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PRESIDENTIAL ADDRESS.

V.—SOME ASPECTS OF ULTRA-VIOLET MICROSCOPY.

By J. E. Barnard, F.R.S.

(*Delivered January* 16, 1929.)

Three Plates and Five Text-Figures.

The subject that I have selected for this, my third Presidential address, is one to which I have devoted a good deal of my available time during the last few years. I did, on one occasion, bring to the notice of the Society some of the results obtained, but I have never yet described in detail the apparatus that I have designed, and in large part constructed, for the production of ultra-violet photo-micrographs. For this failure to describe adequately the methods evolved I have been condemned in no uncertain terms by many, and by some few whose opinion I value. The result has been, I admit, in some respects unfortunate, as it has given an opportunity for descriptions to be launched that are not, perhaps, strictly accurate, and descriptions which would certainly not have been launched had the material that I have at my disposal been available. This, however, will have little effect ultimately on a research that will not be fully justified, much less completed, for some time to come.

A Presidential address is no opportunity for repairing omissions, and I have no intention of making the attempt. It is rather an occasion for a general survey of a subject, enlivened, it may be, with some speculative, if impracticable, ideas as to future developments. Many suggestions have reached me for carrying out most, if not all, of my technique in some better way; but, perhaps because I am now too old to be sufficiently receptive, I have continued along lines that appear to me to have some promise of success. In the hope that I may be able to interest some of those present this evening, I shall only deal with those parts of my subject that have general interest, leaving the technical side for a more general survey which I hope to publish in the Journal of this Society.

There are a good many practical points that are not generally appreciated, the salient one of which, perhaps, is that in ultra-violet work of any sort we are confined to the use of certain natural products—that is, artificial materials such as manufactured glasses, which are generally used in visual microscopical work, are as yet of no real service. We have, therefore, to fall back on substances that occur in Nature, and those are very limited in number. I have on show here to-night just a few samples of the materials that are available, the first of which in importance is quartz. Quartz occurs naturally in the crystalline state, but it is variable, as all natural materials are, and it is doubly refracting. There are two fine specimens of quartz on the table, one of which is clear and colourless, while the other one shows a certain smokiness in mass due to some impurity, probably iron. The result is that if you break it up or look at thin slices, you will, on cursory examination, see no difference between a good specimen and a specimen that is not so good. If you test it for transparency to ultra-violet light, you find the smoky material is less transparent, and it would not do at all for some purposes. In recent years quartz has been fused in sufficiently large pieces to be of use for making small lenses. It is, however, difficult to produce it in homogeneous pieces of any size, and this difficulty is a very real one in the production of microscope objectives for use in the ultra-violet region, where the correction has to be of a high order—how high it is not yet possible to define with accuracy—although it is probable that both in design and construction the necessary precision is at least doubled.

Another remarkable material is fluorite, which, as you know, is used to form one of the components of an apochromatic objective. For the latter purpose it is not so important that the fluorite should be free from fluorescence, but with ultra-violet light work the question of fluorescence becomes a really important one. I have two specimens on the table here which we will illuminate by means of a beam of ultra-violet light. You will notice that one specimen fluoresces brightly enough for all to see, whereas the other specimen is quite free from fluorescence. To an observer in visual light both are fine specimens, but the one that fluoresces would be useless for any part of an ultra-violet microscope; it would convert too much energy into visible light. The slide on which the object is mounted for ultra-violet microscopy is made from crystalline quartz, and the cover-glass is usually of fused quartz. Therefore the difficulty of getting suitable material is greater in the case of the cover-glass than of the slide. Fused quartz is used because its property of double refraction is largely, and in some few cases entirely, eliminated, but there are other faults that can become only too apparent.

Figs. 1 and 2 are photographs of two such cover-glasses taken by projecting a beam through them horizontally—that is, in the plane of the paper if lying on the table. One cover-glass is free from faults, but the other one is full of small flaws. To the ordinary eye, if observed without any means of increasing the apparent size of these defects, little difference is to be seen, but for photographic work in the ultra-violet one specimen would be satisfactory, the other useless.

Fig. 1.

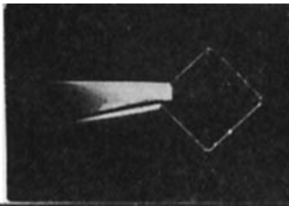
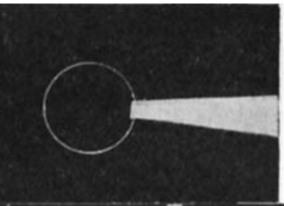


Fig. 2.



