

Observations, &c. (*continued*).

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“Microscopical Researches in High Power Definition.” By G. W. ROYSTON-PIGOTT, M.A., M.D. Cantab., F.R.S. Received May 23, 1879. Read June 19. [The portion between square brackets received November 20.] Part I.

[PLATES 3 and 4.]

In the present communication it is intended to advert to the subjects—

- (1.) Minimum visibility.
- (2.) The effects of excessive angular aperture in obliterating minute molecular structure.
- (3.) Minute measurement and a micrometer gauge.

(1.) *Minimum Visibility.*

Spider-lines miniaturized down to the fourteenth part of the hundred-thousandth of an inch have been made distinctly visible to ordinary good eyesight under proper microscopical manipulation. The question then arose whether an actual thing so small as the millionth of an inch could be descried in the microscopical field of view.

According to recent researches, it would appear from formulæ derived from the undulatory theory of light, that brilliant disks are developed from points of light which vary inversely in diameter with the increase

of angular aperture of the objective employed, and directly as the wave-length of the kind of light employed.*

Professor Helmholtz and Professor Abbè have independently arrived at this beautiful law. And Professor Helmholtz cautiously states, however, there may be some conditions which may modify this law.

It follows from this principle that thin brilliant lines of light can be best separated by glasses of the highest angular aperture; and the separating power can be measured by the sine of the semi-aperture. Now for wave-length $\frac{1}{50000}\dagger$ we may thus tabulate them (when $\frac{1}{2}\lambda = 100,000$):—

The Values of ϵ for Different Apertures.

Aperture.	(ϵ), the limit of proximity of bright lines or disks.
180°	100,000th of an inch.
160	98,480th ,,
140	93,970th ,,
100	76,600th ,,
60	50,000th ,,
40	34,200th ,,
20	17,300th ,,
15	13,000th ,,
10	8,700th ,,
5	4,360th ,,
1	870th ,,
$\frac{1}{2}$	436th ,,

Such values as these have accordingly been generally accepted as limits to the resolvability of close lines with objectives of given apertures. Further, it is said that lines drawn at the rate of a hundred thousand to the inch represent the limit of microscopic visibility.

In a paper on this subject by the author it has been shown that a bright space enclosed between two spider-lines, miniaturised so as to form a bright interval $\frac{1}{53000}$, was distinctly visible, whilst the webs actually were about the $\frac{1}{6000}$ and $\frac{1}{7000}$ of an inch in thickness.‡

Under these circumstances, it was interesting to determine whether

$$* \quad \epsilon = \frac{\lambda}{2 \cdot \sin \alpha}.$$

λ = wave-length; α = semi-angular aperture; ϵ the distance between the centres of the disks in contact.

† Sir John Herschel estimates the wave-lengths as follows, in parts of an inch—

λ Green	47,560th.
λ Intermediate	49,320th.
λ Blue	51,110th.

In round numbers, for bright blue illumination, $\lambda = \frac{1}{50000}$.

‡ "Proc. Camb. Phil. Soc.," vol. iii, p. 217.

real objects could be detected by the microscope of the surprising degree of attenuation represented by the millionth.

For this purpose a long search was instituted for many months, both for the finest attainable defining powers and for suitable objects. Minute mercurial particles have appeared to furnish very interesting evidence. If these be very finely smashed with a small piece of watch-spring, the experiments will be promoted by inserting a minute drop of petroleum beneath the "cover," which should be very thin and clean (fig. 9).

A reflex illuminator is then used to converge rays downwards through the objective upon the preparation.

The most extraordinary minute disks (in a darkened room) may be then observed (of a circular form chiefly). Some of them are irregular, and *upon* some of them will be detected clusters of the most wonderful degree of smallness, consisting of minute black points, visible with a power of 1,000 diameters. And what is so surprising is the entire absence of any diffraction effects at the edges of these black points.

For convenience of ready reference, I have found micrometer spider-lines of service for comparative estimation. There are three thicknesses carefully measured:—

No. 1	1- 5,000th of an inch.
No. 2	1- 8,000th ,,
No. 3	1-10,000th ,,

Comparing these black particles floating on the surface of the mercury with the apparent diameter of the thinnest spider-line, some of them appeared decidedly smaller. This observation has been confirmed by several persons to whom they were exhibited.

Under 1,000 diameters the particle was magnified just one hundred times in the micrometric focus. It then appeared less than the spider-line $\frac{1}{10000}$ of an inch thick. Its real diameter was therefore less than the $\frac{1}{1000}$ of this magnitude, or less than *the millionth of an inch*, a microscopic unit which I propose here to represent by **iii**. Under a power of 2,000 diameters the finest spider-line would represent the quantity $\frac{1}{2}$ **iii**.

The accompanying drawing fairly represents the appearance of the spider-lines seen on a dark field when mercurial particles, as already described, are examined with the micrometer under a power of 1,000 (Plate 3, fig. 9). (The webs appear too coarse in the plate.)

A positive eye-piece utterly spoils the finest defining powers, and a Huyghenian is generally substituted.*

The very beautiful phenomena observed by means of brilliant illumination of these minute mercurial disks, flattened by pressure or

* Definition may be further improved by substituting a crossed lens for the field and an achromatic magnifier for the eye-lens.

moving freely in the fluid, I propose to describe in another portion of the present research. Suffice it to say, that they fairly and surprisingly imitate the starry lustre of a midnight sky—the Milky Way and the various phenomena of the closest double stars.* They display too under the finest attainable amplification the most accurate diffraction rings I have ever witnessed, and as such give admirable indications of quality and correction in the lenses employed. In this case floating mercurial molecules,† uncompressed, reflect astonishingly minute focal images.

From these observations the conclusion may safely be drawn that real objects of unsuspected minuteness may be microscopically displayed, as well as minute miniature images, such as I have elsewhere described.‡

There is only one objection to this result. It may, perhaps, be said that bright diffraction diminishes the apparent diameter of these black points. But they may be observed almost touching each other. The great difference in diffraction in the case of black particles seen on a flat illuminated disk of mercury, and black particles seen by transmitted light, so soon as the objects are illuminated from beneath, is most striking. Besides this, continued observations of another kind confirm the conclusion arrived at. The question of the *minimum visible* regarded as visual angle is of equal importance in telescopic as in microscopic researches, and is well illustrated by the appearance of minute stars in large telescopes of great excellence. There the actual angular aperture is infinitesimally small, as the rays enter the instrument in a state of parallelism. As, however, an object may be supposed to move up towards the instrument, the angular aperture gradually assumes a tangible quantity. For instance, a fly's foot viewed with Lord Rosse's gigantic reflector of 6 feet diameter, formed an angular aperture of $5^{\circ} 44''$, the object being 60 feet distant. At a certain point, as the focus is shortened, a telescope becomes a long focus microscope. The instruments are, indeed, convertible. A very long focussed microscope is in reality a shortened telescope. At this point the laws of vision apply identically, and the angular aperture is the same for each. The question, then, of vision in the microscope, when only small aperture is engaged as in the telescope, may aptly be illustrated by minute astronomical observations. The eye, too, engages a near object at a small angular aperture: the pupil is then the base, and the object the apex of an isosceles triangle measuring the angular aperture, as much perhaps as a couple of degrees in short sight, but infinitesimally small as in the telescope when directed to a star. The

* Great pains were taken to secure brilliant illumination in a dark room.

