## MANIPULATION

OF THE

## MICROSCOPE.

BY

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ILLUSTRATED.

TENTH THOUSAND.

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to the angle of incidence. An opaque stop, which is cemented to the concave surface, prevents the light from passing through the central portion of the paraboloid. The object is thus illuminated on all sides by such an obliquity of light, that it does not pass into the objective; the object stands out in relief, pleasantly illuminated on a dark back ground. In using the paraboloid, the plane mirror should be used, and it is necessary to vary its distance from the object in order to attain the best results.

Cover-Glass.—Thus far no attention has been given to the use of the cover-glass, although it is an important factor in reaching good results. In preliminary examinations of solid objects with low powers it may be dispensed with; but where fluids are used, whether with low, medium, or high powers, it should always be used. A drop or small quantity of fluid placed upon a slide assumes a spherical form, and, on viewing it with a low power, it will be found to give a distorted field, and will cause disagreeable reflections and shadows.

As stated before, medium and high powers have a comparatively short working distance, and the front lenses will be so close to the water, urine, blood, etc., that the capillary attraction will often cause an adherence to the front surface of the objective; besides this, there is such a considerable depth to the-fluid that it obstructs the light, requires a great change in adjustment for the various planes, and is usually in such vibration that a sharp focus becomes impossible;

by merely dropping a cover-glass upon it all these objections are overcome.

The above are merely practical considerations, but there are others of a theoretical nature and of as much importance. After a high power objective has been corrected to a certain thickness of cover. any variation, not necessary considerable, has an injurious effect upon the spherical corrections, and consequently upon the resolving power. It is manifest that the quality of the latter will decrease as the variation increases, and when it reaches a point where no cover is used, it may be so considerable as to destroy an accurate perception of what is sought.

In this connection it is considered important to state what thickness of cover-glass it is best to use. As is probably well known, there are three grades, which are designated as No. 1, No. 2 and No. 3. Although they are classified, there is a variation within the limits of different numbers. The variation is about as follows: No 1,  $\frac{1}{150}$  to  $\frac{1}{200}$  inch thick; No. 2,  $\frac{1}{100}$  to  $\frac{1}{100}$  inch thick; No. 3,  $\frac{1}{150}$  to  $\frac{1}{100}$  inch thick; According to the prices of cover-glasses, when purchased by weight, the No. 1 give the greatest number and No. 3 the least. It may for this reason be thought that the purchase of No. 2 is most advantageous, but it must be considered that there is a greater proportion of breakage by cleaning, as they are very thin and sensitive. Considered only from a optical standpoint, No. 2 should generally be used, as the medium and high power objections are adjusted

to this thickness and give the best results with the thinnest of these. The same thickness is also used on test objects, but they are generally not of as much uniformity as might be desired. Objectives sometimes have such an extremely short working distance, that it is necessary to use the thinnest of No. 1, but as these are usually provided with adjustment for correction, their injurious influence is not so much felt. The thickest covers are most comfortable to handle and may be used with low power objectives without much sacrifice of definition.

The writer takes the liberty of inserting in this connection extracts from a paper which he recently read before the American Society Microscopists and which he hopes will give further information on this subject.

"The cover-glass may truly be called a necessary evil; for, while absolutely required in microscopic investigations, there is no adjunct to the microscope that has been and is productive of so much evil, and has retarded the utilization of benefits made possible by the advance in the construction of objectives so much as it.

"It must be remembered that the majority of objectives will always be dry, and especially so when such improvements, which we hope are still to be made, are accomplished. It is an unfortunate circumstance that with this class of objectives the influence of variation in thickness of cover-glasses is most apparent; but since it is so, we should, if possible, provide an

agency which, eliminating the personal factor of efficiency, will give, under all conditions, results closely equal to those under which the objectives were originally corrected.

"It is surprising to see how little attention is paid to this subject in the large majority of standard works on the microscope. Almost all books give carefully prepared illustrations and descriptions showing the effect on the course of light by the interposition of the cover-glass, and after giving conclusive evidence of its disturbing influence, still, in a general way, say it is of little moment.