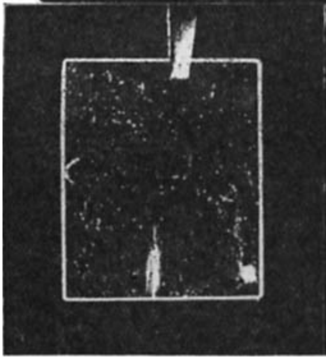


Fig. 3.

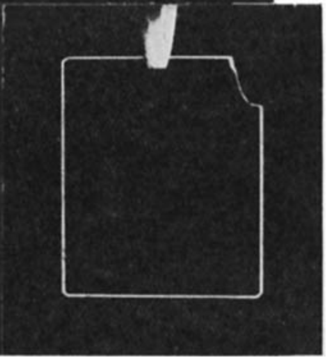


Fig. 4.

Figs. 3 and 4 show slides made from fused quartz which have the same type of defect, but the imperfections are accentuated owing to greater thickness. Objectives are still being made from fused quartz; it is therefore of the first importance to ensure that the material is free from defects of any sort. It is only fair to Messrs. Zeiss to say that when they made their first apparatus, over twenty years ago, they were apparently in a strong position in this respect; they were even at that time able to produce fused quartz of high quality.

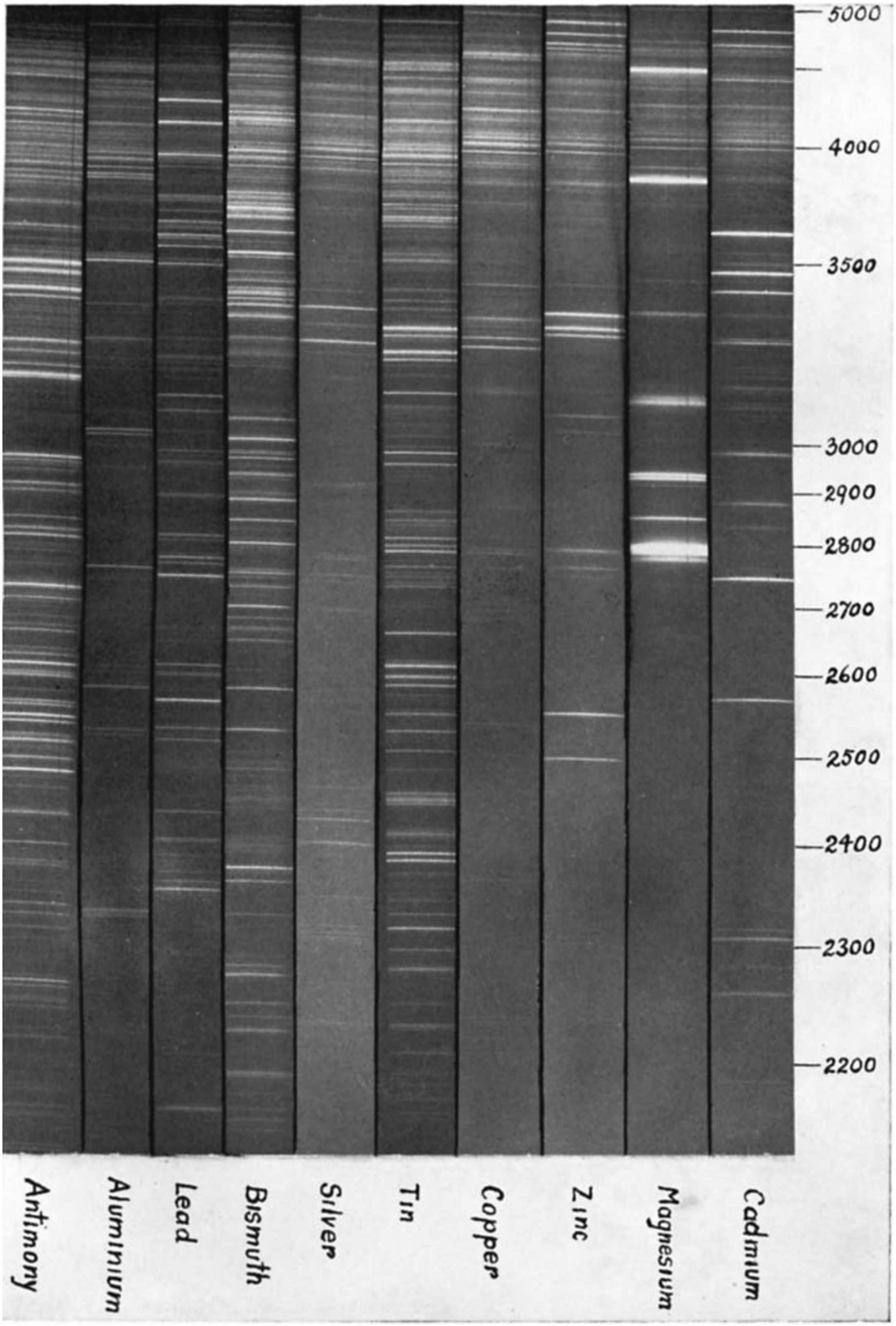
At the present time perhaps the most formidable difficulty in the production of a quartz objective is to obtain material of the necessary quality. When this is available, such objectives can be made in this country—as

