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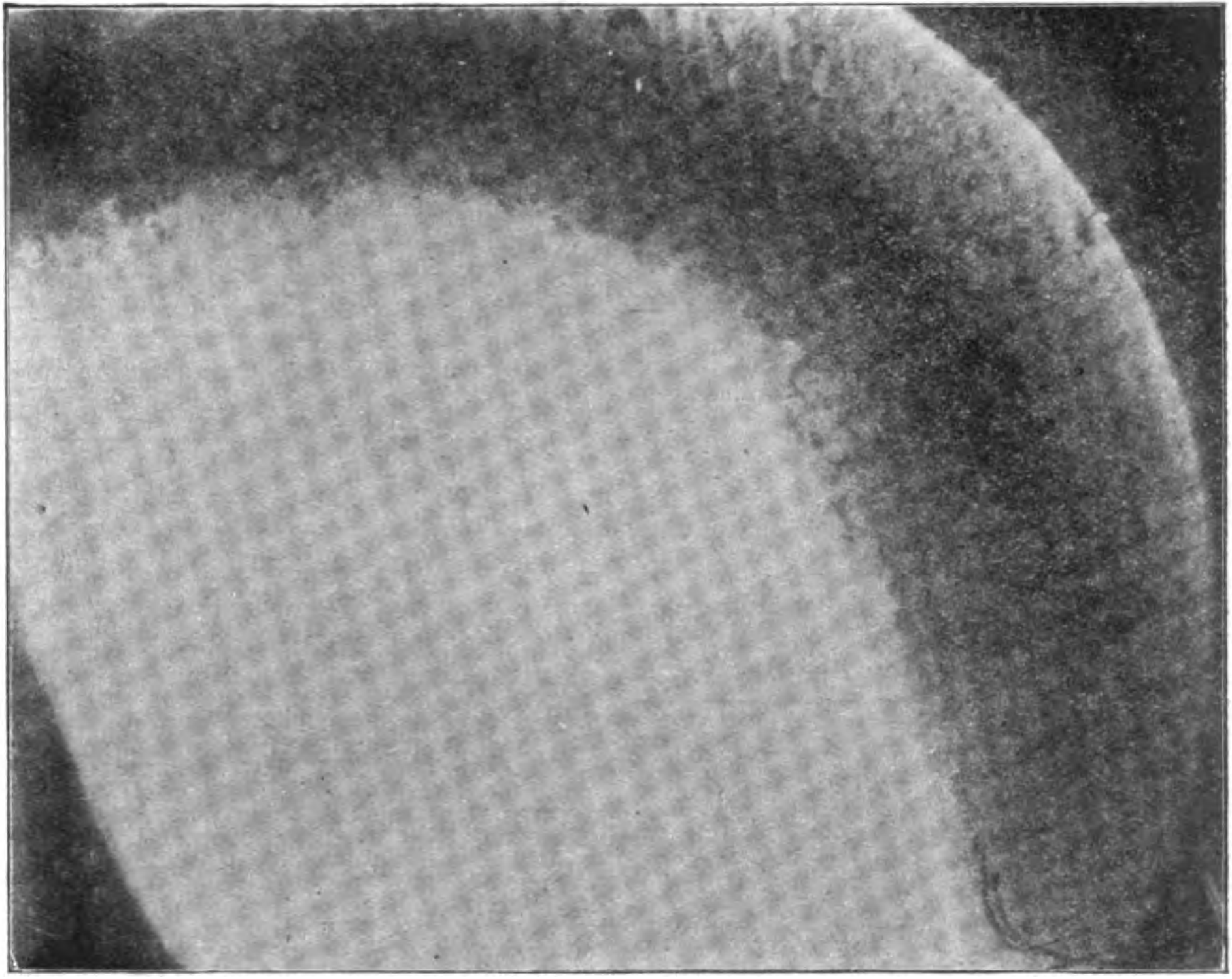


PART OF PLEUROSIGMA OPENING, MAGNIFIED 360,000 DIAMETERS. SEE PAGE 265.

A MEGA-MICROSCOPE.

BY DR. ELMER GATES.

I have been asked to give to the readers of this publication an accurate account of my recent work in microscopy, and judging by the random statements of some of the newspaper clippings sent me, such an account may not be amiss.