† These very minute mercurial globules move freely, as though floating.

‡ "Phil. Trans.," 1870; "Month. Jour. Roy. Mic. Soc.," "Testing Objectives by Miniatures of Mercurial Globules," vol. i, 1875; "Limits of Vision," vol. i and vol. ii, 1876.

limit of visibility measured by visual angle is reached, equally by the eye-lens of a microscope or telescope, or by a simple magnifying glass, when the object subtends too small an angle to be sensible to the retina.* Both instruments require identical eye-pieces. (Two seconds of arc is the limit in general of human vision.)

Comparing, then, the minimum visible in both instruments, and beginning with the telescope, it may be urged that the spurious disks of the minutest stars visible, such as the 17th or 20th magnitude, must subtend an exceedingly small visual angle.

It is then fair to inquire under what visual angle such disks can be glimpsed in an eye-piece, used indifferently in either microscope† or telescope. Great loss of light in defining these minute points is prevented by very accurate curvature in a reflecting mirror.‡ Accordingly the Uranian satellites have been seen in an 18-inch silver glass reflector. The extreme minuteness of these bodies is illustrated as follows:—

A double star between β_1 and β_2 Capricorni may be discovered whose components are of the 16th and 17th magnitude. Yet Sir J. Herschel declares, that in comparison with the Uranian satellites, these two minute stars are splendid objects. The visual angle at which these satellites are seen in Calver's 18-inch mirror must be exceedingly small indeed§ (see mercurial double stars, fig. 9).

Sir John Herschel records an extremely fine observation of the double star Σ_2 , composed of two minute equal stars nearly as small as the 9th magnitude; seen at the Cape with a 20-feet reflector armed with a 24-inch mirror:—

“Charmingly divided with 320; the disks, like two grains of

* The spurious disks of stars of excessive minuteness are very much smaller than those of the larger kind. But the usual mathematical expression for this size is independent of star magnitude, and is in this respect incomplete. Such disks are therefore matters of observation, and their size in some degree conjectural.

† The mercurial illuminated star disks are here alluded to.

‡ The same economy of light is secured in microscopic object-glasses of great perfection, which disperse the otherwise inevitable white fog of spherical aberration. The visibility of these Uranian satellites in Calver's 18-inch silver glass mirror is doubtless due to a similar cause.

§ Compare this with the satellites of Saturn, 280,000,000 distant:—*Dione*: diameter, 500 miles; visual angle, $0''.85$; magnitude, 12. *Tethys* is reckoned magnitude 13; *Enceladus*, 15; *Minas* and *Hyperion*, 17. If the law could be applied, that the light being the same, visibility varies as the square of the diameter, and the next magnitude is half the magnitude preceding, the visual angles subtended by the Saturnian satellites might be guessed at with a power of 500:—

	<i>Rhea.</i>	<i>Dione.</i>	<i>Tethys.</i>	<i>Enceladus.</i>	<i>Minas and Hyperion.</i>
Magnitude.....	10th	12th	13th	15th	17th
Visual angle	160"	67"	47"	23"	16"

And this very nearly agrees with the limits assigned.

mustard seed, separated by one-third the diameter of either. *Vicinæ* according to Struve, but at least *per vicinæ*: dist. between centres 2-3rds of a sec.**

Recurring again to the law of brilliant disks in the microscope diminishing in diameter with the increase of objective angular aperture, I beg to call attention to the observation that in examining miniatures reduced 1,000 times, the details of these miniatures appeared entirely free from diffraction effects so long as the light was subdued. But the instant the sun shone every reflecting point or polished surface became entirely disguised, concealed, or obliterated in its details by the strong diffractions.† It is on this account that mild daylight observations from light received from a white cloud have been found so serviceable in minute research as already described.

Unfortunately, the attainment of the most delicate microscopic

* From these data, if $6x$ be diameter of each, $2x$ is the dividing space or "black division," so that

$$3x + 2x + 3x = \frac{2}{3}''.$$

Whence

$$6x \text{ or diameter of disk} = \frac{1}{3}'',$$

$$2x \text{ or division} = \frac{1}{6}''.$$

Hence the angle subtended by either disk with power 320,

$$\theta = 320 \times \frac{1}{3}'' = 160''.$$

The angle subtended by dividing black line,

$$\theta = 320 \times \frac{1}{6}'' = 53''.$$

The last result corresponds to a black line 1-400,000th of an inch magnified 1,000 times in the field of the *microscope*.

A short table is here inserted for reference. If the millionth of an inch be taken as the microscopic unit of reference (m), and a particle or line of this size be magnified 1,000 times, it will subtend to the eye of the observer, as an image 10 inches distant, an angle *ten times greater than the human limit*, viz. :—

$$20'' \cdot 6265.$$

Diameter of object.	Visual angle.	Power.
1-10,000,000th.	" 2	1,000
1- 1,000,000th.	20½	
1- 500,000th.	41	
1- 400,000th.	51½	
1- 300,000th.	69	
1- 200,000th.	103	
1- 100,000th.	206	
1- 50,000th.	412½	
1-1,000,000th.	41	2,000
1-1,000,000th.	60	2,500

† See "Circular Solar Spectra," by the writer, "Proc. Roy. Soc.," vol. 21, p. 426

research is beset with many difficulties and variable factors, some of which may easily be overlooked or even unsuspected.

As nearly all organic tissues teem more or less with minute molecules, their variable behaviour and optical appearances have received close attention for many years, and I have concluded that—

A.—A refracting molecule changes its appearance and phenomena according to the nature of the fluid by which it is surrounded, also with the fluid in which the objective may be immersed.*

B.—It changes also, in an extraordinary manner, according to the angular aperture of the objective employed, according to the refractions of the media, and the direction of illumination and the parallelism or degree of convergence or divergence of the illuminating pencil transmitted.

C.—Other interesting factors are the residuary aberrations of the compounding lenses of observation and the greater or less intensity of diffraction phenomena introduced by the mode of illumination.

In a short paper, it will be convenient partly to deal with these questions as they arise in the experiments rather than *seriatim*.

(2.) *Excessive Angular Aperture considered.*

The principal and chief, I may say the most valuable, feature in the appearance of a refracting molecule is the extraordinary variability of the blackness and thickness of the *marginal annulus*.

This thickness and consequent visibility is dependent on the value of the refraction into the given media, and the angular aperture of microscopic observation: partially also upon the situation of the focal point of the lenticular illumination.

Example 1.—A glass spherule ($0\cdot1$ diameter) is examined with a pocket lens. An intensely black broad ring is seen against the light. The same black ring is visible in bubbles frequenting plate glass in windows. The angular aperture of the pupil of the eye is about a degree for an image seen at 10 inches distance. Melted glass filaments are also instructive (see figures α , β , γ , δ , ϵ , ζ , η , θ).

* It is not many years since lines on diatoms were all that were searched for. But as these are formed by an aggregation of siliceous spherules, they present extraordinary opportunities for investigating optical characters which must necessarily belong to them—of a degree of minuteness of a highly satisfactory order—such as black annuli, focal points varying with chromatic refraction, shadows varying in contour according to the extinction of transmitted rays by obliquity of illumination: appearances changing with the refractive index of the media in which they are immersed. Only the very finest glasses extant can display the black margin of a diatomic spherule the $\frac{1}{40000}$ of an inch in diameter; and very few, if any, can be obtained capable of displaying focal points and the natural coloured foci at different chromatic foci.