Messrs. R. & J. Beck, Ltd., have shown—which are unsurpassed elsewhere. Among other substances familiar to us as microscopists are calcite and selenite, both of which possess considerable transparency to ultra-violet, but neither are as yet of value in ultra-violet microscopy except for experimental purposes. It is of interest to note that this Society has in its collection a very large and apparently fine block of crystalline calcite, as well as a specimen of selenite of unusual optical quality, both of which are on view at this meeting.

Let us for a few moments consider the question of illuminants suitable for ultra-violet work, as there is a considerable departure from ordinary practice in this essential. It is important to remember that the objectives made hitherto are corrected for light of one wave-length only. It is quite different from the position to which we are accustomed with visual light, where our objectives are corrected for the whole range of the visual spectrum. The reason for this difference will already have become evident to some of my audience. It is an unavoidable result of using one material only for the construction of objectives. Where correction of any sort has to be obtained by variation in curvature of component lenses or by separation, the possibility of combining lenses of suitable optical qualities made from different materials does not exist.

It follows, therefore, that the source of light must be in a practical sense of one wave-length. In the present state of knowledge such an illuminant can only be produced by means of a high-tension electric discharge taking place between electrodes of suitable pure metals. By this method the energy output is confined to single bright lines or small groups of lines separated by dark spaces. It is of some interest to see what that means, as there are few metals that give a suitable spectrum for such work (plate I).

A few spectra are shown to illustrate the most suitable type. Cadmium is the best, as it has bright lines at 227, 257 and $275\mu\mu$, each being separated by dark spaces or by spaces containing faint lines. It is not only necessary to have bright lines of the utmost obtainable intensity, but these must be separated sufficiently to ensure that the image of the spark in one wave-length can be projected into the field of view of the microscope without any overlapping images. It will be appreciated that the method of illumination is to split up the spark image by means of quartz prisms and to project the spectral images so obtained on to the back lens of the sub-stage condenser. The cadmium spark image in wave-length $275\mu\mu$ is still the most suitable for the purpose, and is the one used by Kohler in the original design of apparatus as made by Zeiss. In magnesium at $283\mu\mu$ a very bright group of lines occurs, which can be used for some purposes, but the results are not so good, although the necessary exposure is substantially reduced. The remaining spectra show the distribution of light energy with the intervening dark spaces. Some of them are of use, but others, owing to the large numbers of lines and their uneven distribution throughout the spectrum, are at present of little value. It is necessary to remember that


5000
4000
3500
3000
2900
2800
2700
2600
2500
2400
2300
2200
Antimony
Aluminium
Lead
Bismuth
Silver
Tin
Copper
Zinc
Magnesium
Cadmium

the apparent reduction in intensity of the shorter wave-lengths is largely due to want of sensitiveness of the ordinary photographic plate to that region.

One of the reasons for the use of ultra-violet light in biological work is that no staining is necessary; it would, in fact, defeat the very purpose of the method. The full advantage of this will not become apparent until objectives are available which can be used over a range of wave-lengths; even if this range is short, the gain will be great. The difference of absorption by organic materials even over a range of a few Angstrom units is evident in recent published work, and makes an appreciable difference in the result. It is very difficult, if not impossible, to use screens in the sense that we use colour screens for visual light. There are not many screens that are sufficiently definite in transmission and absorption for use in the ultra-violet; the loss of energy is considerable, and there is the possibility of rapid change in the screen itself, as strictly selective absorption is not obtained easily by using solutions of inorganic salts.

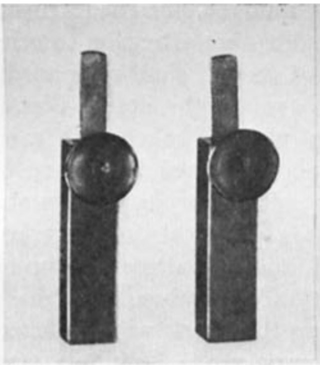


FIG. 5.