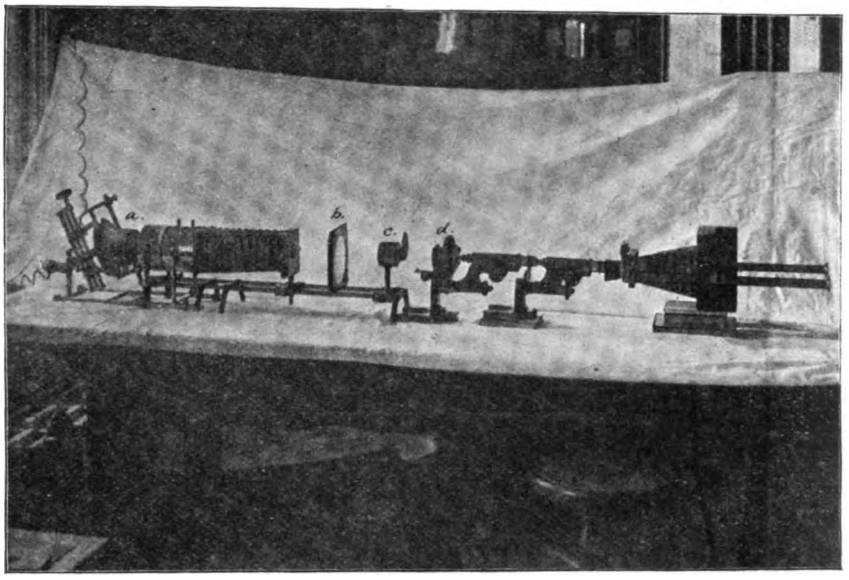
It is known that many attempts have been made to enlarge a photomicrograph so as to get additional details, but such attempts have been failures. On studying the subject, I found why this is so. It is because all the details in the image, which falls on the sensitive plate, that are less than about the ten-thousandth of a millimeter apart, are photographed as a single object, that is, the space between the two details is obliterated by the irradiation of the light over the area between them. Thus, if with a camera that neither enlarges nor diminishes the size of the object, I photograph a line of definite width, I find the line measurably larger in the negative than on the paper from which it was photographed. If I thus photograph two parallel lines which are quite close together, and at each successive picture move the lines closer and closer together, the time soon comes when they cease to be photographed as two lines and they become one line of greater than twice the width of the two lines.

This is perhaps due to two causes: first, to the irradiation of the light from the molecules of photosalt within the area of the image of the line, to the immediately adjacent molecules lying just outside of image-area; and second, to the diffusion of the light through the semi-transparent gelatine or albumen. Everyone has noticed that the edge of an object is not abrupt in a photograph—it gradually fades away. Of course the distance over which this edge spreads itself beyond its actual limits, is small, only about the ten-thousandth of a millimeter, but this is sufficient to cut out of a photograph all details beyond that limit. Hence I saw that it was useless to try to enlarge a photograph in order to get additional details—you cannot do it because the details are not there. It is not, as has often been supposed, a mere matter of photographing on a sensitive surface of sufficiently fine texture or with a photosalt of smaller molecules—it is a matter of irradiation of light from molecules and of diffusion of light through the gelatine.

The fact points out a probable method of improving the sensitive plate, namely to make the gelatine thinner and less transparent so as to diminish the amount and rate of diffusion. In fact, I have just found by experiment that to stain the sensitive film with a dark color composed of substances which are not photosalts, such as aniline, largely prevents this diffusion, so as to permit two lines to be placed closer together than heretofore and still be photographed as two lines This somewhat extends the range of the resolving power of photomicrography. This is of some importance, but it is not the discovery which it is the object of this article to discuss. The important point, so far, is that details closer together than .0001 mm. will not be separated by the sensitive plate.

Hence there occurred to me the idea of still further magnifying a small part of the focal-image-plane of the microscope before photographing that image, and the method which I used was to apply the objective of a second microscope to the focal plane of the ocular of the first microscope. At first I removed the ocular and introduced the objective of the second instrument into the sliding-tube of the first; later, I removed only the outer lens of the ocular and placed the objective of the second instrument about midway between the diaphragm of the ocular and the outer end, and this gives best results. By polishing off the projecting surfaces I can place the objective of the second instrument immediately against the lenticular surface of the outer-lens of the ocular of the first instrument, placing between the two surfaces a small droplet of liquid of nearly the same refractive index, and thus get a fair result, but not as good as the second method. By proper adjustment of oculars, draw-tubes, fine-adjustment screws, condensers and light, a focus is finally obtained which is correct for both instruments—and this is not an easy matter.

The figure is a photograph of the more recent form of the double microscope, or mega-microscope, and has not hitherto been published; it shows to a much better advantage the method of modern photomicrography. Upon the extreme left of this picture, and under the table are three resistance coils used to regulate the five-hundred-volt alternating current with which my laboratory is supplied, so as to reduce it suitably for the arc lamp (*a*), shown at the extreme left upon the table. Next to the right are the condensing lenses, and then the alum filter with its bellows, and after that the lenses used to render the rays parallel. The revolving diagram (*c*) is placed at the right hand end of the parallelizing lenses. Between the bellows of the filter-cell and the parallelizing lenses there is a screen holder (*b*), in which I place stained gelatin films to screen out such rays as may not be desirable. Beyond the revolving diaphragm the light next enters the sub-stage Abbé condenser (*d*) and thence through the microscopic slide to the two microscopes and camera. The light is about 2,000 candle power and the results are extremely satisfactory.



