The Star Test for Microscope Optics

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Summary
The appearances produced by various on-axis and off-axis aberrations of microscope objectives are shown and described. The uses of different sizes of artificial stars are discussed, and the need for several types of test is emphasized.

MANY METHODS have been devised for testing the quality of optical systems, such as microscopes and telescopes, that are required to perform up to the theoretical limits for their apertures:

1. Resolution tests involve examination of ruled gratings or periodic objects of increasing fineness.
2. Interference tests show the shape of the wavefront emerging from the optical system.
3. In the star test and Abbe test, visual inspection of the image of an artifical object under varying conditions of focus and illumination is interpreted in terms of optical aberrations.
4. In the comparison test, images of a variety of objects are produced by the lens under test and compared with those given by a lens believed to be of good quality.
5. The Foucault knife-edge test is regularly used in the production of astronomical optics, but has seldom been applied to the microscope.

All of these testing methods are open to various objections. A graduated series of ruled gratings is difficult to obtain and natural periodic objects are variable. Interference and Foucault tests require elaborate apparatus. It is difficult to quantify the results of star or Abbe tests, and they are really a sophisticated form of comparison. In the comparison test how can the observer be certain that his standard lens is really of good quality? All he can say with certainty is that it is the best he has seen so far. The star test has the advantage of that it can be quickly and easily used without the need for any elaborate apparatus.

The star test originated as a test for the astronomical telescope by observing the focussed and defocussed images of a star, and has been thoroughly described by H. D. Taylor (1946). To a telescope, a star is effectively a point source of light, being smaller than the resolution limit, and the application of similar 'artificial stars' of small size to the testing of microscopes has been described by Slater (1957) and Dade (1958). This paper will discuss the advantages of various sizes of artifical stars.

A star slide for the microscope can be produced in several ways. The opticians of the last century used finely smashed mercury drops with oblique light, but these are not permanent and mercury vapour is toxic. For those with laboratory facilities, aluminium can be evaporated onto a slightly dusty slide, leaving a film with pinholes. A similar result can be obtained using pieces of Sellotape with metallic coating. This is sold for sealing Christmas parcels. The coating will be found to have numerous pinholes and, with persistence, a suitable sample can be found and stuck onto a slide. It is useful to have several pieces mounted under covers of differing thicknesses to test objectives for cover correction. (This was suggested by Dr G. Woolfe). An effective star slide can be produced by negatively stained bacteria. A tooth scraping of plaque is mixed with Indian ink and spread over a slide to dry. The bacteria show as transparent holes in an opaque film. Finely crushed, crystalline calcite mounted in balsam also provides a star test slide. Examined in polarized light between crossed polarizer and analyzer, the fragments appear as bright points on a dark background.

Most descriptions of the test have assumed that the pinhole 'star' should be so small that no structure in it can be resolved by the objective. This implies a diameter of less than 0.3 μm There are serious disadvantages in using such a small star. Critical illumination is needed with the lamp filament focussed onto the pinhole by a corrected condenser to provide sufficient light. The out-of-focus images of such a small source show concentric interference rings and these often hide the effects of zonal aberration in the objective.

The author's experience leads him to prefer a medium size pinhole with a diameter a few times greater than the resolution limit; 1.5 μm to 3 μm for a high power lens. Illumination by a diffuse source and condenser is sufficient, but Köhler illumination should be avoided as it may give anomalous out-of-focus images. Immersion condensers are necessary to fully test oil lenses. Diffraction rings no longer appear and any variations in brightness across the out-of-focus disc can be attributed to the faults of the objective. The eye seems to be more sensitive to slight variations of intensity in a uniform disc of light than to variations in brightness in a series of rings.

A perfectly corrected objective lens will show identical expansions of the image above and below the focus into a uniformly bright circle of light. A sufficiently powerful eyepiece should be used so that the aberrations of the eye do not affect the result. For the majority of objectives, a × 10 eyepiece is sufficient, but with objectives having abnormally large back lenses, a more powerful eyepiece may be needed to reduce the size of exit pupil sufficiently.

Studying the centre of the field, the first thing to look for is the unsymmetrical aberration: coma. This is shown by the defocussed star having one side brighter than the other, the same side being bright both above and below the focus. Central coma indicates that the component lenses of the objective are not correctly aligned, and the effect will revolve with the objective. The slightest amount of coma will destroy the contrast and resolution of the lens. If the brightness appears on opposite sides above and below focus, the illumination is oblique and this must be corrected before proceeding.