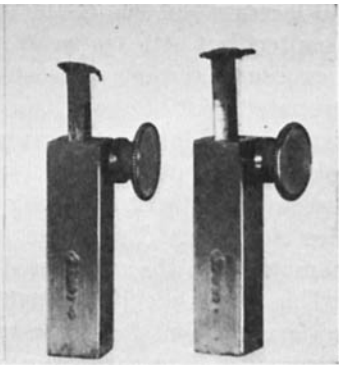
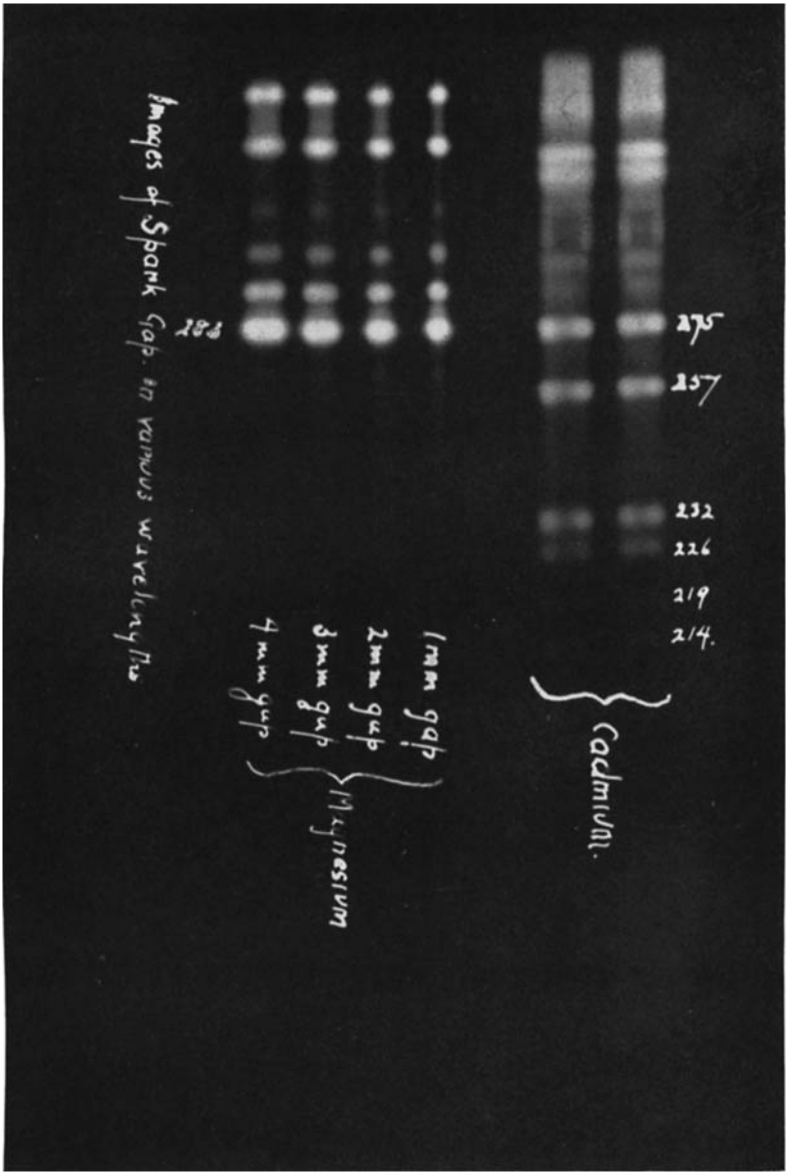


FIG. 6.

