variation in amplitude alone (*37*). Locquin (*38*) and Dufour and Locquin (*39*) used an interferential phase ring instead of the classical type to obtain variable contrast through varying the wavelength of the illumination; it is manufactured by the Société Wild. Variable color (Saylor's system) and comparison with Rhineberg illumination led Gabler (*25a*) to conclude that the best system would use a dispersion medium in the phase plate. Unresolved opaque particles may be measured by phase (*40*). Patents on variable phase have been issued to Kastler and Montarnal (*41*), Françon and Normarski (*42*), Groves (*43*), Taylor (*44*), Osterberg and Bennett (*36*), and Bennett (*45*). A quartz reflecting system for colored phase contrast has been patented by Françon (*46*).

Baker (*47*) recommends that 6-μ unstained sections of snail or slug ovotestis (Zenker fixed-paraffin sectioned) be used as test material for all powers of the phase microscope. He also advises against the use of monochromatic light for phase microscopy. If a green filter is desired, he prefers the Ilford No. 803 for both daylight and tungsten light. Rind (*48*) recommends Agfa X-Ray Fluorapid Film and other European films for photomicrography.

The halos seen around starch grains and other details with the phase microscope are attributed to lens effects by Frey-Wyssling (*49*), and a later paper (*50*) indicates that the images of lenslike objects are formed of mixed brightfield and phase portions. He also notes a reversal in contrast when the index of the medium is more or less than the index of the starch. Barer (*51*) advises mounting starch in cedar oil and using polarized light (*52*). Epstein (*53*), using a point source, concludes that the edge effect is the result of diffraction, and Butterfield *et al.* (*54*) have obtained smaller halos by using unequal areas in the annulus and diffraction plate. B-type dark contrast may be preferable to A-type because of smaller halos (*55*).

Phase Combined with Other Methods of Microscopy

Barer (*56*) discusses the possibilities of infrared phase microscopy and Taylor (*57*) describes the addition of phase to the Cooke, Troughton and Simms ultraviolet microscope. Phase is preferred to darkfield for locating the region to be photographed in ultraviolet. It is possible after taking the picture to shift back to phase to determine any damage from the ultraviolet radiation. King (*58*) describes the addition of a phase searcher to the Burch reflecting microscope. Comparison of the phase and ultraviolet pictures assists interpretation of the specimen, especially in the location of nucleic acids and proteins.

The use of the phase microscope together with other methods of microscopy is now frequent and is yielding considerable information. Richter (*59*) reports using fluorescence and phase microscopy for locating carcinogens within human blood cells and chick heart cultures. Methylcholanthrene, benzpyrene and dibenz-

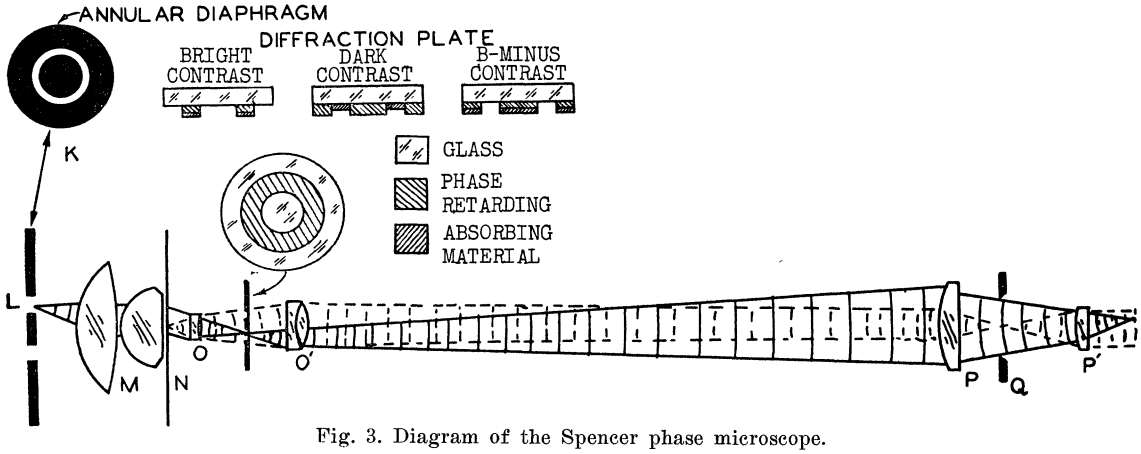

Fig. 3. Diagram of the Spencer phase microscope.

anthracene are soluble in the human and chicken plasma and concentrate in the chylomicrons. Intracellularly, they are located in the cytoplasmic fat droplets and the juxtanuclear Golgi granules. There is no fluorescent evidence of their being in the erythrocytes.