Example 2.—A microscope with ten degrees aperture considerably diminishes the relative thickness of the black annulus.

Example 3.—With forty degrees it is much attenuated and it vanishes altogether when the angular aperture reaches a certain relation to the lenticular refraction.

The vanishing limits vary with the nature of the refracting spherule, and also with the angular observing aperture and the direction of the axis of illumination.*

If it be formed of plate glass whose index of refraction into air is 1.500, the vanishing angular aperture is $83^{\circ} 36'$. But as the index is higher, this angle increases with heavy flint glass ($n=1.988$) to $164^{\circ} 6'$. In both these cases the black defining annulus of the spherule is in these limits attenuated almost to evanescence.

Glasses, therefore, of small angular aperture develop the broadest black outlines in a minute refracting molecule.

The change of appearance of translucent bodies composed of masses of refracting molecules is very finely shown in observing a variety of scales forming the dust of moths and butterflies.

Example 4.—*Featherlets of the death's head moth.* Low angular aperture, 10° . The whole animal bristles with black feathers, armed with three or four long black spines: all of a dark but rich umber colour, tipped intensely black; each spine shows an exceedingly thin line of light running centrally up between two broad intensely black margins. (Exactly what is seen when examining a thread of spun glass with low aperture.) (Fig. 1, Plate 3.)

Increasing Aperture.—Colours pale. Light flashes through. The spherules begin to appear edged with black annuli, which gradually attenuate with higher angled glasses. (Direct light.) (Fig. 2.) (Fig. ϵ , ζ .)

It is remarkable how the colour changes as the aperture increases, through paler shades, until a general sparkling radiance appears to steal through the mass of molecules formerly darkling with the universal presence of black annuli due to low aperture. The black edgings also of terminating membranes extending from spikelet to spikelet, and those of refracting tubules become indistinct.

The obliteration of marginal shadows is well shown by first using low and then large aperture on another very beautiful object, viz.:—

Example 5.—*Plumelets of the Hipparchus Janira.* Aperture 20° . Power 200. The filaments of the plume and their clubbed ends are all intensely black (Plate 4, fig. 13a).

* "The use of a new Aberrameter, for testing Aberration and the Effect or Aperture," "Quart. Jour. Mic. Science," January, 1871. The angles subtended by the black shadow are there calculated and tabulated.

Aperture 44°.—All still intensely sharp and black with the fine definition of a Wray "half-inch."

Aperture 55°.—Wray glass. Margins thinner; the filaments begin to be translucent and at the spherically clubbed ends a focal point of light appears.

Aperture 94°.—A remarkably fine 1-6th by Bénéchè of Berlin. Power 600. Increase of translucency at every part; black margins attenuated. Club margin much thinner (fig. 13*b*).

Aperture 140°.—Fine 1-8th. Power 800. Black margins almost attenuated to invisibility; clubs translucent altogether; no annulus.

It is interesting to state that the thickness of the filaments of this plume vary between $\frac{1}{800000}$ and $\frac{1}{900000}$, yet these are beautifully distinct (aperture 12° and 50 diameters 2-inch objective), and yet under this amplitude a single filament subtends* less than 20 *seconds of arc*.

The exceedingly black and sharp appearance of these filaments doubtless accounts for their actual visibility under this very small visual angle. Now this exactly represents a line 1-1,600,000th thick (considerably less than a millionth of an inch) seen under a power of 1,000 diameters.†

If then the minute fibrillæ of the plume can be clearly distinguished as closely packed black lines, at a visual angle of twenty seconds, with low aperture of twelve degrees, this result is fatally opposed to the popular idea that very close lines, or very minute lines or bodies, can only be distinguished with large angular aperture. These lines were most sharply seen, though less than 1-80,000th thick (fig. 13*a*, Plate 4).

But besides the black sharply defined ring or annulus always developed in a refracting molecule by using low aperture, oblique illumination produces a thorough change in this black ring: it is transformed into various black crescents; and a row of such molecules approximately appears as a continuous black line sometimes notched. A tubule also produces a variety of marginal shadows according to the angular aperture of observation and according to the arrangement of internal molecules, and their combined shadows produce a variety of effects of an important character (fig. 6).

It follows from these considerations that researches upon exhausted structures (those inadequately resolved for instance), must be conducted with especial reference to the development and detection of shadow annuli or bands, or notched black lines, and with special

$$* \theta = \frac{\text{arc} \times \text{power}}{\text{rad.}} = \frac{\frac{1}{800000} \times 50}{10 \text{ inches}} = \frac{1}{16000} \therefore \theta = 13''.$$

$$\dagger \theta = \frac{\text{arc}}{\text{rad.}} \times \text{power} = \frac{1 \div 1600000}{10} \times 1000 = \frac{1}{16000}.$$

regard to the angular aperture of the most effective kind, whether modified by the limitation of illuminating rays or by reduction of aperture; or by the refractive media concerned, viz., immersion fluids and "mountings." (Fig. 10).

Very striking examples of the disappearance of distinctive shadows, and consequent obliteration of structural molecules, are afforded by the coarser *Podura* scales; principally due to the use of excessive angular aperture (figs. 3, 5).

It is now eighteen years since a single observation suggested the present research, which has been followed up almost continuously towards the attainment of transcendent high power definition.

Dark molecules suddenly started into view under accidental manipulation, but were most difficult of reproduction, and in finer objects of the same kind were often utterly unattainable.

The questions naturally arose—are certain optical zones in the objectives more effective; the spherical aberrations existing there as a minimum; or are there other occult causes of occasional yet splendid definition under high powers? This did not appear at all to be a question of the *minimum visible* because the objects exceeded the 1-50,000th of an inch in diameter.

These points may be illustrated by the records of some observations made by the writer.

Although the molecules of the scale from the insect *Podura domestica* are large, the 1-45,000th, a stringlet of these baffles the powers of the finest glasses now extant, as ordinarily employed, whilst those of the finer test scales (the *Podura curvicolis*) are hopelessly attempted by every observer who trusts to excessive angular aperture (*Pod. domestica*, fig. 3, Plate 3).*

Example 6.—*Data for Resolution.* 2-3rd objective as condenser: a U-shaped stop aperture placed on its front lens.† Powell and Lealand's 1-50th objective. Direct light of petroleum lamp.

Result.—A grand display of long rows of whitish molecules in continuous contact (power 2,500), fig. 4, and between these rows are seen closely packed rouleaux of a dark lead-blue colour in a parallel higher plane. The molecules appear like a pearl necklace, in stringlets of twenties and thirties, which can be easily counted, as each molecule appears to be about three degrees of visual angle in diameter.‡

Remove the U-shaped stop, and the whole beautiful resolution disappears (fig. 5, left half). If, now, instead of using this difficult

* See Plates 73, 74. "Monograph of the Collembola and Thysanura," by Sir John Lubbock, Bart. (Ray Society).

† This method causes a slight obliquity of illumination, besides considerably reducing the angular aperture of the objective. Aperture of condenser 30°.