LATEST FORM OF MEGA-MICROSCOPE.

MAGNIFYING 360,000 DIAMETERS. SEE PAGE 270.

In a slightly modified form of the same apparatus, the two microscopes are provided with triple nose-pieces and projection lenses. I have just been using the apparatus shown in the figure, and have placed in it several test slides, and have not found any object that was not easily resolved by a one-sixth inch in the first microscope and a two-third inch in the second. Even a Pleurosigma pellucida is easily resolved. Mr. Bartsch or Prof. W. J. McGee, of the Smithsonian Institution, Mr. H. H. Doubleday, of Washington, D. C., and a very prominent instrument maker of great experience, have seen these tests applied, and can vouch for the verity of this assertion.

The complete form of the instrument now being manufactured, will consist of a solid brass bed-plate long enough to hold the entire system of apparatus, with means for adjusting the various parts from the rear of the camera. The entire system will be enclosed in a box capable of screening out all actinic rays. Dust and aqueous vapor particles will be

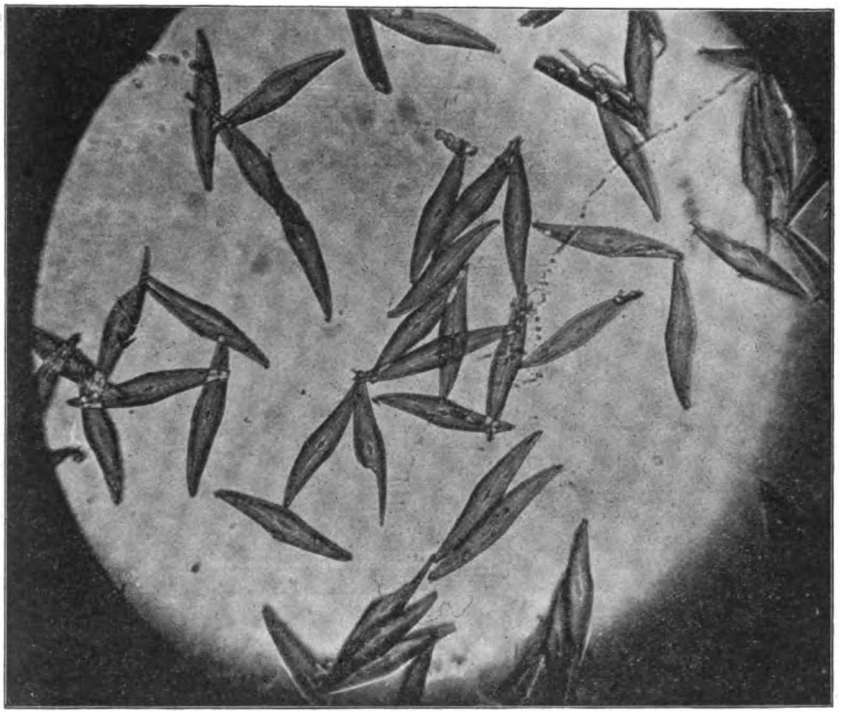
removed from the interior of the instrument by special device which need not here be described. I am having made a similar outfit from which I will have eliminated all the lenses which have hitherto been used for correcting chromatic aberration; also a new apparatus for furnishing parallel rays for monochromatic light of great intensity, and I am satisfied that ten million diameters can easily be photographed.

I am not quite certain as to the explanation of the result. I do not know whether it is more correct to say that the second objective views the "real" image of the first, or whether I had better say that it further magnifies part of the focal-image-plane. The "virtual" image seen by the eye possesses no details beyond the real image which the eye receives through the ocular. The result has been to me a matter of greater moment than the explanation.

The point to be settled, therefore, is this: Can a second microscope be used to view the magnified result of a first microscope so as to give details which the lens of highest power would not give singly. It is necessary to prove this, because it is widely taught that to get greater magnification and *detail* than a given lens, say a one-sixth inch objective, we must use a smaller lens, for example an eighth-inch, and get nearer the object. That this is not true I will at once proceed to demonstrate. First, let me mention to those not familiar with the microscope, that if we take a series of lenses ranging from 1 inch, $\frac{2}{3}$ inch, $\frac{1}{2}$ inch, $\frac{1}{4}$th inch, $\frac{1}{6}$th inch, $\frac{1}{10}$th inch, $\frac{1}{12}$th inch, $\frac{1}{16}$th inch to $\frac{1}{20}$th inch, etc, that the one-inch is the largest and the one-twentieth inch is the smallest of the series; and that the one-inch is farthest away from the object and magnifies the least; and that the twentieth-inch is nearest the object and magnifies the most.

Fig. 1 is a photomicrograph of a few diatoms, Pleurosigma angulatus, made with a $\frac{2}{3}$ inch lens, one inch ocular, 1600 mm. tube-length.