"With such statements to guide the microscopist, it is not surprising that the subject should have received so little attention, and that any efforts to lead to improved methods of manipulating objectives should have almost completely failed because of a lack of the true understanding of their need and consequent failure to create interest. The belief is quite general that any time devoted to this subject is wasted and might better be utilized in other directions. I hope to be able to show that this is entirely wrong, and may here say that, while I may be considered an extremist in the other direction, my efforts emanate from the desire to put it in the power of every microscopist to obtain the highest possible results from his optical battery and equal to those obtainable by the optician.

"Outside of the differences of the lengths of tubes used by different makers, which is also of great bear-

ing on the spherical correction of objectives, one is astounded by the difference in standard cover-glasses used by different makers in correcting non-adjustable objectives. With a thickness of 0.10 mm. for the thinnest and 0.25 mm. for the thickest, it is only too apparent that with the additional variation in lengths of tubes it is beyond the power of the microscopist to obtain even approximately the best results from his objectives. More than this, a large quota of the advance made in recent years in the capacity of objectives has been lost.

"The greatest difficulty is met with non-adjustaleb objectives. As is well known, compensation for thickness may be obtained in the proper adjustment of tube length; but while not all microscopes are suitably provided with draw-tubes, the requisite experience and skill is lacking with a large number of microscopists to properly make the correction in this manner, as well as in objectives specially provided with collar correction. I am sure that microscopists of long experience will bear me out in the statement that results with adjustable objectives depend upon individual skill, and that many such objectives now in use fail to give results corresponding to their capacity. It would seem, therefore, that any system to permit the full utilization of the capacity of objectives should depend on no personal factor—in fact, should be mechanical.

"In an objective corrected for normal thickness of cover-glass there will be spherical over-correction with

thick covers and under-correction with thin covers, the amount of correction varying in a different ratio to the amount of variations from the normal thickness. The chromatic correction will also lose correspondingly, but to not so high a degree. While a devi-

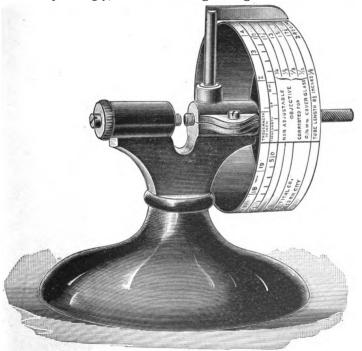


Fig. 18.

ation of a few hundredth millimeters in either direction will, perhaps, not signify, that which occurs in covers classified in price-list under one number is sufficient to seriously affect and the high powers totally obliterate the definition which under normal conditions it may possess. The microscopist is therefore not obtaining such results as his objectives ought to enable him to obtain, and the efforts of the conscientious optician to provide classified objectives of reliability and similar performance is almost entirely nullified.

"The system which I have devised to aid in overcoming these difficulties depends in the first instance upon a micrometer for measuring the thickness of cover glass. See Fig. 18.

"In objectives provided with cover correction the graduation is so arranged as to read to  $\frac{1}{100}$  mm. No matter what the power of objective, the number gives proper correction for a thickness corresponding to it. Thus, with a cover glass of 0.20 mm. the collar of such an objective need merely to be set at 20 to give the proper correction and, consequently, the best results.

"All the other scales give the correct tube length in inches and millimeters for covers corresponding to them, and in this manner offer a ready and definite means of correction. The tube-lengths required for the thinnest and thickest covers are so extreme that probably no convenient means for obtaining them can be practically arranged, but they can be so approximately if not entirely. At any rate, the micrometer will detect the requirements before using the covers,

and those deviating considerably from the normal can be used on objects for use with low powers only, in which case the effect will not be very appreciable.

"In this system I do not overlook the fact that variation in tube length involves a variation in magnifying power; but, except in cases where micrometers are used, I consider this of secondary importance, as it always is in comparison to results obtained in resolving and defining power.

"This system involves four conditions:

First.—That all cover glass be measured before using them, and that the thickness be noted on the preparation.

Second.—That for convenience all draw-tubes be marked in inches or millimeters or both.

*Third.*—That adjustable objectives be corrected according to this scale.

Fourth.—That the same tube length and cover glass thickness be used in all original corrections of objectives."

To Draw Objects.—It is very important that the appearance of an object should be put upon paper, especially of one which is not permanently mounted. To do this does not require any great amount of skill as the lines which are projected upon paper are merely followed out; but it is necessary that those drawings be made truthful. Nothing should be put down which is not actually seen; neither should any-