‡ $\theta = \frac{1}{45000} \div 10 \times 2500 = \frac{5}{90} = 3^\circ$ nearly.

glass, a very fine 1-8th is used, at the same time that very oblique light illuminates the object, by tilting the axis of the condenser, black crescentic annuli are clearly developed (fig. 6). There is no doubt the view manipulated is entirely due to shadow thus developed and the preservation of marginal darkness. In this way the object may be shown with a very fine half-inch objective. The molecules are indeed huge compared with other visibilities: perhaps the explanation here given may enable other observers to confirm their existence in this comparatively easy object.

The magnificent *Oil Immersion Lens*, by Zeiss, of Jena, utterly fails with its full aperture to show the appearances just described. It displays grandly indeed the long corrugations, tubules, or ribs crossing each other irregularly, but entirely misses their beautiful contents: fully charged though they be with spherules (see fig. 7, Plate 3).

The total disappearance of this interesting structure, on the field of such a glass, is both surprising and instructive to the beholder.

Experiment 7.—Data of Resolution. Inferior 1-8th P. and L. 140° Objective, 1862. Aplanatic condenser 44° of aperture (Wray half-inch). February 5th, 1879. Power 800: cloudy daylight. Direct light.

The ribs are interrupted and alternately mottled blue and rose colour, slightly pink-coloured molecules without shadow annuli just discoverable peeping between the interrupted ribs. (The lowest focal plane must here be diligently searched.)

Example 8.—Data. Objective by Powell and Lealand of exceedingly fine quality. (Screw collar has $2\frac{1}{2}$ turns and was opened to 25° from zero.) Cloudy daylight; Same illumination.

Result.—Eidolic black dots very much smaller than spherules discernible between very decisive appearance of spines or exclamation markings (!!!). At a very high plane of focal vision, spines alone, the familiar optical test (fig. 8). *This singular result requires investigation.* Eidolic black dots are generally discoverable on a focal plane above the centre of refracting molecules.

Example 9.—Newest form of P. and L. 1-10th immersion, a glass of remarkable precision.

Lowest attainable focal plane, with “collar” fully open, so that “nose” is in contact with the “cover.” Daylight as before. Aperture of direct illuminating cone 44°, formed by Wray half-inch.

Result.—The spines are broken into *double rows of minute black dots*. Shadowy white beads are glimpsed (with most scrupulous attention to focal and collar adjustments) between the spines. But when the condenser is tilted, strong dark crescentic annuli suddenly appear on the shadowy whitish molecules.

Example 10.—Data. 3 P.M. Sun dimly visible: Powell and Lealand’s diaphragm No. 2, diameter 0.2 to reduce angular aperture of

Zeiss oil lens used as condenser. Objective Wray 1-10th immersion of extraordinary precision of definition; same object as before.

Result.—Whenever the sunshine was dimmed with clouds both spaces between the spines and the spines themselves are thoroughly resolved into molecules at the same instant. (A very difficult operation.) A slotted U-stop replacing the small diaphragm aperture now displayed both black and yellow molecules, the spines having totally disappeared.

As it now seemed only fair to Herr Zeiss' splendid "*oil immersion*" $\frac{1}{10}$, the aperture of the achromatic condenser was reduced to 44° , its axis being coincident with the optical. In place of using cedar oil the glass was immersed in a mixture of Rangoon and olive oil. Long straight chains of red beads appear shaded black at their margins, and yellow ones dotted black and in a lower focal plane (fig. 10).

Shaded ribs filled with spherules finely seen with 1-16th imm. Gundlach. The aperture of this glass was further reduced by using only a five-inch tube, and separating the front lenses as much as possible. This also corrected the spherical aberration (introduced by the tube being shortened) as well as reduced the angular aperture.

In these very difficult resolutions, skill, even in full practice, frequently fails.

Fortune may, however, surpass it, and I may record here a remarkable instance of it:—

Herr Bénéchè, of Berlin, has made for the writer a very excellent glass, which, however, performs best with direct central light (1-16th immersion objective). It will be now seen how important a factor in delicate observations is the thickness of the "cover" glass.*

Example 11.—*Data.* Bénéchè. Sleeting sky—same object. The nose touches the cover; slight action of the screw collar compresses the spherules. The Podura oil, natural to these scales, is seen slowly to permeate within the now flattened sac forming the scale. Rows of molecules vanish one after another. Upon releasing gently the slight pressure, they reappear at will one at a time. A molecule remaining still in contact with the cover appears dark. They lie in rows, by twos and threes, and here and there form V's by anastomosis. Placing the correction collar at the most favourable point for finest definition, intensely dark, almost jet-black, spherules appear like shot to occupy each rib. But the scale appeared utterly spoiled by pressure. The next day, however, it had entirely recovered its former appearance by the force of capillary attraction. Again, the native oil was squeezed gently along its many channels at a venture, by means of the fine focal adjustment.

* Bénéchè particularly insists, very properly, upon this: as, after all, the collar adjustment is but a coarse compensation for the peculiar aberrations introduced by a thin plate of glass of varying quality and thickness.

Result.—Many V-shaped rows are visible: double molecules here and there cross between the ribs. Rapid but delicate changes of pressure cause minute oil globules to flash up and down with focal points.

An interesting demonstration of the molecules completely filling up the scale, may be given by rotating the object so as to receive oblique pencils successively. The black crescentic shadows of the molecules may then be seen in different positions relative to the ribs of the scale (fig. 6). Fig. 10.

I must confess that in my earlier attempts to account for the extreme and often insurmountable difficulty attending the demonstration of minute crowded molecules in many translucent organic forms, it was erroneously concluded that residuary aberration was the only cause of their obstinate obscuration. I am now able to amend this conclusion by adding another obstacle, *excessive angular aperture, which I found attenuated margin.*

Indeed, upon patiently reducing the residuary errors to a minimum, by means of supplementary lenses, as described in the Transactions of the Royal Society,* it was further discovered that the mode there described very considerably reduced also angular aperture. This result necessarily followed the very considerable shortening of the posterior foci of the objectives employed: at the same time, notwithstanding the full aperture of the lenses is engaged, the residuary aberration of the whole is reduced also, in consequence of angular aperture being at the same time reduced: this large aperture being indeed the great source of spherical error.

The angular aperture may be reduced in the same objective by introducing stops between the back sets of lenses. It is better, however, to have a series of objectives of excellent quality of graduated apertures. Aperture can always be reduced by shortening the eye-tube, or by using low power objectives and deeper eye-pieces to get the same power. It may also be reduced in a manner by using an illuminating cone of light, whose aperture can be diminished at will.

There is, it may be said, something unnatural in the mode of vision intrinsic to very high angled glasses. It is undoubtedly true that such a glass presents an *all-round vision*. It really conveys visual rays from a given brilliant particle, at every inclination in azimuth and altitude; and this too at one and the same instant.

To illustrate this position, a minute die may be imagined the hundred thousandth broad. The highest angled objective really enables the

* "Phil. Trans.," vol. 160, 1870. "On a Searcher for Aplanatic Images," by the Author.

observer to collect rays emanating from *four sides*, and the top at the same instant. The human eye could at most view *three sides* at once. Doubtless, the effect of this angular vision all round the corners, causes particles to look spherical when sufficiently minute, even if cubical.