Pfeiffer (*60*) uses a Polanret system for the study of starch, combining polarized light and phase. After questioning by Barer (*61*) he admits (*60*) obtaining the idea from the work of Osterberg. Dolejsi is also using polarized light with phase microscopy. The advantages of phase microscopy with birefringent objects are pointed out by Barer (*62*) and the problems of combining phase and interference microscopy are discussed by Barer (*63*) and by Françon (*64*).

The phase and electron microscope combination is proving particularly fruitful. The phase microscope gives basic orientation and the electron microscope considerable detail (*65*). Ornstein and Polister (*66*) use thin sections for the electron microscope and slightly thicker ones (1 μ) for phase microscopy. With good fixation they find no need for staining, for phase shows adequate detail. Gey and Bang (*67*) also are using the combination to study the fibrous components of the hyaloplasm and the character of intracellular traffic in a typical and normal cell. Comparable work on bacteria is reported by Winkler *et al.* (*68*). Yamaguchi (*69*) used phase to examine the extent that replicas are faithful representations of the sources from which they are drawn. Phase and electron microscopes are elucidating the life cycle of fowl plague virus for Flewett and Challice (*70*).

The use of dispersion staining for the localization of free silica has been proposed by Crossmon (*71*). Koenig *et al.* (*72*) report photomicrographs of living cells obtained with phase and with ultraviolet microscopy. Focusing was done in visible light to minimize damage from the ultraviolet exposure. The ultraviolet pictures provide information on the morphologic distribution of nucleic acids and protein in living cells and show alternation from fixation which can be compared with the living by phase and by staining in brightfield.

Investigations using phase and electron microscopy on nervous tissue are in progress (*73, 74*). Dalton and Felix (*75*) are working in the epithelial cells of mouse and rat epididymis, and other combined applications will be described later. Phase is of aid in orientation for detailed electron microscope study, for instance with the nebenkern of the grasshopper spermatid (*76*).

Motion Pictures and Tissue Cultures

Cliffe (*77*) describes a simple motion picture apparatus for use with the phase microscope and Frederic discusses cytologic techniques with phase microscopy. The motion picture equipment designed by Michel (*78*) is manufactured by Winkel-Zeiss according to Frederic (*79*). Laporte and Roehrich-Goussu (*80*) describe zenon or krypton electronic flash for illumination. Twelve pictures were made per second with exposures of about 30 μsec (8 joules) with the 85X immersion objective. Locquin's varicolor system required 32 joules. The equipment can provide 100 flashes per second.

Chevremont and Frederic (*81*) report motion pictures of tissue culture and Haselmann (*82*) is using phase motion pictures to study the fixation process. A slide has been designed by Chevremont (*83*) for tissue culture phase microscopy.

Pomerat (*84*) has motion pictures of cultures of skeletal muscle and nervous tissue. Ruffled membranes were seen in cultures of cat nervous tissue along the course of the cell processes, and they postulate that perhaps *in vivo* similar activities may obtain and assist in the propulsion of interstitial fluid in brain tissue. Rhythmic pulsations have been observed by time-lapse motion pictures on the oligodendrologic cells from the corpus collosom of the rat and cat, as well as the human cerebral cortex. Rotation of the nuclei of human nasal mucosa is shown by motion pictures of the cultures (*85*).

Richter (*59*) has filmed the reactions between leucocytes and crystalline methylcholanthrene, and Policard and Collet (*86*) report a motion picture showing the phagocytic reaction to quartz and carbon particles. Besis and Bricka (*87*) and Bländau *et al.* (*88*) use phase motion pictures to study blood cells. Pseudopod formation in phagocytosis is described briefly by Frederic and Robineau (*89*). Lettré pictures fibroblasts with phase and with Ehrlich-Bondi stain (*89a*).