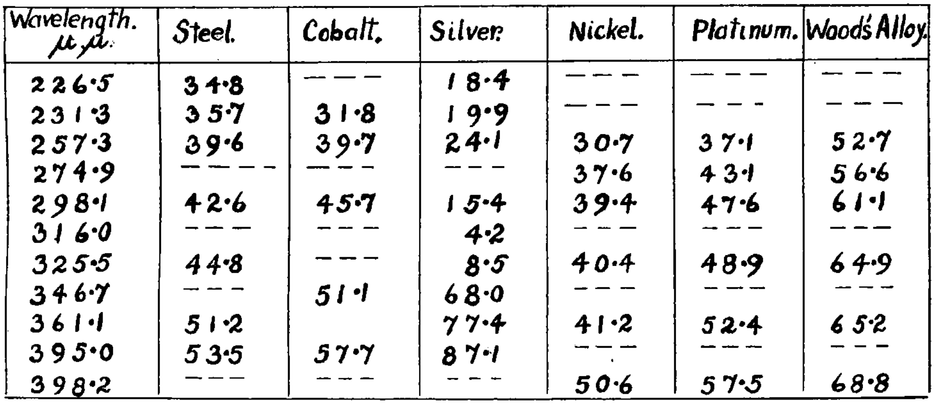
There is one rather important point about spark illumination that will appeal to all microscopists, and that is the actual nature of the spark image. In visual work we like a circular illuminant of uniform luminosity, so that the image of the radiant may be projected, if desired, into the field of view, but this state of affairs is not secured so easily with a spark. The difficulty is increased when various metals are used, as the necessary current, density and length of spark vary with the properties of the metals used. Thus cadmium and magnesium, which are the two most useful metals, have quite different standards of behaviour. Figs. 5 and 6 illustrate one of these differences, and show that while magnesium burns away steadily, maintaining a fairly constant end form, cadmium, presumably owing to its being more ductile, spreads out into a mushroom-like excrescence. The result is that with cadmium a spark is obtained with two intense areas, one at the end of each electrode; it is, in fact, a pole effect with an intervening gap of less intensity. If spark length is reduced too much, the intensity falls off and other electrical troubles appear; but under suitable conditions, which can only be determined experimentally for each metal, a round, uniform and intense light source is produced. From a microscopical point of view it is almost ideal. Plate III shows the difference sufficiently clearly to need no further explanation. This variability in the light source has given us much trouble; no definite rule can be laid down except by determining experimentally the necessary current and spark length for each metal used. We should like to get a very much more intense source of light, so that our exposures could be reduced by at least one-twentieth, but there seems little immediate probability of this being achieved. All our efforts in the direction of increasing current density, or the potential difference between the electrodes, have not yet proved sufficiently useful to warrant any drastic change of method. It seems that one can, as with the ordinary carbon arc, increase the electrical current density without any substantial increase in intrinsic brilliance. It is possible to increase the size of the illuminant, but the intrinsic brilliancy on the whole alters but little, so we are devoting attention to other methods of reducing exposure, and one at least of these I shall refer to directly. It will be appreciated that the spark images are the actual illuminants, not bright lines as shown in spectrograms (plate I) taken with a slit spectrograph. The images so obtained and photographed by means of a pinhole camera are seen in plate II, and the result of varying the distance between the electrodes is shown. This again is a practical difficulty, as the spark differs in character with the metal used, and also alters in form as the spark lengthens. This has made it necessary to introduce into the ultra-violet apparatus an arrangement for observing the spark with sufficient accuracy to appreciate small differences in the spark gap. A pinhole camera is used for observation purposes, and is sufficiently accurate to enable a spark image of almost constant length and form to be secured if adjustment is made at short intervals of time.

I have referred to the transmission of ultra-violet light by certain transparent substances, but have not said anything about materials that are good reflectors. In view of the importance of dark-ground illumination in visual work, and the substantial advances that have recently been made, it is to be expected that work would be attempted on the same lines with ultra-violet light. So far as I know, my own work in that direction still stands alone, yet it represents the most important advance in microscopical method of recent times. As you know, a modern dark-ground illuminator is essentially a reflecting system relying on spherical silvered surfaces for its production of an annular beam of light by reflection from such surfaces. It might at first glance appear possible to use such an illuminator for ultra-violet work, but by an unhappy chance silvered surfaces are quite unsuitable. In fact, one can go further and say they are the least suitable for such work of all known reflecting surfaces. A silvered quartz plate does, in fact, make quite a satisfactory screen which will reflect a large percentage of visual light while


Images of Spark Gap in various wavelengths
283
1mm gap
2mm gap
3mm gap
4mm gap
Magnesium
275
257
232
226
219
214.
Cadmium

Face p. 96.