Fig. 2 is a photomicrograph of same diatoms made with a smaller lens which gets closer to the object and consequently magnifies more than the one just described. It is made with a one-sixth inch lens and same ocular and tube-length and camera-length. The magnification is about 450 diameters.



1. PLEUROSIGMA, WITH 2-3 INCH OBJECTIVE.

Fig. 3 is a photomicrograph with a one-twelfth inch, oil-immersion lens; it is still smaller and gets still nearer the object than the sixth-inch lens. The magnification is about 1,450 diameters. These pictures are made in a hurry with objectives that had been frequently taken apart and hurriedly readjusted and not sufficiently cleaned, and with a leaky camera. The light was reflected to the microscope by an ordinary mirror. Moreover, the cover glass over the diatoms was broken and some alcohol and cedar oil had leaked in.

Now if a widely accepted theory be true, the only way by which I can get a better magnification than with the above one-twelfth inch objective is to use a one-sixteenth inch, because no other lens would be smaller and nearer the object. But instead, I took a one-sixth inch objective—(the one with which I made Fig. 2)—for the *first* microscope, and the two-third inch objective—(the one with which I made Fig. 1)—for the second microscope, and focussed the objective of the second instrument upon the focal plane of the image in the ocular of the first instrument, the outer lens of ocular having been removed, and then to the ocular of the second instrument, I adjusted my photomicrographic camera, and the result was Fig. 5, in which the magnification is about 6,000 diameters.

This proves that with two lenses low down in the series—a two-third and a sixth—I have obtained a better result than with an expensive twelfth!

Then I tried a still lower lens in the second instrument—an inch lens—and a twelfth in the first instrument. The twelfth is the one with which I obtained Fig. 3. I used shortest tube-lengths and two-inch oculars, and the result is Fig 6, which was a little out of focus. The magnification is nearly 10,000 diameters.

So far I have demonstrated that better work can be done with low power lenses by using a double-microscope than with the highest power lenses, if but a single microscope be used. This augurs well for the popularization of the study of the higher and more interesting domains of microscopy, because an instrument can now be constructed with a few cheap objectives capable of doing better work than has hitherto been possible with objectives costing twenty times as much. With a sixth-inch objective in the first microscope and a one-half inch in the second, I have resolved many markings and details in well-known microscopic objects that could not be resolved with the best sixteenth-inch apochromatic of high aperture.

When higher objectives than a sixth and a half are used the eye can no longer see the image, because of its faintness. But where the eye fails, the sensitive plate comes to our aid, and photographs the otherwise invisible image. The sensitive plate acts cumulatively and gathers into one concrete result the continuous action of the faint rays, for seconds and minutes of time, and thus records the higher magnifications. By this means, I succeeded in using a sixth upon a sixth, and secured Fig. 7, which is a poor picture of one corner of a single *one* of the "lattice-work" openings shown in Fig. 5 and 6. The magnification enters what has hitherto been the ultra-microscopic domain. The magnification is about 360,000 diameters! This makes the opening 40 inches in diameter. and it would be necessary to take over 200 photographs 3 inch by 3 inch, and paste them into one map to show this one hole!

A blood cell under such a magnification would cover a map 600 inches square! A sixth on a sixth is probably the limit with an ordinary camera in an ordinary dark-room, and ordinary photographic technique. Photomicrography has hitherto, so far as I know, obtained no results much beyond 10,000 diameters or 100,000,000 million times the area of the original object. Any given detail in such a photomicrograph is made with the $\frac{1}{100000000}$th the amount of light that comes from the

corresponding part of the object observed.

But I have succeeded in getting a picture with only the $\frac{1}{1800}$th of that amount of light. How did I do it? What advantage is there in using one microscope focussed on another microscope? There is this notable advantage:—the old way required the use of lenses whose apertures were smaller and smaller as the magnification was more and more, thus admitting less and less light than through the larger lenses of the double microscope. But the old method has another fatal defect, namely, when the hole for the admission of light through the lens gets too small it diffracts the light, the same as when a slit through which light is admitted is too small; it breaks up the light into various colors or wave-lengths, thus interfering with the formation of an image.

