techniques, especially to a greater "discipline" in schools. Presumably this is to be obtained with some form of punishment, to be administered either with certain classical instruments of physical injury—the dried bullock's tail of the Greek teacher or the cane of the English schoolmaster—or as disapproval or failure, the frequency of which is to be increased by "raising standards." This is probably not a feasible solution. Not only education but Western culture as a whole is moving away from aversive practices. We cannot prepare young people for one kind of life in institutions organized on quite different principles. The discipline of the birch rod may facilitate learning, but we must remember that it also breeds followers of dictators and revolutionists.

In the light of our present knowledge a school system must be called a failure if it cannot induce students to learn except by threatening them for not learning. That this has always been the standard pattern simply emphasizes the importance of modern techniques. John Dewey was speaking for his culture and his time when he attacked aversive educational practices and appealed to teachers to turn to positive and humane methods. What he threw out should have been thrown out. Unfortunately he had too little to put in its place. Progressive education has been a temporizing measure which can now be effectively supplemented. Aversive practices can not only be replaced, they can be replaced with far more powerful techniques. The possibilities should be thoroughly explored if we are to build an educational system which will meet the present demand without sacrificing democratic principles.

References and Notes

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7. This material was prepared with the assistance of Susan R. Meyer.
8. Dr. Homme prepared sets of frames for teaching part of college physics (kinematics), and Mrs. Meyer has prepared and informally tested material in remedial reading and vocabulary building at the junior high school level. Others who have contributed to the development of teaching machines should be mentioned. Nathan H. Azrin cooperated with me in testing a version of a machine to teach arithmetic. C. B. Ferster and Stanley M. Sapon used a simple "machine" to teach German [see "An application of recent developments in psychology to the teaching of German," *Harvard Educational Rev.* 28, 1 (1958)]. Douglas Porter, of the Graduate School of Education at Harvard, has made an independent schoolroom test of machine instruction in spelling [see "Teaching machines," *Harvard Graduate School of Educ. Assoc. Bull.* 3, 1 (1958)]. Devra Cooper has experimented with the teaching of English composition for freshmen at the University of Kentucky. Thomas F. Gilbert, of the University of Georgia, has compared standard and machine instruction in an introductory course in psychology, and with the collaboration of J. E. Jewett has prepared material in algebra. The U.S. Naval Training Devices Center has recently contracted with the University of Pennsylvania for a study of programs relating to the machine instruction of servicemen, under the direction of Eugene H. Galanter.
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The Microfluoroscope

An x-ray microscope for direct visual or photometric measurements in biological specimens is described.

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The x-ray microscope in several forms has become increasingly valuable as a complementary instrument to light and electron microscopes. It is especially suited to quantitative *in situ* measurements of the mass or thickness of microscopic structures, and to specific types of elementary microchemical analysis. Present methods of x-ray microscopy may be grouped into four classes: (i) contact methods in which the x-ray image is initially recorded or detected at unity magnification, (ii) true focusing systems in which mirrors produce images by convergent x-rays, (iii) point-projection systems in which the image is formed as a geometrical shadow cast by divergent x-rays, and (iv) scanning systems in which a likeness is recreated by a time-sequential light pattern representing the x-ray absorption in the object. These methods and their applications are described in detail elsewhere (*1*).

In all x-ray microscopes the problem of obtaining adequate intensity is serious, especially at the long wavelengths (2 to 20 angstroms) which are necessary for observing most biological material at high magnifications. This is a consequence of the inefficiency of x-ray production at the low excitation voltages and atomic numbers of targets which are practical for producing these long wavelengths. The point-projection x-ray microscope can form a reasonably bright fluorescent image at about 1 micron resolution with 5 or 10 kilovolt x-rays, but as the source diameter and excitation voltage are reduced below this, the image becomes too dim for direct viewing. The scanning system may offer some advantages in the display intensity, but it is limited ultimately by the photon noise, in the same way that the point-projection method is. Order of magnitude estimates of this ultimate speed of point-projection and scanning systems do not give much hope for direct viewing at high magnification and useful field width, especially at soft wavelengths (*2*). In reflection x-ray microscopes, some gain in intensity at long wavelengths may result from an increase in useful mirror aperture, but this increase is not enough to overcome the inherently low intensity of reflection systems in comparison with other methods of x-ray-image formation. In most cases, as we shall show, the proper contact image geometry can still provide the highest x-ray intensity at the detector for a given resolution and width of field. It has the further practical advantage, that the specimen may be mounted in air, even for viewing with ultrasoft x-rays, since the total x-ray path may be made short enough to prevent appreciable atmospheric absorption.