To these considerations we may add the complete attenuation of annuli (which are black in refracting molecules with low angular aperture), and the comparative invisibility of masses of molecules.

It may be remarked here, that the resolutions described have chiefly been accomplished by placing the objects in a condition to develop black annuli or crescentic shadows, and chiefly by reducing the aperture. It is also remarkable that the popular markings of the Podura scale lie in a higher plane than the molecules, and having been sought hitherto in a wrong focal plane, were naturally missed.

In cases, however, where the composite spherules are much more crowded and much more minute, mere reduction of aperture is inadequate to discover their existence. Their excessive delicacy of form and translucency of substance, the difficulties introduced by unmodified diffractions, and by searching the wrong focal planes, mismanagement of shadow-phenomena and uncorrected residuary aberration combine together to present such formidable obstacles to success, that few can congratulate themselves on the complete resolution of this celebrated test object.

[It having been pointed out that the apparent beadings on gnat scales have been degraded by the application of the term spurious, it may be said in reply that no proper explanation of these appearances has been given. On the other hand, spherules much finer may be distinguished on several delicate scales.

Mr. De La Rue pointed out many years since the beaded character of insect striated scales;* Mr. Stephenson has minutely described those of the gnat scale; and Mr. Slack other beaded scales, in "Student," vol. v.

In order to place the matter beyond reasonable doubt, and to clear up the structure, diligent search has been made among a considerable variety of these objects, and in the 2nd Plate drawings are given from

* "Trans. Mic. Society," vol. iii, December 20th, 1848. In this paper Mr. De La Rue was able to show that with a 1-12th Ross, the ribs of the butterfly scales of *Amathusia Horskfeldii*, were from the 1-6,000th to 1-10,000th apart centre to centre, and that cross striæ, under a power of 850, showed protuberances on beaded lines, and the latter focussed at their summits appeared as brown dots. The scale viewed from the under side exhibited the lower membrane as slightly undulating, probably from being dry, p. 36. He proceeds: "Some of my friends thought the constricted appearance of the cross striæ is due to overlaying pigment cell: this, in my opinion, is not correct, as I have convinced myself by repeated examinations, more especially from the under side, that the striæ themselves are really beaded."

the objects in view under powers varying from 1,000 to 3,000 diameters, by the aid of the camera lucida.

Scales seen in profile "end on."

Figs. *a, b, c*, represent the fortunate vertical appearance of a scale of the *Petrobius maritimus*, a Podura frequenting the coast limestone rocks. The scale was luckily caught between the slide and cover during the process of remounting (*a*). A slightly deeper focus showed appearance (*b*), deeper (*c*); a little more pressure caused the scale to turn over and lie nearly flat, recovering its vertical position on the removal of the pressure. This phenomenon solved the problem once for all, that the under side is smooth, the upper ridged. Black beads could be detected in (*c*) at the tips of the ridges. Having secured this fact, attention was next turned to examination of the tips of the spikes with a very exquisite definition of 2,000 diameters. On searching many specimens, a full-grown scale was found and accurately drawn from the microscope by an assistant. Never was a search better rewarded, for not only were the great ribs found beaded on both sides, but up to the very tip (fig. 12*d*); while transversely three or four spherules stretched across, in short rows, apparently supplemented by another parallel set placed in contact in a lower focal plane. Increased pressure being applied the scale was ruptured; the scale oil then flowed out, but returned, insinuating itself along some of its old channels; but while pressed out, the whole molecular structure became visible in the obliterated part; attempted to be shown in the engraving. Besides these a great number appeared to have burst forth from the disruption.

Podura macrotoma.—This little dark-brown leaping insect is very commonly found in the early spring under old timber and brick-bats.

A young scale is represented faithfully in fig. 14, a full grown one in fig. 15, and the cross beading under a power of 3,000 in fig. 16, the ribs being quite out of focus in an upper focal plane. In fig. 15 several spherules have escaped and lie irregularly upon the surface and outside the spikelets, each showing a bright focal point and *annulus*.

It should be here stated that an object-glass was used as condenser of only 15° angular aperture. Large aperture renders the spherules faint and almost invisible. Direct light was employed and *lard oil* with a Seibert 1-32nd, or No. XI objective of excellent quality. Great niceties were also attended to, such as—

(1.) Varying the length of the observing tube between 5 and 16 inches, changing the eye-pieces and especially altering the aberrations by the screw collar.

(2.) Varying the immersion fluid.

(3.) Varying the illumination by means of a series of objectives as condensers having angular apertures between 10° and 85°.

The full and brilliant display of these minute spherules is, therefore, attended with no little patient research. (Direct light used only.)

I wish to call attention to the appearance of a fine full-grown scale of the *Podura macrotoma* under from 2,000 to 3,000 diameters in three different focal planes.

Fig. 18 represents the upper focus.

Fig. 19 represents the middle focal plane.

Fig. 20. The lowest.

The *Podura macrotoma* appears very much to resemble in its scales those of the *Petrobius maritimus*. Younger scales show single rows of spherules on the ribs, and also between them.

Fig. 21. A strongly-marked scale, showing the appearance of large spherules.]

Method of Estimating very Minute Bodies by means of the Micrometer Gauge.

A series of fibres of spun glass is arranged according to thickness, with free ends projecting into the middle of the field of view of the eye-piece, also spider-lines placed across the stop:—

Thickness of No. 1 accurately measured 1- 1,750th of an inch.

„	No. 2	„	„	1- 2,000th	„
Spider-line	No. 3	„	„	1- 6,000th	„
„	No. 4	„	„	1- 8,000th	„
„	No. 5	„	„	1-10,000th	„

If a positive eye-piece be employed magnifying ten times with an objective of 1-10th focal length, the posterior focal image of the glass presents an image magnified 100 times, the total power being 1,000. If, then, No. 1 appears to gauge the size of a magnified object really 100 times less—

(No. 1.) Its size = $\frac{1}{1750} \times \frac{1}{100} = 1-175,000\text{th}$.

(No. 5.) The size would be $\frac{1}{100000} \times \frac{1}{100} = 1-1,000,000\text{th}$.

The micrometer gauge is not difficult of construction, as the projecting glass fibres can readily be cemented to the stop within the eye-piece. An object is much better compared by bringing it apparently to the free extremity of the fibre, so as to be in juxtaposition.

*Examples of Measurement:—*1. *Fibrillæ of the H. Janira Moth.* 1-80,000th to 1-90,000th of an inch thick. Black margin of the stem of the fibril about one-fourth of this: thickness of black margin between 1-300,000th and 1-400,000th. (Fig. 13b.)

2. *The delicate Membrane uniting the Minute Spikelets of the Scale of the Gnat.*—It may be observed that whenever a membrane is duplicated and flattened a dark black line is formed; the edge is, in fact, semi-cylindrical, and transmits no light.

If the spider-lines be employed, careful microscopic examination should be made and their actual thickness measured. When they cannot be exposed, a very long focus objective must be employed with a deep eye-piece and a long tube. It should be noted whether they are simple or complex in structure.