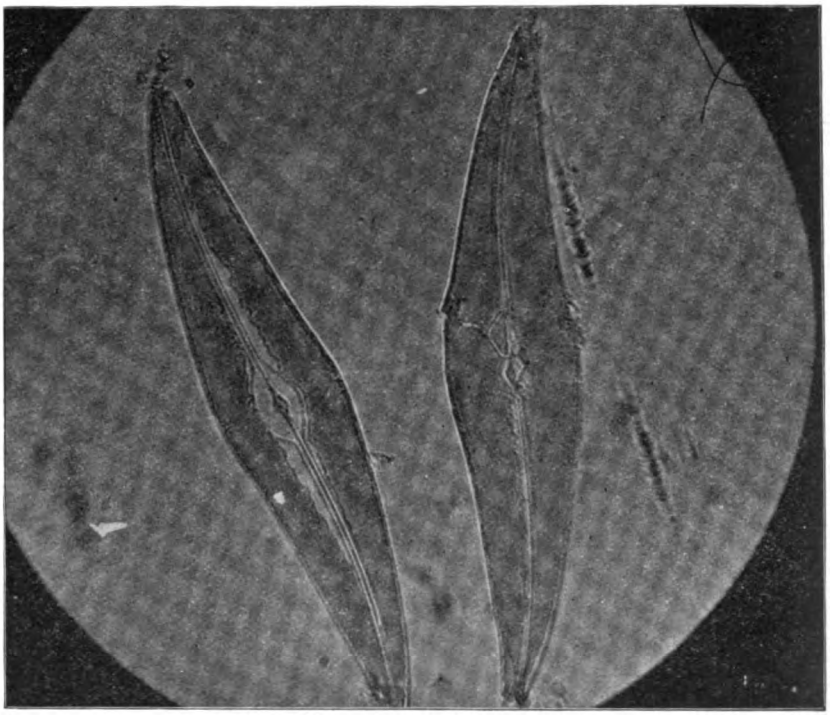
If any such breaking up of the light is admissible, it must be due to the object at which we are looking and not to the size of the hole for the admission of light through the lens. In the double system the lens may have a large aperture, thus eliminating this source of imperfection. But a small hole also forms an image of its own apart from the image formed by the lens—it is a faint one, but nevertheless it interferes with photography.

Under special conditions the eye can see the image of a sixth on a sixth. The microscopist must go into a completely darkened room and remain there an hour or more until the glare of entoptic "after-images" of light have entirely died out of the eyes. Having previously arranged the microscopes so that no light can enter except through a suitably arranged hole in the wall, with a porte lumiere in the hole, he can then try to see the image. No light must enter the room except through the microscope. Under these conditions the eyes behold a new world—a realm far beyond the ken of the present scope of bacteriology and histology.

To use still higher powers, a new photographic technique is required which will enable us to make photomicrographs with fainter light than has hitherto been thought possible. The apparatus which I am now constructing for this purpose is based upon some experiments which I made to determine the influence of dust and aqueous vapor globules upon the rays which form the image. I found that in order to use fainter light than hitherto, I had to take these substances out of the path of the rays between the objectives and the sensitive plate, for the reason that these particles intercept the rays, polarize and diffract them, and thus not only destroy clearness and definition, but these particles become luminous centers and are photographed more rapidly than the details of the faint image, and completely drown the image so that no picture is obtained. I find by experiment that when extraneous rays cross the path of the rays from the objective to the sensitive plate, the image is less perfect, and general diffused light resulting from the reflection from particle to particle of dust utterly prevents photomicrography with light beyond a certain faintness.

In the photomicrographic apparatus now being constructed, I have arranged to exclude from the interiors of the microscopes and camera all dust particles and aqueous vapor globules. Then the light can act cumulatively hour after hour and day after day if necessary, and the photogenic changes made on the sensitive plate will result wholly from the action of the image. From some tests already made I think I am safe in saying that owing to this device I shall be able to photograph with less than the one-hundredth part the intensity of light formerly considered necessary. This improvement is applicable to photography in general, but especially so to photomicrography. First by using the wider lenses of the double microscope I photographed with the 1-1,300th the usual amount of light, thus making a magnification of 360,000 diameters or 129,000,000,000 times the *area* possible. Exclusion of dust particles promises to permit the use of a hundredth part of this latter amount of light, that is, it makes possible the photography of over *three and a half million diameters* or over 12,000,000,000,000 times the *area*.

But I found another source of trouble in the use of such very faint light, namely, the leakage of actinic rays through the wooden and leather walls of the camera and through the imperfectly-fitting sliding joints and connections of the microscopes. With reference to ordinary photomicrography this leakage is but a small percentage of the amount of light which reaches the sensitive plate, but when we get beyond a sixth-inch objective in the first microscope and a sixth-inch in the second, we are dealing with quantities of light much less than the amount of leakage in the best cameras probably ever before constructed. A sixth-inch objective can magnify, with proper oculars and tube-lengths, at least 600 diameters, which is an area of 360,000 times that of the object. Hence any point of the magnified image has only the one-three-hundred-sixty-thousandth the intensity of light of the corresponding part of the object. But when on this already faint part of the image I focus another sixth inch objective I still further spread that light over an area 360,000 greater and I get the 1-360,-000th of the intensity of light with which such photomicrography has hitherto been accomplished, that is, I have the 1-360,000th of the 1-360,000th the amount of light coming from the corresponding part of the object.