transmitting a substantial portion of ultra-violet. A silvered surface, therefore, is of no value, and we must look for some other metal which will take a high polish and that has a high reflecting value for light of short wavelength. Much work in this direction has been done in my own laboratory, with the result that we now have samples of magnalium, the most suitable metal for the purpose, which does in certain wave-lengths reflect as much as 81 p.c. of the incident light. A short table has been compiled from various sources and is reproduced here (fig. 7). to indicate the large variation that occurs in reflecting power, and it also shows that certain metals, such as nickel, which reflects well in the visible, are not so suitable for our purpose. It is well to point out that all these reflecting values are dependent on the production of a polished surface of high quality, a by no means easy thing to secure. The difficulty is, however, now being overcome. My colleague Smiles is now able to polish magnalium satisfactorily, and Messrs. R. & J. Beck have produced an ultra-violet dark-ground illuminator of fine quality. The first one made by them was of similar design to their high-power illuminator, which is of outstanding merit for visual work. The reflecting surfaces are of magnalium, and the top lens, the one that is in immersion contact with the under-side of the slide, is of fused quartz. Homogeneous immersion is then secured by a water glycerine mixture similar to that used with a quartz immersion objective. A quartz objective of about 1–o N.A. in terms of visual light can be used, and the results obtained are, in my opinion, a striking advance, at least with biological material, on anything hitherto used. One disadvantage we are faced with is that the necessary exposure to obtain a satisfactory photograph is still rather too long for many objects, a disadvantage that also occurs very generally even with visual light, unless



Wavelength. $\mu\mu$.	Steel.	Cobalt.	Silver.	Nickel.	Platinum.	Wood's Alloy.
226·5	34·8	---	18·4	---	---	---
231·3	35·7	31·8	19·9	---	---	---
257·3	39·6	39·7	24·1	30·7	37·1	52·7
274·9	---	---	---	37·6	43·1	56·6
298·1	42·6	45·7	15·4	39·4	47·6	61·1
316·0	---	---	4·2	---	---	---
325·5	44·8	---	8·5	40·4	48·9	64·9
346·7	---	51·1	68·0	---	---	---
361·1	51·2		77·4	41·2	52·4	65·2
395·0	53·5	57·7	87·1	---	---	---
398·2	---	---	---	50·6	57·5	68·8

Magnalium. 283$\mu\mu$ 81% (Smiles)
Reflection-factors of Metals (in per cent)

Fig. 7.

a light source of high power is used. In ordinary work an electric arc can sometimes be used, but with ultra-violet light the spark source, with all its limitations, is the only one available. We are experimenting in several ways to overcome this difficulty with the object of ultimately obtaining instantaneous photographs. If we succeed in reducing the necessary exposure to one-tenth of a second, it will be possible to attack many biological problems, particularly those in connection with the study of the filterable viruses, in which my interest is greatest.

One effort at present in progress I will describe briefly, as it has some element of novelty. It is hardly necessary for me to say that all dark-ground methods are founded on illumination by means of an annular beam, and that this involves considerable loss of light, as only a portion of the image of the radiant is brought into use. The arrangement now under trial is shown in principle in fig. 8. Instead of an annular opening, the full beam is received by a magnalium cone which reflects the whole of the light from the radiant on to the internal reflecting surfaces, and from this passes through the fused quartz front and is concentrated at the position on the slide occupied by the object. The diagram is sufficiently clear for anyone conversant with such appliances to appreciate the arrangement. A further improvement on this provisional form has been evolved in my laboratory, and Messrs. Beck are now making an illuminator which, from the experimental evidence we already possess, appears likely to be of considerable value. As I have already said, this is not the only factor we are considering, but from its very nature it must be the most important one, and will be of great value should it realise our expectations.