Method of Estimating the Size of a Minute Molecule or Line.—Contact is apparently made; first, with the outside of the spider-lines, the micrometer is then read. Inside contacts must now be noted. The mean will be the true size of the molecule, whether the spider webs are of the same thickness or are of different thicknesses.*

If a fine membrane be folded over tightly, the line of flexure appears exceedingly black and straight, and somewhat thicker than the membrane. The folded edge may then be considered at any point as a refracting semi-cylinder, of special optical beauty and precision of form unrivalled in organic life.

To ascertain roughly the nature of this black line, gold beater's skin was folded over and pressed; at the line of flexure a very sharp black line appeared, when examined with a pocket lens. This line appeared about *one-third* thicker than the free edge of the skin forming a section. Gold leaf exhibits similar dark lines of flexure,† upon the malachite green of the gold under transmitted light, if there be but little alloy present. On the whole, this line is thicker than the leaf, or about 1-100,000th, but it is not nearly so sharply displayed as in the flexure of insect scales; in many cases of which the breadth of this black line is even less‡ (particularly in the finest gnat and *Podura* scales).

Example.—In the gnat's scale the membrane, including the molecules of its structure, stretches in graceful little curves from spikelet

* If R be the first, R' the second reading δ, δ' , the thickness of the webs, it is evident, if Δ be true diameter of molecule,

$$\begin{aligned} R &= \Delta + \frac{1}{2}\delta + \frac{1}{2}\delta', \\ R' &= \Delta - \frac{1}{2}\delta - \frac{1}{2}\delta', \\ R + R' &= 2\Delta, \\ \Delta &= \frac{R + R'}{2} \text{ the mean,} \end{aligned}$$

which is independent of the size of the webs.

† One grain of gold can be hammered into leaves sufficient to cover $56\frac{1}{2}$ square inches; its thickness will then not exceed 1-282,000th of an inch.—“*Encyclopædia Britannica*,” Article “Gold.” The skin is really a tough French paper.

‡ This line has in general resisted every attempt at resolution; yet, when a corrugated scale is folded over, not too sharply, a serrated edge must necessarily exist. I have never been able to define this serration perfectly, except with object-glasses of the highest excellence and reduced aperture, which of course darkened and sharpened the defining shadows. A very fine view was obtained by means of the posterior focus of a 1-50th being shortened to about two inches, which very considerably reduced the aperture and admitted considerable separation of the front lenses, to correct the aberration thus introduced.

to spikelet, and is very black and distinct at the edge with a first-class glass, the objective aperture being reduced by using a condenser of about 15° aperture.

One of these terminators was placed between the spider-lines of a delicate micrometer in such a way that the lined edge was apparently half way between, and the interval on each side between them equal to the thickness of the lined edge or terminating curve. Neglecting the semi-thickness of the spider-lines, the distance between the latter was read 15 millionths (15m); one-third of this is 5m, or the 200,000th of an inch, whilst the general black margins of the ribs appear very much less.

As against transmitted light, causing diffraction, the extremities of the spikelets were barely visible and did not appear one-third the thickness of the black line edge or margin, or about the 600,000th of an inch. Between the ribs forming the spikelets rows of about four molecules could be seen stretching across like the rounds of a ladder. The ribs were 1-50,000th thick; the molecules about one-third of this in diameter.

On one occasion a scale was discovered "end" on. The ribs then appeared rounded off and prominent, and of an intensely blue-black colour; projecting nearly the same amount in relief as they are separated by the corrugated channels between them as semi-cylinders (fig. 22) on one side, *the other being quite smooth*.

The most surprisingly minute lines I have been able to detect were seen in examining an extremely small species of gnat. Mounted fresh and dry, the plumule hairs, about the 1-100,000th thick, were themselves hairy!—ciliated hairs.

The antennæ displayed central grooves also ciliated. And the secretory organ, whence the plume hairs issued, formed a boss or cup, around which a fringe of cilia of very minute magnitude was erected. Comparing these with the familiar images of spider-lines reduced in miniatures 140 times, I should not hesitate to estimate these objects at a size approaching the millionth or microscopical unit (m). An extraordinary instance of accidental magnification of these usually invisible cilia with perfect distinctness, indeed, I might say, with the most vivid definition (probably a power of 10,000 diameters) was discovered to-day. See description of Plates, fig. 17.

DESCRIPTION OF PLATES.

- Figures 1-2. Insect scales. Death's head moth under very low and high aperture.
 Figure 3. Ordinary appearance of *Degeeria domestica*.
 Figure 4. Resolution of *Podura domestica* (same object as fig. 3) into dark and light molecules, 1-50th Powell and Lealand objective.
 Figure 5. The approved appearance of the spines of the *Podura domestica* (left half).

Figure 6. Crescentic and tubular shadows. *Degeeria domestica*.

Figure 5*a*. Same object obliquely illuminated, after Mr. McIntyre.

Figures α , β , γ . . . θ . Refracting globules of melted glass filaments and refracting spherules. The black annulus varies in thickness, as described according to the conditions of refraction and the degree of angular aperture.

Figure 9 represents the spider-lines in the field of view. Minute black dots appeared on some of the mercurial mirrorlets, but too small for the writer to depict. Where the spider-lines crossed the minute reflecting circlets they appeared intensely black, but otherwise were seen plainly black upon a dark grey ground. The experiment is difficult of successful performance.

Figure 10. Highly magnified *Degeeria* spherules, with 1-50th object-glass.

Figure 11. Spherules of *Degeeria*, after Mr. McIntyre. "Monthly Microscopical Journal," 1870, p. 5, vol. iii.

Figures *a*, *b*, *c*. Successive views of spikelets of scale *Petrob. maritimus*, "end on," and in different focal planes, an extremity being one spherule. Plate 4.

Figure 12*d*. Black beaded appearance of spikelets and ribs. Same scale.

Figure 13*a*. Plume of Meadow-brown seen with low aperture, 1-80,000th inch thick being size of the fibrils. (*Hipparchus Janira*.)

Figure 13*b*. The same, with large angular aperture.

Figure 14. Young beaded scale of *Podura macrotoma*.