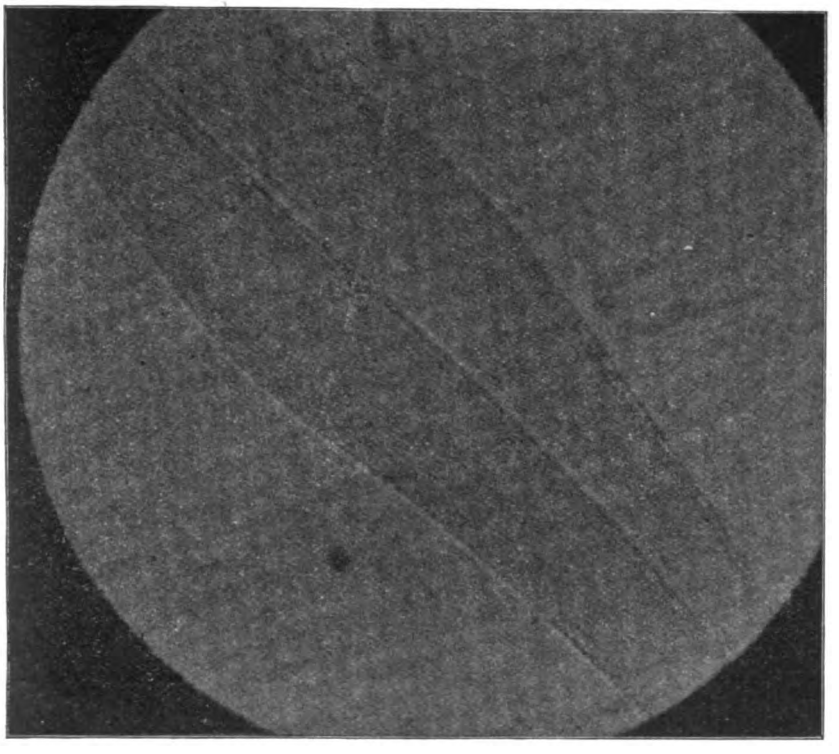


2. PLEUROSIGMA MAGNIFED 450 DIAMETERS.

If I use a one-twelfth in the first microscope and a sixth in the second, the magnification will be 2,000 times 600 diameters or 1,200,000 diameters which equals an area of 1,440,000,000,000 times that of the original object. This seems incredible, but I have already obtained evidence of being able to photograph a magnification of over three million diameters, or over twelve trillions of times the area. But if I mistake not, the limit is far beyond this, and that limit is one of photochemistry and not of photography as hitherto known. Beyond a certain point the rays will doubtless grow too weak to effect chemical changes. The energy may not be sufficient to decompose the molecule, no matter how long the ray acts. We do not know where that limit is, but it can be shown to be very

far beyond three and a half million diameters.

If in addition to using the larger lenses of the double microscope and the exclusion of dust and aqueous vapor, we also put the entire apparatus inside of an actinic-proof box, we shall still further extend this capacity to a fainter light. If only one-tenth less light can be used it will make possible ten-million diameters or one hundred trillion areas. If one-hundredth the amount of light can be used then 100,000,000 diameters are possible. So far, I am quite sure of being able to effect more than 3,5000,000 diameters; how much more we shall never know unless we find out, and that is what I am busy doing.



3. PLEUROSIGMA, MAGNIFIED 1,450 DIAMETERS.

There is another source of leakage and interference that has not, so far as I know, been hitherto corrected, namely, that which occurs in and between the objective and the condenser. Light from all directions impinges upon the condenser, upon the glass-slide, upon the tissue being examined, upon the cover-glass, and upon the front surface of the objective, thus distorting and weakening the rays from which the photographic effect is to be obtained. This produces a vast amount of useless interference of waves. I find that when I protect all those parts from light, a much better result is obtainable with a given amount of light. Hence in all cases where the light is transmitted through the object the use of such a light-shield from objective to condenser will still further extend the probable limit of magnification.

But there is a limit not so far away as that of the before-mentioned photochemic sensitivity of light, namely, the destruction of the energy of those rays within the perfected camera by their mutual interference. This will probably place the practical limit somewhere between about ten million diameters and the much higher limit of photochemic sensibility.

By using monochromatic light this "interference" limit will be at a much higher magnification than with white light. If we could get light of only one wave-length it would certainly be quite useful to the new microscope, but a narrow range of the best actinic portion of the spectrum will do much better than polychromatic light.

But monochromatic light is desirable for quite another reason. It enables a much greater amonnt of light to be concentrated upon the tissues being examined upon the slide without acting as a burning glass to destroy the object. Rays near the upper limit of the spectrum do not so rapidly heat an object as the lower rays or as white light. Hence I am arranging to focus the blue rays upon a large prism, parallelize them, and then transmit them through the objective. By starting with a large area of blue light I hope to get a much greater intensity of light into the microscope than has hitherto been attempted. As far as I know I shall have at least one hundred times as much. This will extend the heretofore assigned limit of photomicrography magnification by the new method.