Lumsden and Pomerat (*55*) report the B-type dark contrast preferable to the A-type dark contrast for photography of tissue cultures because less detail is obscured by halos. Phase pictures of fibroblasts growing in an air bubble have been obtained with dark contrast phase by Slabeycius and Fisher (*90*) with a combination of Carrel and Lewis techniques for tissue culture. The effects of beryllium on the mitotic growth of tissue cultures have been examined by Chevremont and Firket (*91*). Applications of phase microscopy in tissue culture are summarized by Richards (*92*). The culture chamber of Christiansen *et al.* (*93*) should be useful in phase microscopy.

Microorganisms

Phase microscopy as reported by Linhardt (*94*) shows the gelatinous material in blue-green algae without reagents. Numerous studies of bacteria with phase microscopy have appeared. Techniques for the examination of unstained living bacteria are summarized by Poetschke and Bommer (*95*). A series by Delamanter (*96*) and his associates (*97*) suggests a mitotic division in bacteria. Their 1952 paper gives a review of their work on *T. pallidum* and *Borrelia anserina*. The formation of "large bodies" has been observed in *Proteus vulgaris* by Stempen and Hutchinson (*98*). Many of their pictures were made with low bright contrast phase and the stained and fixed preparations were examined with the medium dark A-type phase objectives.

Doglioni (*99*) found that many cells can be identi-

fied in sputum with phase microscopy. Cultures of avian tubercle bacilli show long filamentous mycelia, branches of which develop into long strands of bacilli. All observed types of growth resulted in the ultimate formation of acid-fast bacteria (*100*). Phase microscopy is reported to be about twice as good as bright-field for finding tuberculosis bacteria in some 2028 examinations of sputum, stomach washings, and so forth, by Poetschke *et al.* (*101*). Unstained tuberculosis bacteria are examined in phase and darkfield by Kolbel (*102*) to avoid the shrinking, coagulation, and destruction of the staining procedures, and Tokiwa (*103*) believes he has seen budding from a refractile body formed at one end of the tubercle bacillus.

Morphologic changes to penicillin in *P. vulgaris, E. coli* and *B. anthraxis* have been observed by Levaditi *et al.* (*104*) and Eisenstark *et al.* (*105*) have used phase and electron microscopy in the study of a pleomorphic strain of *Azotobacter*. Héden (*106*) used phase and a special chamber to mount *E. coli* with the phase microscope.

Phase microscopy proved helpful to Browning *et al.* (*107*) for the examination and timing of nuclear cytoplasmic and cortical divisions of *Tetrahymena,* and inclusions were studied by him (*108*) in colonies of the organism mounted in immersion oil.

Cells and Tissues

Passano (*109*) used phase for examining teased, living cell bodies from the crab *Sesarma.* The cells, from the organ forming the molt-inhibiting eye stock hormone, resemble steroid systems similar to the Golgi apparatus of Baker and Thomas. Good bright contrast phase pictures of mitochondria in the flight muscles of insects have been published by Watanabe and Williams (*110*). The association of mitochondria and the Golgi net is discussed by Taylor and Adamstone (*111*). After comparing phase with hematoxylin-eosin stained sections, Kludas and Maibauer (*112*) conclude that in many cases, but not all, phase reveals more structural detail, but they advise further comparison.

The refractive index of living cells has been obtained by Barer and Ross (*113*) and Crossmon (*114*) by immersing them in known solutions, for example bovine plasma albumen, and matching under the phase microscope. By combining this technique with the use of interference microscope, they claim that the total dry weight, the concentration of solids and water, and the cell thickness and volume can be determined. Methods for studying the third dimension of cells and tissues including the use of phase and interference microscopy have been summarized by Richards (*115*). Interference is useful also for the study of thickness, surface effects, and phase for making stereophotomicrographs. Changing contrast by varying the refractive index of the mounting fluid has been rediscovered by Hirsch (*116*). Refractive index data for some mounting mediums are given by Lillie (*116a*).

Further work on the effects of killing and fixing fluids has been reported. Rather and Brice (*117*) recommend Goucher cells from impression preparations of spleen for this work and report comparison with phase on unstained, and after hematoxylin and eosin staining. The effects of formaldehyde fixation have been analyzed by Crawford and Barer (*118*). Haselmann (*82*) has studied structural changes, using a circulation cell for tissues, and measuring area changes with a planimeter to assess shrinkage.