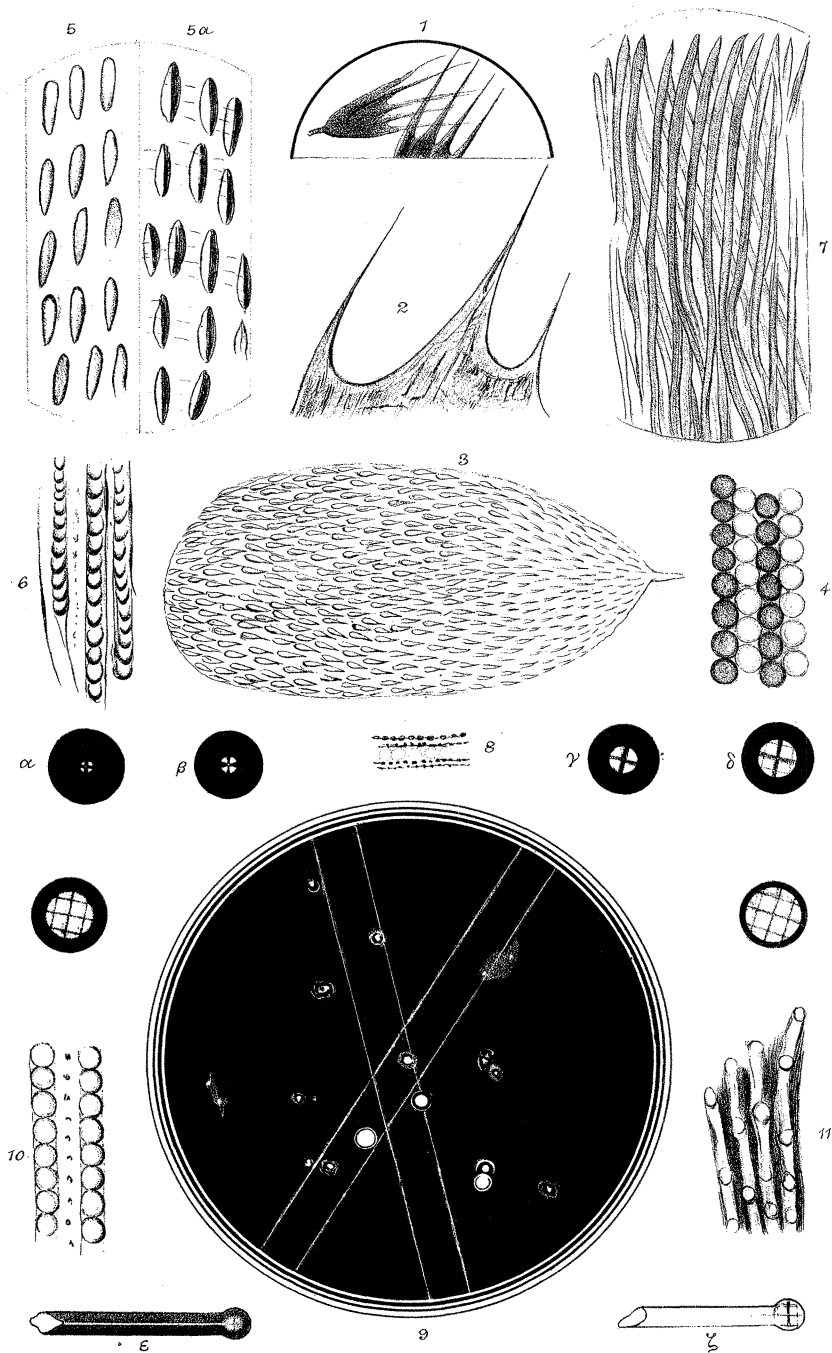
Figure 15. Mature scale; cross rouleaux of molecules developed; loose spherules escaped after pressure.

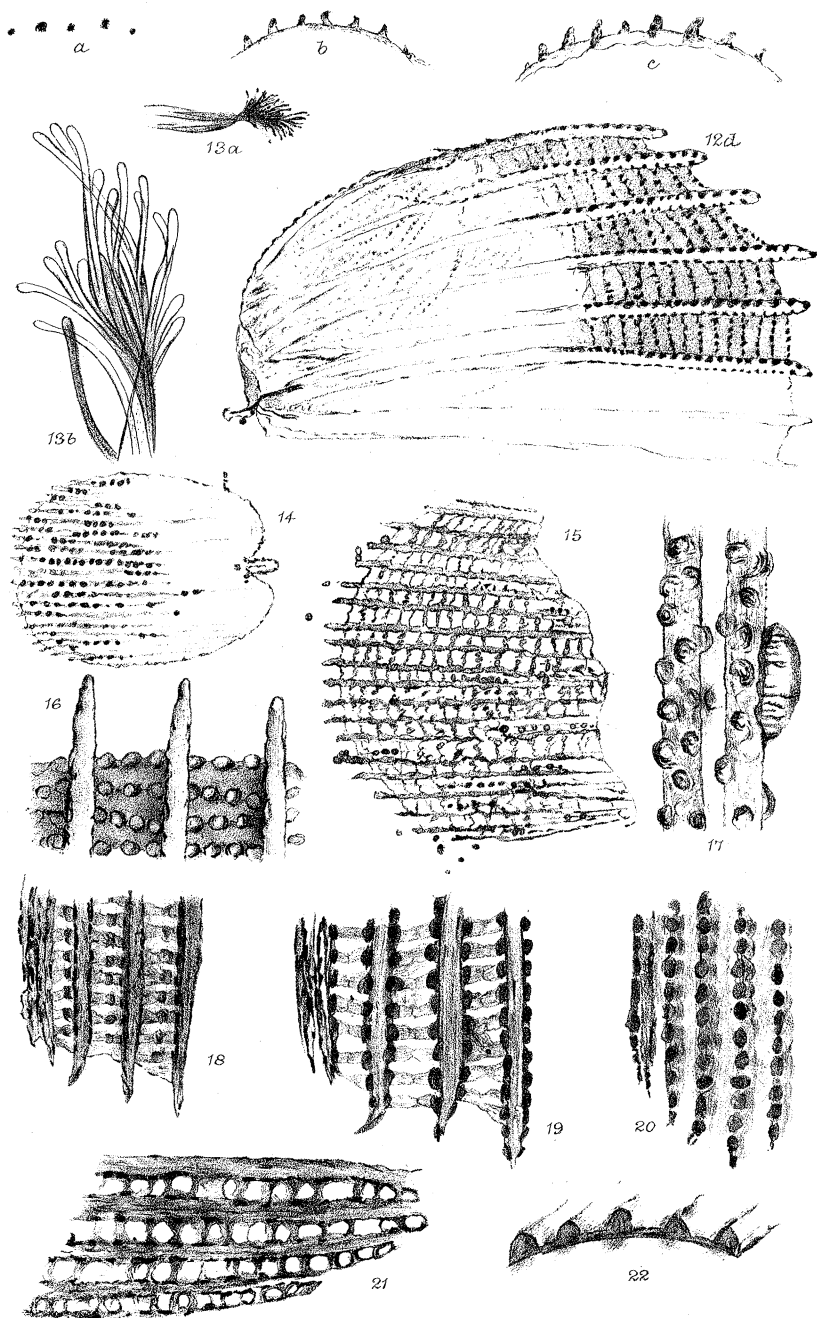
Figure 16. Highly magnified spikelets and transverse spherules in lower focal plane. Power 3,000, Seibert of Berlin 1-32nd, No. XI objective.

