NOBERT'S TEST PLATE, MOLLER'S DIATOM TYPE SLIDE, AND MODERN MICROSCOPES.

By Francis Abbott, F.R.A.S., F.R.M.S.

Nobert's Test Plate, as an instrument for trial, is admirably adapted for comparing the quality of object-glasses, supplied by different makers,and every microscopist who possesses a first-class instrument naturally desires to know what it is capable of doing; for when the power and quality of his lenses are known to him, he is convinced it would be only a waste of time to try them further.

One of the most important tests that the properties of a microscope can be tried by is to clearly "resolve" the very fine lines of the frustules of the *Diatomaceæ.* The value of minute *Diatomaceæ,* is, in their surface markings, which are admirable test objects for the higher power of the microscope. This test was first made known by Messrs. Harrison and Sollitt, of Hull, but unfortunately as a standard measure, a great difference exists in the fineness of the markings in specimens of the same species obtained from different localities. In the *Amphipleura-pellucida,* the number of *striæ* range as low as 34, and many specimens present 60, 70, and 80 in 1·1000 of an inch. *Naviculæ-rhomboides,* range between 30 and 50 in 1·1000 of an inch, so that some specimens may be resolved with a 1-5th, a 1-4th, a 4-10th, or even a half-inch objective, while others require a 1-8th, or a 1-10th, or even a higher power. The first of the *Diatomaceæ* on which the lines were seen, was the *N. Hippocampus,* of Ehrenberg, by Pritchard and Queckett, in the year 1841. In 1844, Mr. Sollitt was in London, and showed the lines on the *N. liniata,* of Harrison, to the late Mr. Ross, with a 1-8th of Nachett's; at that time Mr. Ross could not bring them out with a very fine 1-12th he had just finished. This same Diatom *N. liniata,* has an average of about 60,000 *striæ* to the inch, which can now be well seen with a good $\frac{1}{4}$-inch objective.

For the purpose of testing this resolving power of microscopes, F. A. Nobert, of Pomerania, has adopted a method only known to himself, of ruling lines to a known scale of exceeding fineness, so close that physicists have declared it impossible they ever can be seen, and all trials hitherto have resulted in failures to resolve the finer lines of these plates.

Nobert's test is a series of groups of fine lines ruled parallel on thin glass, such as used for covering, and will admit of the focusing of high powers when reversed and secured on the glass slide; different plates contain a different number of groups, ruled to different scales, some have 30 bands or groups, the coarsest having its lines 1-1000th of a Paris line apart, and the finest being 1-8000th—each group or band being about 1-2000th of an English inch in width, and the whole 30 occupying a space perhaps a little more than 1-50th of an inch.

The plates now most in use have 19 bands, the first or coarsest being ruled to 1-11,240th of an English inch, and the finest to 1-112,668th of an inch. It is a difficult matter for the mind to appreciate such minute divisions, yet it is essential it should conceive them to prepare it for estimating the difficulty of seeing and counting such lines.

Is it possible for human art ever to make an instrument capable of rendering lines 112,668 to an inch visible. Is there anything in the laws of light which renders it impossible to see lines so close, and is it therefore uselesss for the optician to attempt to improve the microscope beyond a certain point? Or, for the naturalist to try any further investigations on the structure of tissues beyond what the present existing instruments have shown. This problem is the exact parallel of that in testing the quality of the telescope for separating double stars. In 1855, Professor Queckett, asserted that "no achromatic has yet been made capable of separating lines closer together than 1-75,000th of an inch." "Mr. Ross found it impossible to ascertain the position of lines nearer than 1-80,000th of an inch." "Mr. De la Rue was unable to resolve any lines on Nobert's test-plate, closer than 1-81,000th of an inch." "Dr. W. B. Carpenter says, that no objective will, probably, ever be able distinctly to resolve lines closer than 1-84,000th of an inch." If these observations are correct, the existence of lines finer than this is a matter of faith rather than sight; there can be no reasonable doubt, however, that lines do exist, and the resolution of them would evince the extraordinary superiority of any objective, or any system of illumination, which would enable them to be distinguished."