Policard *et al.* (*119*) have examined a number of fixing fluids, and conclude that osmic acid is the best because it causes no distortion other than slight shrinkage of the nucleolus with polymorphonuclear and thrombocytic cells. Other fixing fluids gave a mediocre result on nuclear sap, coagulating it in large masses, except bichloride of mercury and potassium bichromate which produced a fine reticulum. With these compounds the cytoplasm appeared well enough fixed. Almost all the fixatives thickened the cell membrane. Freeze-drying is useful but needs improving.

The phase microscope is being used to study the effects of various physiological agents. For instance, phase photographs have been made of cells of rabbit ovarian granulosa cultivated after having been cooled to a very low temperature (*120*). Phase microscopy has been applied to the Sabin-Feldman reaction by Lelong and Desmets (*121*), and to the Gomori reaction by Hancox and Nicholas (*122*). Zollinger (*122a*) discusses intravital phase microscopy.

The effects of a microbeam of protons on dividing cells are photographed in motion pictures by Bloom and Zirkle (*123*). Pomerat (*84*) describes a chamber for continuous perfusion of cells for environmental studies.

Giant chromosomes swollen in 0.14*M* sodium chloride have been examined by Ambrose and Gapal-Ayengar (*124*) with phase microscopy and by birefringence with a microscope arranged as a flicker dichroscope. An orientation of large molecules within the chromosome is indicated. Denues (*125*) disrupted chromosome fragments from chicken erythrocytes with a Waring Blendor for comparative pictures with phase and electron microscopes.

Tahmisian and Adamson (*126*) saw the chromatin in the resting grasshopper nucleus mounted in Belar's solution with dark medium phase. Pycnosis from x-ray effects appeared as a diminution of the viscosity in the nucleus. The effects can be delayed by placing the grasshopper embryos in cold storage, but when they are brought back to 25°C, pycnosis occurs. Roto-oscillating motions of the chromosomes were found in grasshopper eggs (*76, 127*).

New information on the surfaces of the bile-conducting cells in mammals is reported by Ralph (*128*). Thin 1-μ sections were used without stain, and contrast was varied by changing the refractive index of the mounting mediums. Evidence of spiral internal surface sculpturing was seen in the walls of the bile capillaries, and filamentous structures were seen on the interlobular bile ducts, hepatic cells, gall bladders, cystic ducts, and common bile ducts. Size and number varied in different species. Liver cells show nuclear walls with B-type contrast, Golgi apparatus with low

A-type contrast and mitochondria with medium A-type contrast using a Tiyoda Phase Microscope (*23, 129*).

Morris (*130*) reports that the phase microscope is satisfactory for the examination and evaluation of bull semen without fixing and staining, and Fossel (*131*) recommends the phase microscope for evaluation of spermatozoa. By varying the refractive index of the mounting medium, Blandau (*132*) saw more detail in rat spermatozoa taken from the epididymis than with previous methods. The perforatorium divides into two prongs which fit neatly along the rostral-ventral part of the elongated nucleus, and the axial filament was observed to be composed of 7 to 9 continuous fibrils that extend throughout the middle piece and tail. The fate of these parts in fertilization is being investigated.

The detailed description of the rat's spermatid is given by Austin and Sapsford (*133*) from preparations obtained by wiping a slide onto a cut surface of testis or from a bit of the macerated tissue. Van Duijn (*134*) has published a detailed description of the human spermatozoon from study with special differential stains, darkfield, phase, and fluorescence microscopy. Pictures of spermatogenesis in the grasshopper are published by Cleveland and Winchester (*135*), and by Winchester (*136*).

A slide with an aluminum spacer has been described by Smith (*137*) for culturing rabbit eggs for examination with the phase microscope. The phase and ultraviolet microscope were used by Austin (*138*) to determine the location of nucleic acids within the nuclei of living segmented rat eggs. Time relations and form changes of fertilization and first segmentation division in rat ova have been described by Odor and Blandel (*139*).

Incinerated blood cells examined by Kruczynski (*140*) revealed less potassium and iron in senile cells than in cells from younger individuals, although analyses showed considerable variation. Cellular and nuclear membranes leave mineral deposits composed of calcium and perhaps phosphorus.

Considerable interest has been shown in the counting of platelets by phase microscopy. Cazal and Izarn (*141*) recommend phase for counting of both platelets and reticulocytes. Brecher *et al.* (*142*) find that "Phase is the easy and certain recognition of individual platelets, and it is believed that this accounts for a satisfactory reproducibility even by inexperienced technicians." They use a diluting fluid without cocaine, and their work has been summarized (*143*). Zuckor (*144*) has used the phase microscope to study and classify platelets which vary in shape from smooth disks to others with spiny projections.