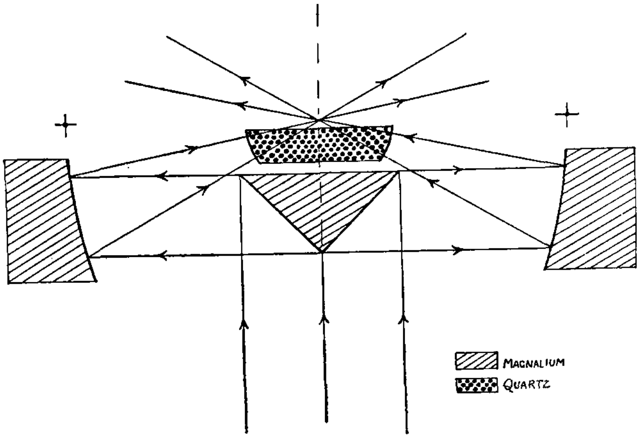
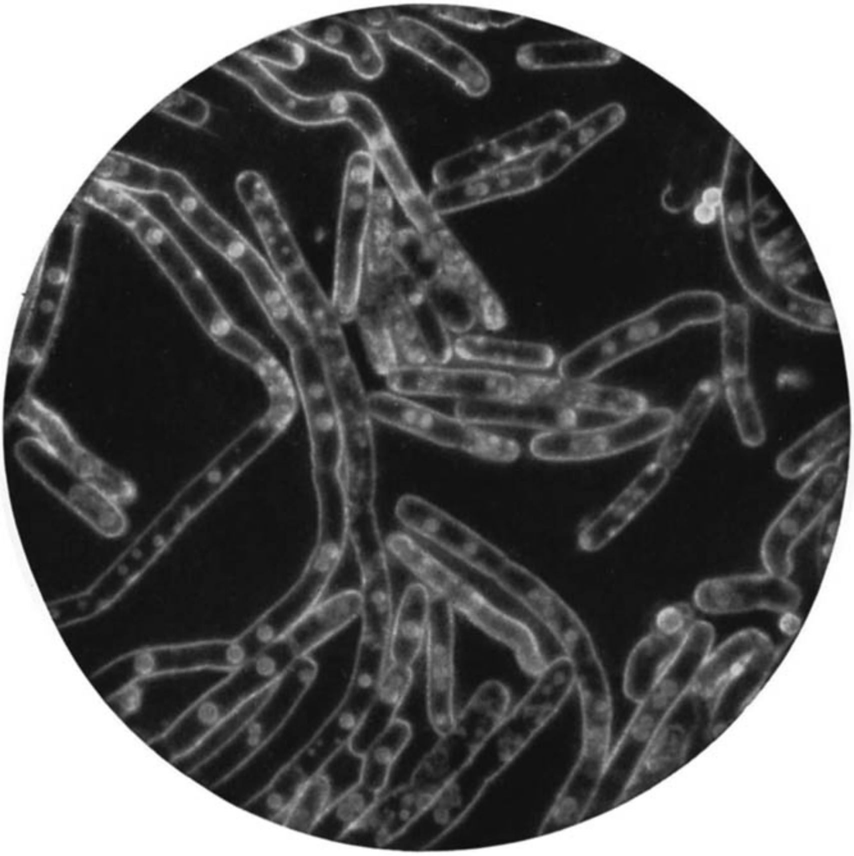
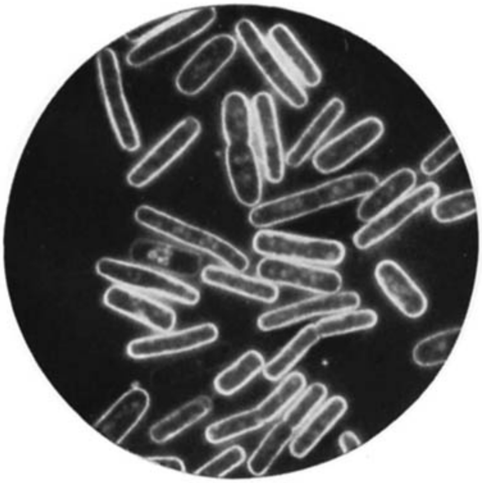


FIG. 8.

Even at the risk of telling you an old story, might I draw your attention to the increase of resolution expected as the result of using ultra-violet light. It is embodied in fig. 9, and shows the resolution we should get on



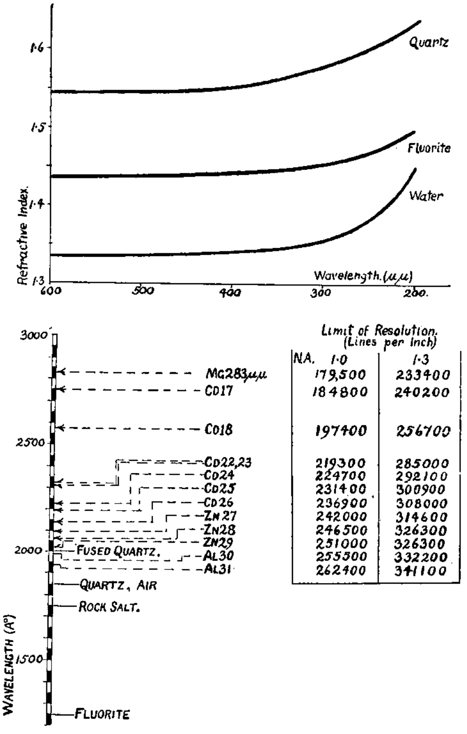
a



b

c

theoretical grounds. The N.A. is given in terms of visual light; it takes no account of the increase that occurs as the result of using shorter wave-lengths. On the left is a wave-length scale in Angstrom units. The next vertical column sets out certain bright lines in the spectra of cadmium, magnesium, zinc and aluminium. Below that the limits of transmission are indicated, and we see that quartz and air are broadly of the same transparency—in fact, the limit in dry air comes very close to that of crystalline quartz. Fluorite, however, is much more transparent than air, and selected pieces will transmit down to nearly 1,200 Angstroms, a most remarkable physical fact for which there are few parallels in nature. As fluorite has been used in the manufacture of objectives for many years, it is of interest to know something about it, and it is only the limitations of time that prevent me from saying more.