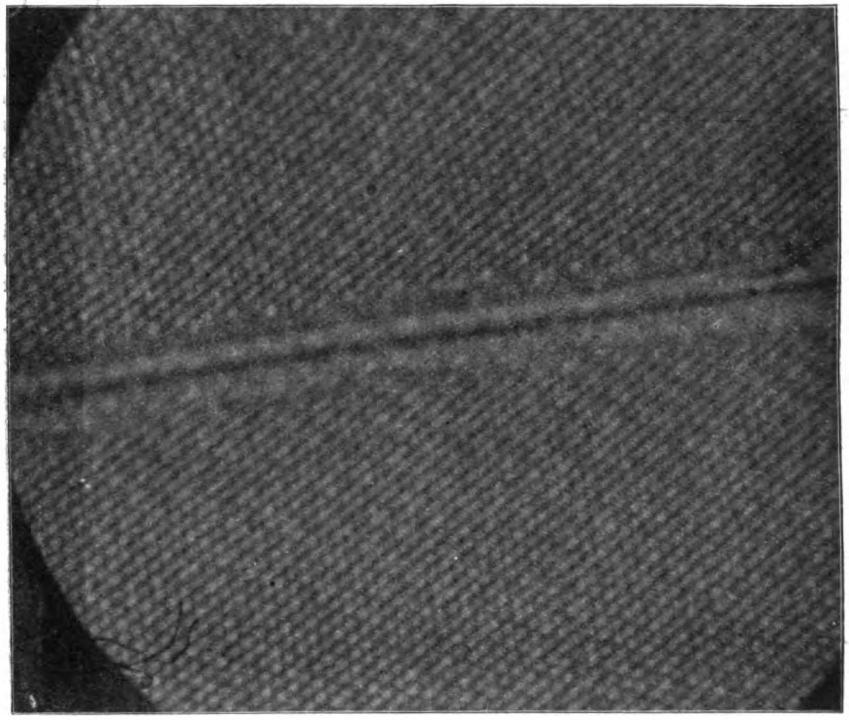
Monochromatic light makes possible another important improvement. I do not mean the use of the well-known sub-stage spectroscopic attachment, but of large prisms in series so as to give a large area of actinic rays of one color, and then the focussing of these rays to a diameter several hundred or thousand times less, then making them parallel, and sending them into the substage condenser as you would a beam of sunlight from the heliostat. Such rays obviate the necessity for the usual additional lenses for correcting chromatic aberation. As is well known, the different colored rays come to a focus at different distances from the lens. It requires several lenses, in addition to the ones required for resolution and magnification, to correct this and make the rays come to one focus. If rays of one color are used only, then no corrections need be made for the rays of the colors not used. This requires less thickness of glass for the rays to pass through, and consequently the light will be stronger, and this again slightly extends the formerly assigned limits. As far as I know the microscope which I am now having built is the first one that has been made especially for one-colored light. It requires correction for only one color, and for spherical aberration, and this latter presents less difficulty for larger than for smaller lenses.

I have also used a third microscope focussed upon the ocular of the second, using my lowest power oculars and objectives, and the result is conformable to the laws of magnification for the double microscope. With two-inch oculars and one-inch objectives I secured a magnification more than equal to a sixteenth-inch objective in a single microscope. But the defects and the striæ in the glass of the lenses become painfully apparent.

Truly this is a marvellous universe. The earlier anatomists were astonished to find that the fibers and tissues of the animal body are composed of smaller units, called cells. Later, it was found that putrefactious and infectious diseases are caused by minute unicellular organisms. We may now soon hope to learn that these seemingly simple cells are composed of units approximately as much smaller than a cell as a cell is smaller than a man; and then find that these units are built up of still smaller units, and so on, until the molecule is reached. Chemically we conceive the molecule to be made of atoms, and the atom is doubtless a highly complex structure made of still smaller components, and so on, towards an inconceivable infinitude of infinitessimals.

It should be added that I am having made a new microtome, by means of which I have taken thin slices out of the center of the nucleus of a blood-cell. Upon a narrow slip, one-fourth inch wide, of celluloid, I deposited a thin layer of a mixture of beeswax and rosin. I dissolve the beeswax in turpentine and then add a very small amount of rosin and coat the plate with the solution. When the turpentine evaporates it leaves a thin layer of cement. Upon the waxy surface of this plate I place a layer of blood-cells by spreading out very thinly a droplet of blood previously stained. The plate is then warmed until the wax melts and then is allowed to cool. Then I place on top of this plate another waxed

celluloid slip, warm it and allow it to cool. The cells are thus placed between two thin layers of wax and are cemented to



5. PLEUROSIGMA, MAGNIFIED 6,000 DIAMETERS.

those layers. Between the two celluloid slips I introduce a very thin sheet of copper or platinum, made as thin as possible and sharpened in the following manner: The edge is observed under a twelfth inch objective while it is being polished by a piece of soft wood moved rapidly to and fro along the edge of the blade by machinery. This produces an edge very much finer than can be produced upon razor-steel by the usual methods of sharpening a microtome blade. Passing this blade between the two celluloid strips it slices many of the blood-cells into two parts. Then I take one of the slides and lay upon it another thinly coated celluloid strip and again pass the slicing-blade between the two strips, and the result is that the half cells are again sliced thus giving me a section out of the midst of a cell of exceeding thinness,—very much thinner than has ever before been produced.