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Comparison with Other Methods

We may easily compare the intensities of point-projection and contact x-ray microscopes operating at the same resolution and width of field. In both methods the width of field will be limited by the obliquity of the peripheral rays passing through the edge of the specimen, and also by the lower intensity at the edge of the field caused by the longer path length and increased target absorption of these oblique rays. A practical maximum width of field may be taken as one-half the target-to-specimen distance (A in Fig. 1). For the point-projection system the minimum geometrical resolving distance, at large magnification, is equivalent to the x-ray source diameter, s. In the contact image the minimum geometrical resolving distance at the surface of the specimen farthest from the detector is given by $R = sB/A$, where B is the specimen thickness. Points on the inside of the specimen, closer to the detector, will have correspondingly better geometric resolution. Consequently, for a given width of field and geometric resolution, the contact image may utilize a source diameter larger than the source diameter of the point-projection system by a factor A/B. For example, if we wish to obtain a geometrical resolution of 0.2 micron over a 100-micron field, the point-projection system will require a source diameter of 0.2 micron located at least 200 microns from the specimen. The contact image of a 5-micron-thick specimen with the same resolution and width of field could utilize an 8-micron source diameter. Since the maximum permissible specific loading on targets of these dimensions is roughly inversely proportional to the source diameter (*3*), the total energy on the detector in the contact system will be about 40 times greater than in the point-projection system, in this typical example.

For the long x-ray wavelengths it is also necessary to consider the diffraction resolution. We shall use as the diffraction resolution criterion the width of the first Fresnel fringe at the edge of an opaque screen, divided by the image magnification. This is given by

$$r = [\lambda ab/(a+b)]^{1/2}$$

where a is the source-to-screen distance and b is the screen-to-image distance. For the point-projection case where the magnification is much greater than unity and $b \gg a$, we may call $r^2 \cong \lambda a$; and in the contact method where $m=1$ and $b \ll a$, we may call $r^2 \cong \lambda b$, where b now corresponds to the maximum specimen thickness. If we set the diffraction resolution, defined by this criterion, equal to the geometric resolution, defined earlier, then in the example where $a = 200$ microns and $r = R = 0.2$ micron, we find that to keep the diffraction within this limit for a point-projection image, the wavelength cannot exceed 2 angstroms. This wavelength is too short to give adequate contrast in most thin sections of biological material. In order to use the point-projection system with softer radiation at this resolution, the specimen would have to be located closer to the source, thereby reducing the useful width of field. For the contact method with a specimen thickness of 5 microns, the corresponding maximum wavelength which would allow 0.2-micron resolution is 80 angstroms. This indicates that Fresnel diffraction will seldom be a problem in contact x-ray images even in the ultrasoft x-ray region, or with electron-optical enlargement.

In principle, therefore, contact-image formation stands comparison with other existing methods of x-ray microscopy very well. Not only is the contact method capable of much higher image intensity but, in the ultrasoft x-ray region useful for high-resolution biological studies, it is also capable of a higher diffraction resolution over a wider field of view than is possible with other existing systems.

As a practical matter, of course, the ideal grainless detector which is essential for making use of the contact-image capabilities does not exist. The most common materials in use for contact microradiography are the fine-grain Lippmann emulsions, such as Eastman Kodak's type 649 spectroscopic plate and Gevaert Lippmann film. Although the so-called grain size of these materials is below the resolution of the light microscope, the final developed image suffers from nonuniformity, which is clearly visible at high optical magnification. Recourt (*4*) has tried enlarging contact images made on this fine-grained film in the electron microscope in order to take full advantage of the possible resolution, but the film grain structure still presents a serious limit to this method. Ladd, Hess, and Ladd (*5*) have also used the electron microscope to improve the resolution of contact images. Their image-recording process depends upon a change of solubility of certain materials upon exposure to x-rays. A topographic image corresponding to the x-ray transmission of the specimen is thereby produced and then replicated for observation in the electron microscope. Similar investigations have been carried on by Warnes (*6*) in this laboratory, using only optical enlargement.