Limit of Resolution.
(Lines per Inch)

N.A. 1·0	1·3
179,500	233400
184800	240200
197400	256700
219300	285000
224700	292100
231400	300900
236900	308000
242000	314600
246500	326300
251000	326300
255500	332200
262400	341100

FIG. 9.

The resolving power is set out opposite to each wave-length, and needs no further explanation, but it does reach a very high figure at or near to the limit of transmission for fused quartz. This substance is used in the construction of ultra-violet objectives, and at least for the present it limits the path of progress. The relative refractive indices of quartz, fluorite, and water are also shown in the upper part of this diagram, and it is of interest to see that water alters most in shorter wave-lengths. In the design and construction of lenses in the future it is possible that this property may be of value, as the difference between quartz and fluorite is not great enough for correction to be secured easily. Attempts at new computations are, however, now being made both by the Department of Scientific and Industrial Research and in my own laboratory. It remains to be seen what the results will be, but I see no reason to be pessimistic—rather do I think that there is much ground for optimism. Whatever may be the outcome of this work, it is worth doing, and much is being learnt during its progress. In general terms it is essential that we should be able to secure visibility of microscopical objects before other optical factors are fully worked out. I have little doubt that such visibility will not satisfactorily be obtained by any staining method, so far at least as the observation of the smallest living things is concerned ; it is more likely that observation of living material will lead to a solution.

I can hardly conclude this rather scrappy address without reference to the application of some of these physical methods to biological research—particularly to those problems in which my interest is greatest—connected with the smallest living things. However interesting it may be to improve microscopical method and instrumental resources, it does appear to me that such things can only be justified when they extend our knowledge in some direction. It may be said with truth that the physical side of microscopy is by far the easier ; it is when attempts are made to apply any new method that the difficulties really begin. For that reason I think it is justifiable to conclude that such work can only be carried to a successful issue when improvements in apparatus are carried out at least in close collaboration with those who are attacking definite problems that await a solution. In my own case it would afford me the greatest satisfaction if I should be able to contribute to the knowledge already accumulated on such a subject as the filterable viruses. Micro-organisms of many varieties have now been photographed in my laboratory by means of this new method of dark-ground ultra-violet microscopy, and I venture to express the opinion that by no other method can comparable results be obtained. I am selecting three photographs only from those I am showing this evening, as I am afraid that the inevitable loss that will occur as the result of reproduction will detract from their value. In some of these photographs the wealth of fine detail cannot possibly be seen except in the original negatives, and it is just this fine structure and its satisfactory delineation that constitute the value of the results. Many of the details seen are of as small an order of size as some viruses must be, that is if we accept the theory that they are particulate and behave in some ways at least as larger micro-organisms do. That we have been successful in obtaining photographs of organisms to which the term " virus " is applicable, I think is probably true, but there is so much to do to confirm this to the satisfaction of other interested workers that I hesitate to refer to it further at this stage. Still, the work proceeds, slowly, it is true, but unless it did hold out some hope, the feeling of discouragement and disappointment would be strong enough to annihilate the chance of progress. I can, in conclusion, only express the wish that I may be able at no distant date to communicate to this Society some results that are of value and that have been arrived at by the methods I have from time to time described. If I should be so able, I am sure that I shall receive that sympathetic hearing and that tolerance for shortcomings that the Fellows of this Society have so consistently extended to me in the past.

DESCRIPTION OF PLATES.

PLATE I.—Spark spectra of metals. These show that the spectra of certain metals, such as cadmium, magnesium, zinc, silver, lead and aluminium, are suitable for use in ultra-violet microscopy, as their lines are separated sufficiently. Others, such as bismuth and tin, are unsuitable, because the lines are too numerous and not separated sufficiently.

PLATE II.—Spark images in magnesium and cadmium, showing the effect of differing spark gaps.

PLATE III.—Photo-micrographs of bacteria taken by ultra-violet dark-ground illumination.

(*a*) Bacillus megatherium. × 3000.
(*b*) Bacillus mycoides. × 3000.
(*c*) Streptococcus pyogenes. × 3000.