A successful application of the microscope to the telescope, giving assurance of great results, is omitted here for lack of space.

Anilin Colors for Vegetable Sections.

The anilin colors of to-day are so varied and numerous, that by observing a few well-established rules, one may obtain the most exact differential results, and at the same time have mounts of rare beauty. For this purpose the anilin may be broadly divided into three classes (as demonstrated by Vinassa), to wit:

1. Those which stain parenchyma only.
2. Those staining liginfied tissues, collenchyma, vessels and nuclear sheaths.
3. Stains that simply differentiate parts.

In the first class are safranin, Congo red, benzo-purpurins.

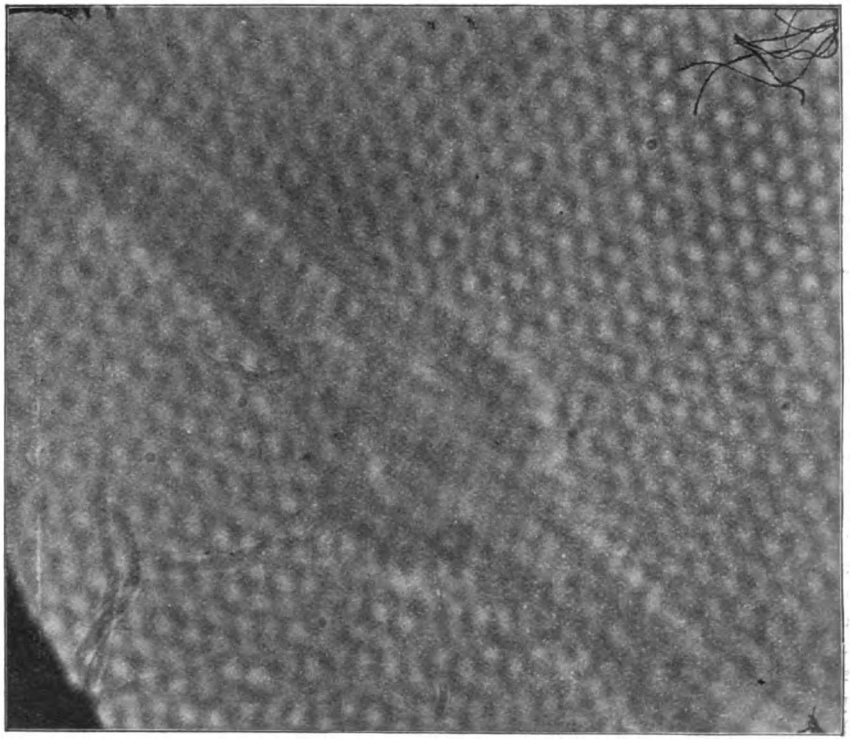
In the second are vesuvin, Victoria green, chrysoidin, violet, methyl green, fuchsin, etc.

In the third are the Victoria blues (B, RRRR, and BB.,) which color the thickened cells darker than the surroundings and thus render them conspicuous.

To get good results all protoplasm must be got rid of by boiling with caustic soda in solution, washing with plenty of water, acetic acid, and finally draining thoroughly. (Boiling in Labarraque's solution will effect the same end) The stain is used at once, dehydration being unnecessary at this stage of the process. Use weak solutions of the anilin colors—1 to 1½% being strong enough, and let the sections remain in them from two to three minutes. On removing, wash under a gentle stream of water until the latter comes away clear. Glycerin jelly makes a good mounting medium and its use renders dehydration, etc., unnecessary. Preparations mounted in it keep much better when balsam or dammar is used.—Nat. Drug.

Bacterial Mud.

An instance of oxide of iron associated with bacteria occurring on a large scale has been investigated by Prof. Manabu Miyoshi, who holds the chair of botany in the University of Tokio, in Japan. The material examined by him was a ferruginous mud from the hot springs of Ikao. This mud consists entirely of bacteria. The bacteria seem to resemble the leptothrix ochracea, of Kütz, but further observations will be necessary in order to determine whether they constitute a new species or not. From experiments made with hydrochloric acid, Prof. Miyoshi is led to believe that the oxide of iron is not deposited on the walls of the cells but is more or less intimately incorporated with them. No others of the more common iron-containing bacteria, such as Crenothrix kühmana, were found in this particular mud.—Lancet.



6. PLEUROSIGMA, MAGNIFIED 10,000 DIAMETERS.