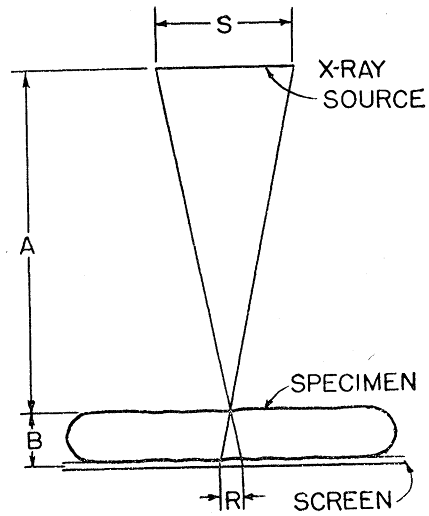


Fig. 1. Geometry of contact image formation (see text).

In an attempt to eliminate the intermediate x-ray recording material altogether, Huang (*7*) has used direct x-ray–to–electron conversion in a special photocathode which is placed at the object plane of an electron microscope. The resolution of this type of system is limited by the chromatic aberration of the photoelectrons. Again, the intensity presents serious restrictions.

Design of Microfluoroscope

The microfluoroscope under discussion is designed to take full advantage of the intensity which is attainable with contact-image formation in order to produce a direct-viewing x-ray microscope useful for the ultrasoft x-ray region. In principle, the microfluoroscope is no different from the ordinary fluoroscope. The x-rays diverge from their source, pass through the object under study, in which some are absorbed, and fall on a fluorescent screen which converts the x-ray energy to visible light which may be viewed directly. In the microfluoroscope, the size of the x-ray source, the distance from the source to the screen, and the grain size of the fluorescent screen have been scaled down by several orders of magnitude, and the final fluorescent image is then viewed with a high-power light microscope. The wavelength of the x-rays, on the other hand, has been increased to obtain higher contrast in very thin microscopic specimens.

A schematic diagram of this microfluoroscope is shown in Fig. 2. The three basic components are (i) a high-intensity, microfocus x-ray tube; (ii) a thin, fine-grained fluorescent screen; and (iii) a high numerical aperture viewing microscope. At the top of the figure is the electron source, which is imaged on the x-ray target by the electron lens. The demagnification is about times 20. The target is a metal foil (aluminum, copper, tungsten, or other metal) several microns thick, glued to the outside of a heavier metal disk, which in turn is sealed to the vacuum system with a small gasket. A septum valve at the target mount permits rapid replacement of targets without opening the column to air. The fluorescent screen, on a quartz cover slip, rests on the mechanical stage of an inverted metallurgical microscope. This stage can be positioned vertically so that the screen is within 50 microns of the x-ray source. The viewing-microscope objective is focused independently of the target-to-specimen distance adjustment. The incident light illuminator is useful for the initial alignment of the specimen, for the measurement of target-to-screen distance, and for observing the condition of the target foil. The microscope itself is arranged for either visual observation, photographic recording, or direct photometric measurement of the fluorescent image.

The principal operating dimensions may be determined by the following considerations: Given the specimen thickness, *B*, the minimum resolving distance, *R*, and the width of field, *F*, the first two lengths determine the maximum allowable angle which the x-ray source may subtend (see Fig. 1), and the width of field determines the minimum distance from the specimen at which the x-ray source may be placed. Since the maximum permissible specific loading increases approximately inversely with the source diameter, it is important to place the source as close to the specimen as is consistent with the desired field width, and to make it as large as is consistent with the given specimen thickness and desired resolution. Only in this way can the maximum intensity benefits of the high specific loading of the microfocus source be utilized. These requirements make it advisable to have both the diameter of the target spot and the target-to-specimen distance easily adjustable under operating conditions. The minimum target-to-specimen distance should be as small as 50 microns in order to obtain maximum intensity when viewing under the highest optical magnification.

The optical components should, of course, have the highest possible numerical aperture. Oil-immersion objectives help reduce internal reflection at the surface of the cover slip supporting the screen. Since the object is self-luminous, there may be an undue amount of stray light entering the microscope which will reduce the image contrast and cause errors in photometric measurements. This can be controlled by the correct placement of stops in the objective. Some gain in optical intensity is produced by evaporating a reflecting layer of aluminum on the polished surface of the fluorescent screen. This also reduces back reflection of light from the outside of the x-ray target foil.