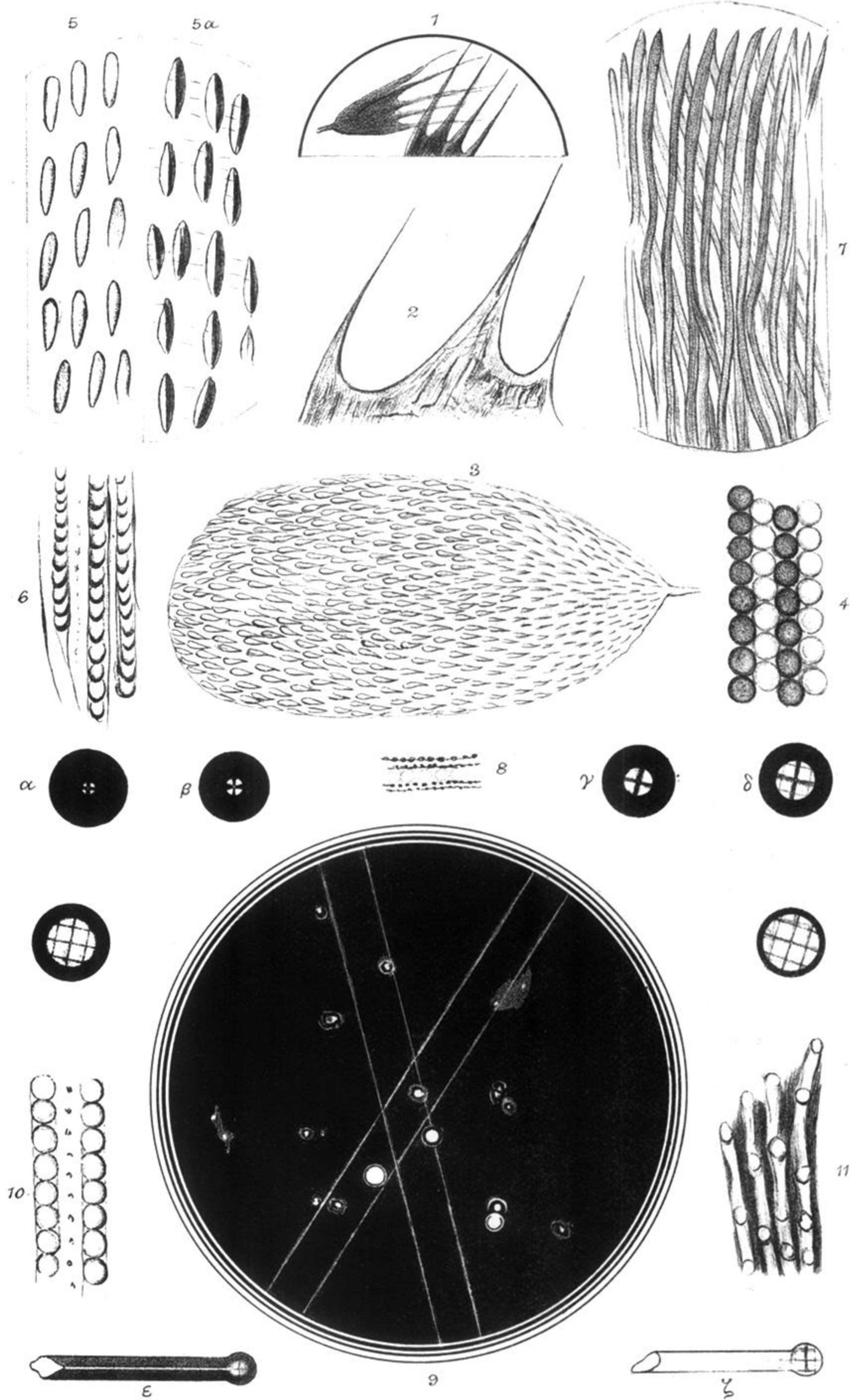
Figure 17. Extraordinary double or compound amplification of ciliary hairs growing on antennæ of male gnat; produced by an irregular oil globule adhering to the stem. The most wonderful power ever seen by the writer, and suggestive of further experiment, the cilia here being otherwise invisible.

Figures 18, 19, 20, and 21. Different appearances of the *Pod. macrot.* in higher, middle, and lower focal planes, 21 being a strongly marked beaded scale.

Figure 22. Gnat scale fortuitously discovered, and one similar to the scale shown, figs. *a*, *b*, *c*, Plate 4. One surface being ribbed, the other perfectly smooth. The intense blue-black is not shown: caused by the arrest of rays of light and the beauty of the definition as in lunar shadows.







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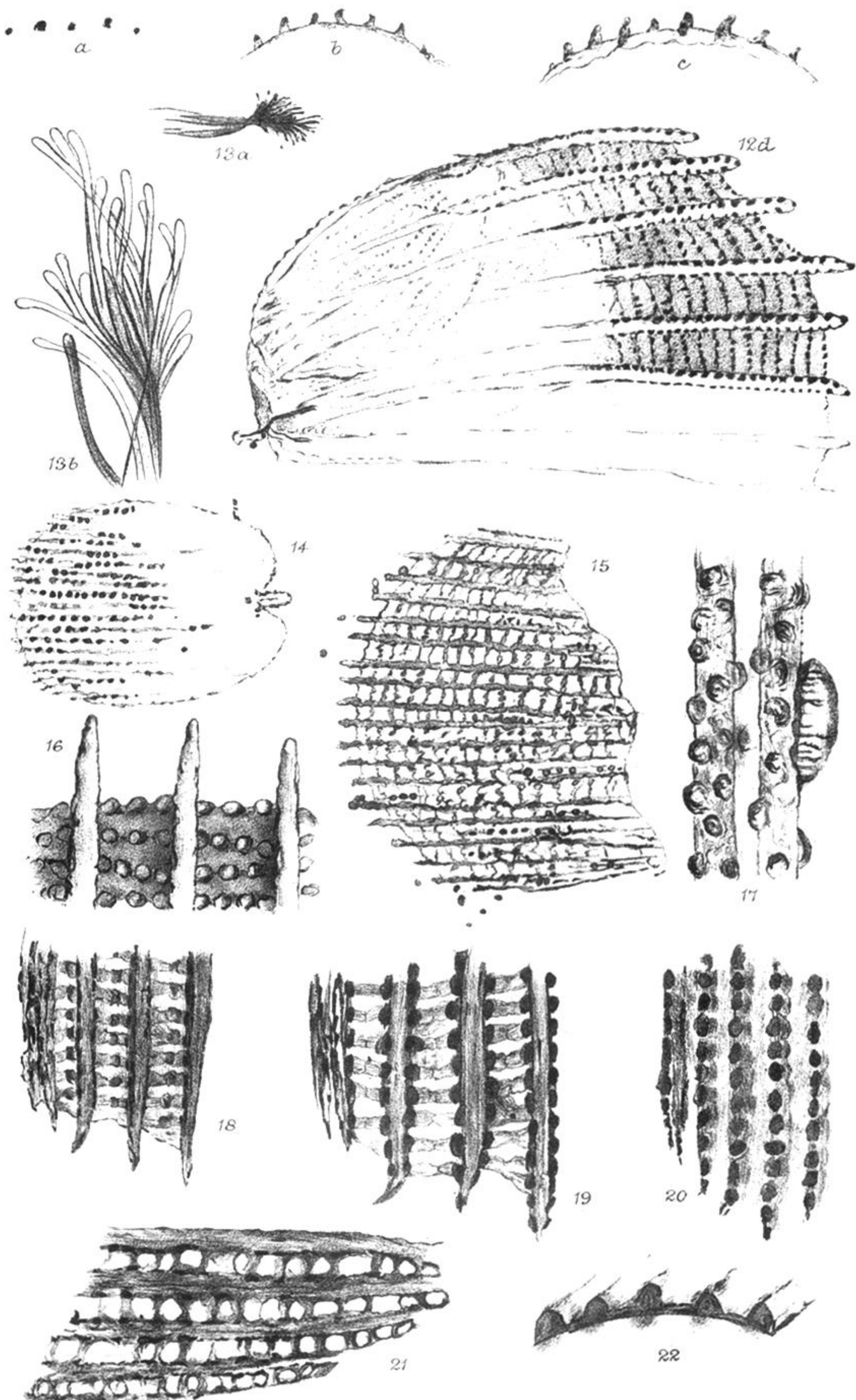
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