Weis-Fogh (*145*) has described a flicker phenomenon, or change of optical density, in erythrocytes which was thought to be a possible test for activity. A further study by Parpart *et al.* (*146*) showed that it could be seen with a change of mediums and in hemolyzed cells, and concluded it is not related to metabolism but to the Brownian movement of the cell. Tompkins (*147*) reports that the internal movements of the erythrocytes are lost in saline washed cells, but return when the cells are returned to the serum.

The movement of leucocytes is being investigated by Neuman and Kreis (*148*) and phase is recommended by Crossmon (*149*) for counting white blood cells. Phase microscopy is used by Shelton *et al.* (*150*) for counting mitochondria, and they include formulas for estimating errors. The storage of hemoglobin in kidney cells after intraperitoneal injections of hemolyzed blood has been examined with phase by Zingg and Zollinger (*151*).

Bright contrast phase shows hyaline membranes and amyloid deposits at the capillary wall and the intercapillary space of kidney glomeruli (*152*). The structure of skin and the detail of the sweat glands is described by Takagi (*153*) who used phase microscopy and fresh material. The lumen of the duct was found to be lined with epithelial cells throughout its length.

The transparent tissues of the eye can be examined to advantage with phase. François and Rabaey (*154*) find that epithelial cells of cataractous human lenses appear differently from those of normal lenses in vacuolation and pigment changes. No inclusion bodies were seen, although the normal structure was clearly seen in the cellular elements of the conjunctiva and corneal epithelium in epidemic keratoconjunctivitis (*154a*). The endothelium of the cornea is composed of large cells with 2 nuclei each and a granular cytoplasm. The structure of the cornea after the epithelium was removed is illustrated by a replica. Yamamoto (*155*) saw a membrane in unstained rabbit cornea resembling Boman's membrane. Böke has studied the lens epithelium (*156*).

Asayama and Nakashima (*157*) show stellate cells in the normal capsule and in cataract they found perinuclear vessicles.

The vitreous body shows definite fiber structure under the phase microscope. Bembridge (*158*), Swartz and Schuchardt (*159*), Grignolo (*160*) and Richards (*161*) have published pictures showing structure. Swartz and Schuchardt used phase and darkfield and an immersion ultramicroscope in their study. Grignolo shows that the fibers split into progressively smaller elements which anastomose to form a fairly extensive network posterior to the equator. In man, this is connected anteriorly with fibrous bundles that are firmly attached to the vitreous base. The vitreous is limited by a homogeneous membranelike transparent layer. Rossi fails to find fibers in very fresh rabbit vitreous and believes the fibers are formed postmortem (*162*).

An examination of a sympathetic ganglion with Sudan black and with phase led Gatenby and Moussa (*163*) to conclude that Baker and Owen were wrong with regard to the Golgi apparatus. Cole (*164*) has some pictures and comments on the study of rabbit muscle cells. In the mouse the Golgi apparatus is characteristically wrapped around the nucleus, but as the mouse ages, it breaks away from the nucleus. Green (*165*) found a copious innervation in the adenohypophysis of man and the rabbit using a controlled phase

and impregnation technique. Höpker (*166*) believes the "inner body" to be a physical product of the nucleolus. He used phase microscopy and a series of mammalian brains. Globules and filaments are found in brain tissue from mentally sick people and Papez (*167*) reports a life cycle for these "organisms."

Owen's lines of dentine and Retzius streaks in the enamel of the tooth have been observed with the phase microscope in 80- to 300-μ sections of tooth by Bodingbauer (*168*), and Gustafson (*169*) has made a detailed study of the structure of human dental enamel by means of incident light, polarized light, phase microscopy and micro-hardness tests. The epithelium around the teeth of vitamin-A deficient rats shows aplasia of the basement membrane, degeneration of the tonofibrils and parakeratosis of the surface layer according to Baum and Frandsen (*170*). A study of replicas from tooth surfaces by phase microscopy led Frank (*171*) to conclude that the initial step in caries is proteolysis. A fine cell structure in the organ of Corti is described by Katsuki and Covell (*172*).