A photograph of the microfluoroscope is shown in Fig. 3. The viewing microscope can be translated horizontally on a short optical bench in order to provide space in which to manipulate the specimen and view it under transmitted light, as well as access to the target area, which is necessary for replacing target foils. The microfocus x-ray tube is a greatly modified Metropolitan-Vickers diffraction tube. The electron gun and anode have been altered to produce a beam of circular cross section. The objective was converted to a pinhole electron lens after Liebmann (*8*) by the addition of a pole piece (designed by W. C. Nixon). This type of lens produces "strong" electron focusing but at the same time permits a relatively long working distance, so that the target foil may be located outside the magnetic field. Under normal operation this tube can produce a focal spot 10 microns in diameter with a specific loading of 1 or 2 megawatts per square centimeter, depending upon the excitation voltage. For observation with 8-angstrom wavelength, when an aluminum target foil 3-microns thick is used, it is possible to obtain adequate intensity for direct viewing at magnification of several hundred with 9-kilovolt excitation and target current of 60 microamperes. The optical microscope is a Unitron model BMEC-3 inverted metallurgical microscope.

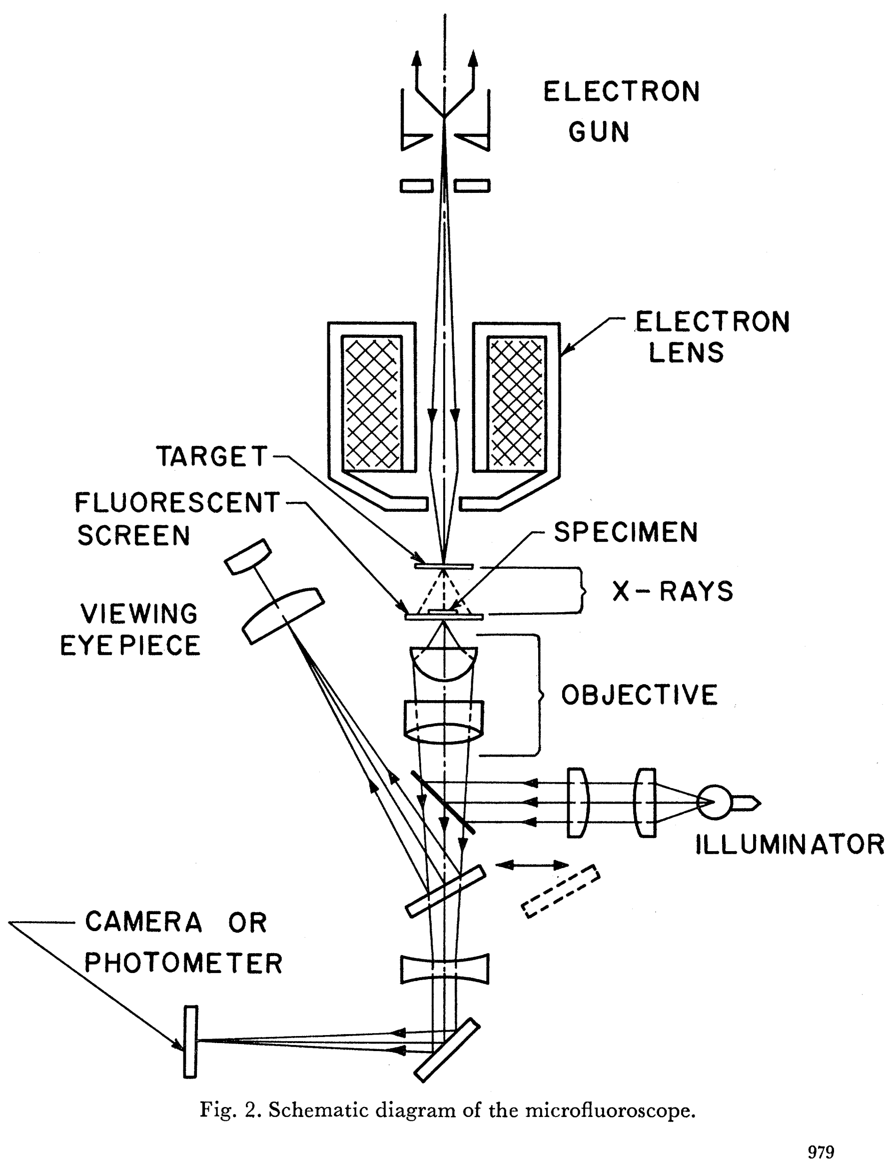


Fig. 2. Schematic diagram of the microfluoroscope.