The late Professor J. W. Baily, claimed to have seen lines as close together as 1-100,000th of an inch, and Messrs. Harrison and Sollitt of Hull, claimed to have measured lines on the Diatom *Amphipleura-pellucida*, as fine as 120,000, to 130,000 to the inch. R. C. Greenleaf of Boston, and Mr. Charles Stoddard, were well satisfied that they saw the lines 90,000 to the inch, with a Tolles 1-5th, and afterwards Mr. Greenleafe saw the same lines very distinctly with a Tolles 1-12th. Dr. J. J. Woodward, of Washington, states that with mono-chromatic light, and Powell and Lealand's 1-50th, and 1-25th, and 1-16th objectives a Hartnach's immersion No. 11, and Wales 1-8th with amplifier, he satisfactorily resolved the 29th and 30th bands of Nobert's test plates, the lines of which were 1-83,917 and 1-86,334 to the inch.

Dr. Woodward lately received a Nobert's test plate of 19 bands, the photographs procured from which were lately brought under the notice of the Royal Microscopic Society by the hon. secretary, Mr. Jabez Hogg, the covering glass on this test-plate was too thick for a 1-50th objective, but with all the others he was able to resolve the 17th band, 101,000 to the inch, the 18th and 19th he was unable to resolve. Both Dr. Curtis and Dr. Woodward have photographed the 16th, 17th, 18th, and 19th bands with a Powell and Lealands' 1-25. In these photographs the lines of the 16th and 17th bands can be counted with some difficulty, but the lines of the 18th and 19th cannot.

Dr. Barnard found the counting of the lines to be attended with a great difficulty, in addition to which there is another trouble, the whole width of a band is not in perfect focus at the same time, and the slightest change in focal adjustment renders it extremely difficult to fix, even with the cobweb micrometer, the last line counted.

One way of verifying the accuracy of the divisions of Nobert's test lines, is by counting them in a measured space. If 46 equidistant lines are ruled in the space of 1-2,000th of an inch, the inter-spaces must be at the rate of 90,000 to the inch. An error of one line more or less in counting the whole on such a band, would decide the rate of interspaces to be either 92,000, or 88,000, instead of 90,000 to the inch.

The foot note to Mr. Stodder's paper in the "American Naturalist," deserves notice. Speaking of Dr. Woodward's photographs, the author says, "The first fifteen bands are sharply and clearly resolved into the true lines; the fifteenth band, however, (which is ruled to the 90,000 of an English inch), requires a hand-glass magnifying four or five diameters, to show its lines distinctly." Dr. Woodward gives the following directions for counting the lines in the highest bands that can be resolved. "If a cobweb micrometer is used, the micrometer eye-piece should be firmly clamped in a stand screwed to the table, so that the eye-piece is close to the end of the microscope-tube, but not to touch it, a piece of black velvet being used to complete the connection. The motion of the micrometer-screw now communicates no tremor to the microscope, and all difficulty in counting the lines seen (whether real or spurious) disappears." Still better than this is the following method:—"The microscope being set up in a dark room, as though to take a photograph, and the eye-piece being removed, the image of the band to be counted is received on a piece of plate-glass in the plate-holder, and viewed with a focusing-glass, on the field-lens of which a black point is marked; as the focusing-glass is moved on the plate from side to side, the black point is moved from line to line. The lines may thus be counted with as much ease and precision as though they were large enough to be touched with the finger."