Medical

The use of the phase microscope is gaining in the medical profession. Hansen *et al.* (*22*) and Matilla *et al.* (*173*) summarize the main applications as follows: (i) clinical analysis of urine sediments, vaginal exudates, feces, semen, sputum, cerebrospinal fluid, blood cells, and red, white, reticulocyte and platelet cell counts; (ii) protozoology for cysts and active animals; (iii) bacteriology for unstained, living organisms, agglutination, virus and inclusion bodies; (iv) helminthology, identification of eggs and diagnostic details of adults; (v) biopsy specimens, especially bone marrow and spleen; (vi) identification of fungi and fungus infections; (vii) normal and pathological histology, tumor diagnosis, phagocytosis, transplantation and regeneration; and (viii) measurement of unstained, living microorganisms.

Discombe (*174*) states that the diagnosis of leukemia can sometimes be made more rapidly with the phase microscope. Mizuhira (*175, 176*) discusses the advantages of phase for the examination of connective tissue and the arrangement of the fibers within it. Merklen and Cottenot (*177*) recommend phase microscopy for venereal dermatology. Other articles in this field are by Bommer (*178*), Brodskii (*179*), Fritze and Streufe (*180*), Kostic (*181*), Mannigault (*182*), and Pulvertaft (*183*). Lash and Antonow discuss cervical and endometrial carcinoma (*184*).

Phase motion pictures of a chemically induced rat tumor tissue show living leucocytes *within* the tumor cells (*185*). Seyfarth (*186*) suggests that the small granules in tumor cells are endospores, from which microorganisms develop. Ludford and Smiles (*187*) report on a malignant cell polymorphism. The phase microscope shows a canalicular system which enlarges in the giant cells to fill all but a small ectoplasm. The cytoplasm changes during mitosis with disorganization of the canalicular system, but remnants persist as vacuoles.

Martinez (*188*) concludes from a study of eight gliomas with H and E, and with silver staining and with phase that the phase microscope gives the same diagnosis more quickly and more easily than the classical methods. Staining methods also were reported cumbersome, damaging to tissue and time consuming, as compared with phase microscopy by Siering (*189*). Tumor cytology is the subject of a paper by Adehold and Siering (*190*) and ascites tumors are examined by Makino and Nakahara (*191*). Tumor diagnosis can be made from renal cells in the urine when biopsy is impractical (*192*).

Policard and Collet (*86*) call attention to the advantages of phase motion pictures for the study of phagocytosis with respect to pneumoconiosis.

Industrial

Phase microscopy has been reported by Camus *et al.* (*193*) as useful for the examination and evaluation of ghosts in gratings. Graininess in photographic emulsions has been examined by Falla (*194*); particle sizes of 0.1 to 0.4 μ were estimated with the phase microscope from the color of the photographic emulsion. A paper in Japanese by Tajima, Yamaguchi, and Hosoya (*195*) includes pictures of a number of photographic emulsions.

Seidenberg and Benford (*196*) describe the addition of a phase system to the Bausch & Lomb Metallograph and the advantage it gives in showing more detail, particularly of pearlite, stainless steel and Monel. The Reichert metallographic phase microscope is described by Gabler (*25*). Greater useful contrast occurs with particles in steel, chalcopyrite, coal, ores and refractories when examined with phase vertical illumination (*197*). Perryman and Vernon-Smith (*198*) are investigating the correlation of observations of metal surfaces with light and electron microscopes. Kehl (*199*) reports that phase microscopy is helpful in revealing prices and boundaries on metal surfaces.

The darkfield produced with a too large annulus image of the phase microscope is used by Yamaguchi (*200*) to examine the sharpness of razor blades. Crossmon (*71*) finds that phase microscopy is useful for the identification and counting of dust particles.

A brief summary of the possibilities of phase microscopy in the brewing industry was published by Richards (*201*).

Horst (*202*) discusses the study of coal. Replicas were made with glycerine jelly, and photomicrographs show algae, pollens, *Sclerotium,* and megaspores and microspores of *Triletes.* The article discusses the application of phase to the study of coal bogs.

The surface membrane was removed from wool fiber by Mariner (*203*) and studied with phase and polarization microscopy. With phase they could see a differentiation of intensities representing 300 to 400 A. In a summary of the proceedings of the conference on optical and electron microscopical properties of textile fibers, Drummond (*204*) discusses the use of different kinds of microscopes indicating that polarized phase is useful with viscose cross sections.

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