Fluorescent Screen

The fluorescent screen itself is the most critical component of the microfluoroscope, since it must fulfill several functions and satisfy many conditions in order to provide a useful image. In the first place, the structure of the screen must be sufficiently microscopic to allow image resolution comparable to that possible with the light microscope (about 0.2 micron); secondly, the thickness of the fluorescent material must be less than the depth of focus of the light microscope if the image is to appear crisp and if photometric measurements on small objects are not to suffer from extraneous light produced outside of the focal plane of the microscope; thirdly, the screen must be made of a material which absorbs only those x-ray wavelengths which produce adequate contrast in the specimen under observation; and finally, the screen must be optically homogeneous and smooth in order to reduce light scattering. To these essential requirements we may add other desirable features of the screen such as high sensitivity to soft x-rays, uniformity over the field of view, and both chemical and mechanical stability.

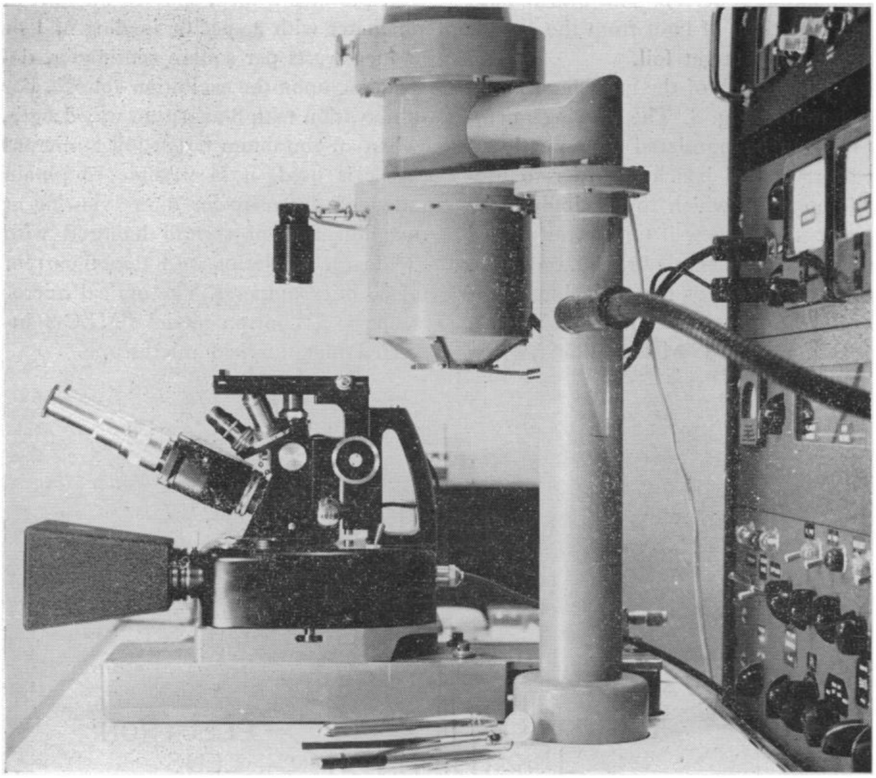
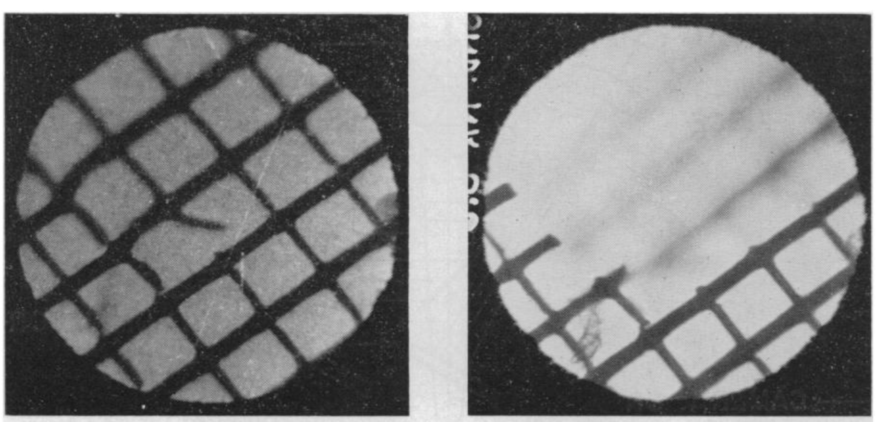