These photographs support an opinion given by Mr. Wenham many years ago, that the time would come when photography would reveal minute detail much plainer than it can be seen in the microscope. The reason of this is obvious. In photography the object can be illuminated by highly condensed sun-light, which will produce intense black shadows, quite unendurable to the eye, and it was with such illumination that these photographs were obtained with a Spencer 1-12th, and a Tollis 1-5th, *both dry objectives.* Dr. Woodward made five counts of the 19th band, which gave him a mean of 110,820 to the inch. The number, according to Nobert, is 112,668. Dr. Barnard counts for the 15th band 91,545; Nobert 90,074. There is a similar alleged variation in the striation of Diatomaceæ, among individuals of the same species, *Pleurosigma fasciola,* has been specially designated by Dr. Wallich, as very inconstant in its markings, as well as *Pleurosigma strigosum, Navicula-rhomboides,* and *Nitzschia sigmoidea.*

With the 1-8th and the 1-12th by Ross, and the 1-20th by Smith, in the possession of the Royal Microscopic Society, and with a 1-8th, a 1-12th, a 1-16th, and a 1-25th, by Powell and Lealand, all dry objectives, on a new 19-band plate, all the bands beyond the 12th seemed imperfect, the lines were not separated. But with a 1-10th and a 1-18th by Hartnarch, of Paris, a 1-16th by Merz, of Munich, and a 1-20th by Nobert, all *immersion objectives,* straight and well-defined lines were separated as far as the 15th band inclusive. Several trials by sunlight proved that the lines were thus rendered more visible, but the immersion objectives maintained their superiority by all methods.

Continental opticians and men of science have been aware of the merits of the immersion system for some years past; and, from its having received so little attention in England, continental makers do not scruple to say that the English no longer take the lead, either as opticians or microscopists.

The advantage claimed for immersion objectives are:—greater working distance between the object and objective, increase of light, and superior definition and clearness in the optical image, which image is obtained by much simpler illumination than that required in using high-power, dry objectives.

In its construction the objective is immersed in water, that is, there is a film of water between the front of the objective and the object, or thin glass covering it. An *amplifier*, is an achromatic combination inserted in the compound body of the instrument, to increase the power of the objective and eye-piece. *Angle of aperture* is the angle in the surface of the front lens, at which light will enter the objective ; the greater the angle of the aperture, the more light, and to a certain extent greater resolving power ; there is no evidence, however, for the statement that the resolving of test-diatoms and Nobert's test-lines is a question of *angular aperture*, since the 1-12th inch object-glass may have its *angle of aperture* extended to 170 deg., the utmost limit compatible with the reception of rays from any object ; nothing is therefore gained in this respect by a reduction of the focal distance.

Before entering upon the investigation of test-objects, it would be well for every microscopist to ascertain the true magnifying powers and properties of his own instrument, English, American, and Continental European makers differ in distinguishing their instruments ; each maker has generally his own system. The theoretical power of a microscope is usually measured from an arbitrary standard of 10 inches ; thus a one inch is said to magnify ten diameters ; a $\frac{1}{4}$ inch, forty diameters. If the standard is taken at five inches, as it is by some, then the power is but one-half as much. The power of the microscope is that of the objective multiplied by that of the eye-piece ; if the objective magnifies ten diameters, and the eye-piece ten, the result is one hundred diameters.

In a scientific point of view, and next to Nobert's test plate we have a marvellous specimen of art in Herr Möllers, diatom type slide ; this single slide contains over 450 diatoms, the whole are arranged and scientifically classified into four series by Herr Grunow, of Vienna, and within such a compass that the whole can be examined simultaneously with a two inch, objective, and under dark ground illumination they present a singular and beautiful appearance.

There is issued with the slide a "Systematic Catalogue," which admits of easy reference. Thus, there are nineteen objects belonging to the genus Epithemia the same number of Eunotia, five of Merediones, thirteen of Synedra, &c., &c. In the catalogue they are classed in families,—as *Epithemiæ Meridionæ. Diatomeæ Tabellariæ, Surireleæ, Nitzschiæ Amphipleuræ, Cocconeidæ, Achnantheæ, Cymbelleæ, Gomphonemeæ, Naviculeæ, Isthemiæ, Biddulphiaceæ, Eupodisceæ, Melosireæ,* and *Chaeto-cereæ.* From this it will be easily seen how to compare the different families together, and how they differ from other groups.