Fig. 3. Photograph of the microfluoroscope. The microfocus x-ray tube is shown at the top center. The viewing microscope is in position for mounting the specimen. For x-ray viewing, the microscope moves on the short optical bench until its objective is coaxial with the microfocus tube.



a b

Fig. 4. (*a,* left) Microfluorograph of the resolution test grid with 1500 bars per inch. The smallest bars are 2 microns wide. Note depth of field compared with that of the light micrograph (*b*) of the identical specimen. The x-ray wavelength is 8 angstroms. (*b,* right) Light micrograph of the identical grid shown in (*a*), photographed through the fluorescent screen. Both pictures were taken with a Leitz × 90, N.A. 1.32 oil-immersion objective.

The fluorescent screen from which the microfluorographs shown in Figs. 4*a* and 5*a* were taken was made by vacuum evaporation of manganese-activated zinc orthosilicate phosphor by means of a technique similar to that used by Feldman and O'Hara (*9*) for producing transparent cathode-ray tube screens. The substrate is a quartz cover slip thin enough to allow viewing with a 100 × oil-immersion objective when mounted in the microfluoroscope. After evaporation, the cover slips are placed in a covered porcelain dish and air-baked at 1100°C for a few minutes (*10*). In order to obtain a good optical surface on the screens, so that the specimen may be viewed with transmitted light without loss of quality, we follow the baking with optical polish, using a pitch lap and tin oxide abrasive. The screen thickness may be determined by interference methods or by direct measurements with a sensitive mechanical gauge. Screens have been made by this method in thicknesses ranging from 0.2 to 1.0 micron (*11*). Figure 4 illustrates some of the characteristics of the fluorescent screen. Figure 4*a* is a microfluorograph of a silver test grid with 1500 bars per inch. The smallest bars are 2 microns wide. The grain of the fluorescent screen is clearly visible in the background. However, the resolution is not seriously affected by this structure, as can be seen by comparison with Fig. 4*b,* which is a light micrograph of the identical section of grid mounted on the same fluorescent screen. Both micrographs were taken with a Leitz 90 × N.A. 1.32 oil-immersion objective. This figure also clearly illustrates the great depth of field of the x-ray image in comparison with the light image. The portion of the grid which is broken and bent out of the focal depth of the light micrograph shows clearly in the fluoroscopic image. This great depth of field allows stereoscopic photography at the highest magnification which provides an excellent method for directly measuring the thickness of structures within a specimen.

Figure 5*a* illustrates the quality of the x-rays producing the fluoroscopic image. One quadrant of the field is covered by a 6-micron Mylar sheet while one half the field is covered with a 3-micron aluminum foil. Photometric measurements indicate that the wavelength of the x-rays producing this contrast corresponds to 90-percent aluminum K radiation. The light micrograph of the identical specimen (Fig. 5*b*) shows practically total transmission in the Mylar and total absorption in the aluminum foil. A more significant difference between the x-ray and light image is the relatively large amount of scattered radiation in the light micrograph from dust particles and, especially, from the edge of the Mylar. This scattered light, which is essential to the formation of the image, nevertheless produces large errors in light absorption measurements, especially in inhomogeneous objects or on structures with dimensions comparable with the wavelength. Scattering in x-ray images at this wavelength is entirely negligible.

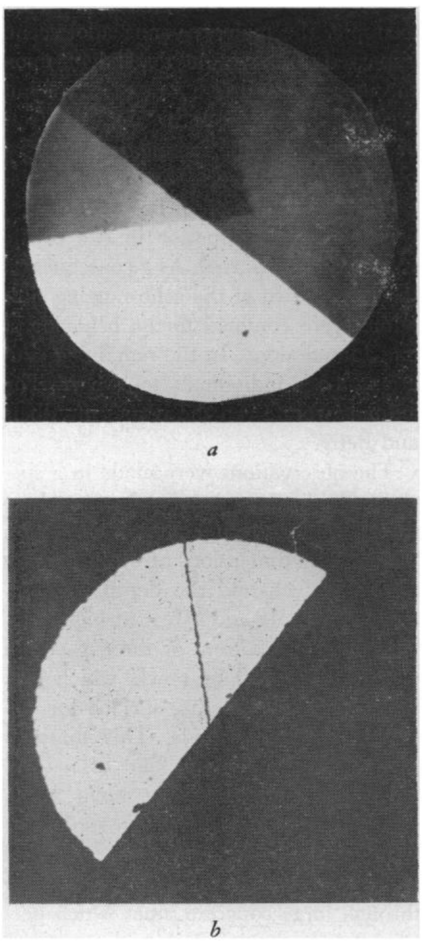


Fig. 5. (*a,* top) Microfluorograph of a contrast test object. Upper right quadrant is a Mylar sheet, 6 microns thick. Half the field is covered with aluminum foil, 3 microns thick. The x-ray wavelength is 8 angstroms. The contrast results only from absorption. (*b,* bottom) Light micrograph of the identical object shown in (*a*), still mounted on fluorescent screen. The edge of the Mylar sheet is visible because of scattered light, which may cause errors in absorption measurements.

A further indication of the contrast produced by 8-angstrom x-rays is shown in Fig. 6, which is a microfluorograph of cellulose fibers ranging in thickness from 2 to 20 microns.

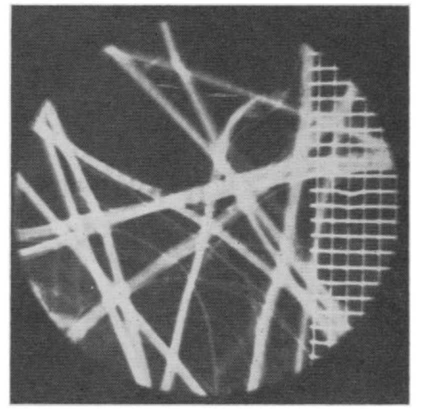


Fig. 6. Microfluorograph of cellulose fibers ranging in thickness from 2 to 20 microns, indicating the contrast range of 8-angstrom radiation. The 1500 bars-per-inch test grid indicates scale.

These evaporated fluorescent screens have distinct advantages over the fine-grained photograph emulsions which have been used to record contact x-ray images for many years. First of all, they can be made exceedingly thin, so that the light image is produced entirely within the depth of focus of the light microscope which is used to view the image. Secondly, by proper choice of the screen thickness, the target material, and the excitation voltage, it is possible to produce the x-ray image with a relatively pure, soft x-ray line spectrum without additional monochromatization, since the harder continuous components pass through the screen with little absorption. Thirdly, the excellent linearity of the fluorescent screen allows direct photometric measurements of x-ray absorption without the many difficulties usually associated with the photographic process and subsequent microdensitometry. Finally, the fluorescent material itself is comparatively durable and insensitive to light, so that the problems of specimen preparation and mounting are greatly reduced. For these reasons, such screens may be useful for other types of x-ray microscopes as well as for electron optics, for which they were originally developed.

Although improvements are being made in the quality of fluorescent screens for microfluoroscopy and in photographic emulsions for contact radiography, both methods have now approached very near to the resolution of the light optics by which they are usually viewed, and no large gain in resolution by these methods may be expected. On the other hand, enlargement of contact images by electron optical systems is a demonstrated possibility, and with the contact image geometry, diffraction and intensity limits at the ultrasoft wavelengths do not appear to be such insurmountable obstacles as they are for other methods of x-ray microscopy. It would appear likely, therefore, that any large improvement in the resolution of x-ray microscopes in the ultrasoft x-ray region would come from contact images enlarged by electron microscopy. However, the relative simplicity and speed of the microfluoroscope will make it uniquely suited to many quantitative microscopic investigations which do not require electron optical resolution. By eliminating the necessity for intermediate image recording, and by allowing specimen mounting in air and under normal illumination, this instrument approaches the simplicity of operation which is essential for routine observations and measurements (*12*).

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10. Thomas A. Midford, research assistant in physics, contributed valuable advice in the initial stages of work with fluorescent screens.
11. Some excellent evaporated fluorescent screens have been obtained through the courtesy of Dwight Barkley of the Liberty Mirror Division of Libbey-Owens-Ford Glass Co., Brackenridge, Pa.
12. This work has been supported in part by grants from the American Cancer Society (grant p-PH-15E) and from the National Institutes of Health (grant RG-4937).