The objects are attached to the covering glass, like Nobert's test lines, which is very thin, so that it allows the use of high powers; both sets of lines on *Navicula Rhomboides* are resolved with a one-twentieth objective, so that each Diatom may be separately examined with deep lenses. The thickness of covering-glass required for focusing under high powers will explain the difficulty of using them. For a 1-12th and 1-16th objective the covering-glass must not exceed ·004 in. For 1-20th, 1-25th, and 1-50th, ·005 in. It was my intention to have shown Möller's Diatom Typen Plate, and Nobert's bands of Test Lines, under the microscope, at the annual soirée which the Council have fixed for the 16th of November, but from a correspondence by the last mail, I learn that they are not to be had, except at a very great price. I have received, however, from members of the Royal Miscroscopic Society, some specimens of diamond writing, &c., equally marvellous and interesting, and much better suited for the purpose, which were engraved and exhibited at the International Exhibition, class 13. The following is a specimen:—" A micrometer with divisions of 1-100th, 1-1,000, 1-2,000th, 1-4,000th of an inch, with 40 parallelograms, each of 1-100,000th of an inch; 40 parallelograms each of 1-200,000th of an inch; 80 parallelograms each of 1-400,000th of an inch; 200 parallelograms each of 1-2,000,000th of an inch; 400 parallelograms each of 1-4,000,000th of an inch; and 400 parallelograms each of 1-8,000,000th of an inch; with squares of 1-10,000th of an inch; 100 squares each of 1-1,000,000th of an inch; 100 squares each of 1-4,000,000th of an inch; and 400 squares each of 1-16,000,000th of an inch. The Lord's Prayer, the Creed, God Save the Queen, Rule Britannia, a geometric coil, the 4,137 letters in the 51 verses of the 1st Chapter of St. John, a poem upon the International Exhibition, from copper plates engraved in the Exhibition, each in a space from 1-1,600th to 1-2,000th of an inch. A Freemason's sign, which can be covered by a pin's point, &c.

There are various means employed for measuring these delicate objects, the best is undoubtedly the position micrometer, the same as the one used with the telescope for measuring the distance and angle of double-stars; but this instrument is both expensive and complicated. The method now in general use is the micrometer eye-piece, this is an eye-piece either positive or negative, having a divided glass micrometer fitted into a brass frame and placed in its proper focus. If the positive eye-piece is used, the micrometer is placed below the field-glass, but if the negative eye-glass is used, then the place for the micrometers is between the two lenses.

To find the value of the lines in the eye-piece micrometer, a glass micrometer is laid on the stage of the microscope, which is divided into 1-100th and 1-1000th of an inch, when the two micrometers are brought into focus with a low power, say a one inch objective, then if every division of 1-100th of an inch in the stage micrometer coincides with ten in that of the eye-piece, it is clear that the lines in the eye-piece micrometer are 1-1000th of an inch, the stage micrometer can now be removed, and every object corresponding to the eye-piece micrometer, under a one inch object glass can be measured to 1-1000th of an inch.

If one of the 1-1000th of an inch of the eye-piece micrometer be divided into four parts by the stage micrometer then every one of these divisions would be 1-4000th of an inch. If with any other power it is found that 1-1000th of an inch on the stage micrometer coincides with ten spaces of the eye-pieces micrometer, then the value of each division in the eye-piece micrometer would be 1-10,000th of an inch. In practice it will be found when high numbers are being observed, and the stage movement too coarse, it will be necessary to use the small milled-head screw at the end of the eye-piece micrometer, so as to bring the lines in each micrometer accurately over each other.

For the information of those who take an interest in this subject, I lay on the table the two volumes of Professor Smith's Synopsis of British Diatomaceæ. In these volumes all the objects are drawn to a scale of 10,000 to the inch, multiplied 400 diameters. Also papers containing some particulars of Möller's Diatom Typen Plate, and Nobert's Band of Test Lines, with Mr. Webb's description of diamond writing and engraving.