Spherical aberration is indicated by non-uniform intensity of light in the expanded, defocussed image. This is affected by the cover thickness, tube length and collar setting of adjustable objectives. These must be corrected before the quality of the objective can be assessed. With undercorrected spherical aberration, the focus of the lens decreases towards the outer zones of its aperture. Consequently, when the objective is moved closer to the test slide, the star expands into a disc showing a bright rim and a relatively dim centre. Above the focus, we see a light centre with the light fading gradually away to an ill-defined edge. At the best focus, a small amount of spherical aberration will leave a well-defined image with a surrounding flare of light; resolution is scarcely affected, but contrast is reduced. With more aberration, no sharp image can be found. Undercorrected spherical aberration may be caused by too short a tube, too thin a cover, a correction collar set for a thick cover, or possibly a pushed-in front lens, and these points should be considered before condemning the objective.

Over-corrected spherical aberration results in a longer focus for rays passed at the edge of the lens, and the appearances and causes are the reverse of the above.

As we adjust the tubelength or collar to minimize the spherical aberration, it will be a rare objective which does not show at least a trace of zonal aberration. In the usual form, rays through the centre and edge of the objective focus at the same distance, but rays passing through an intermediate zone come to a focus at a shorter distance. Above the focus, we see an expanded disc with a bright edge and centre, and below the focus a bright ring with dim centre and fuzzy outer edge (Fig. 1, Zonal 1). An alternative form of zonal aberration has the centre and intermediate zones well-corrected, but a rapidly increasing focus towards the edge (Zonal 2). In normal use, the condenser diaphragm cuts off the edge rays and the images with this type are more or less satisfactory. Closing the condenser iris with an objective having zonal aberration Type 1 produces the appearance of spherical undercorrection. Large aperture fluorite and apochromatic objectives often show more complicated types of zonal aberration with several long and short focus zones.

The colour correction of the objectives can also be examined. Achromatic objectives are designed to have a minimum focus in the centre of the spectrum and increasing focus towards the red or violet. In modern objectives, the minimum focus is in the green, but old objectives were intended for use with oil lamps and are overcorrected for colour with the minimum focus in the yellow or even orange. By raising the objective above a central star, this preferred colour will appear as the outer rim of the expanded disc. Different designs of eyepiece can have surprisingly large effects on this colour correction at the centre of the field, a point to be considered for photographic work. Apochromatic objectives should ideally show no colour effect here.

Examining star images at the edge of the field of view reveals a lot more aberrations. Here, the combined action of the objective and the eyepiece is involved and it is usually necessary to use an eyepiece designed to work with the particular objective. Colour effects in the focussed image show that the overall magnification is larger or smaller at the violet end of the spectrum than the red. Apochromatic and fluorite objectives give more magnification in the violet, and this is balanced by compensating eyepieces magnifying more in the red. Adjustable eyepieces with variable compensation can control this effect for a range of different objectives, but may not match other off-axis effects.

ABERRATION	ABOVE FOCUS	BELOW FOCUS
Oblique Illumination		
Coma		
Under-corrected Spherical		
Corrected		
Zonal 1		
Zonal 2		

FIG. 1. Intensity variation across the images of a medium-size pinhole formed by objectives with various aberrations.

The asymmetrical aberration coma may be seen towards the edge of the field, causing a flare of light either inside or outside the focussed star images. Modern objectives and eyepieces are usually well corrected for coma, but until the end of the last century, the theory of coma correction was not well understood and in the effort to give good spherical and chromatic correction in high power objectives, coma was often left to chance. This resulted in good images being confined to a very small region in the centre of the field.

Flat field objectives especially should be tested for field curvature. Stars at the centre and edge of the field should come to a focus at the same setting. Many objective-eyepiece combinations will show astigmatism at the edge of the field. At one side of the focus, the star will appear as a short radial line, and on the other side of the focus, the image becomes a tangential line. For under-corrected astigmatism, the focus is shorter for the tangential than for the radial. Watson Holos objectives were designed to give strongly under-corrected astigmatism. This is compensated by over-corrected astigmatism of the Holos eyepiece.

A complementary method of testing is provided by using a large pinhole covering an appreciable fraction of the field of view. For small pinholes, the transmitted light is diffracted out of its incident direction, but with a large pinhole, a beam of light can be directed at a particular part of the objective by arranging stops or diaphragms below the condenser. If the objective has a defective outer zone, the maximum useful aperture can be determined by progressively opening the substage iris from a small size. When the light reaches the defective zone, a flare of light appears at the edge of the pinhole, and grows as the iris is opened. A sensitive test for the outer zones is provided by using oblique light. An eccentric stop can be used below the condenser to direct light to the edge of the objective; any resulting displacement of the image indicates that rays passing near the edge of the objective are not correctly focussed. This test is similar to the Abbe test.

As an example of the need for the two complementary methods, an old Ross 0.25 inch objective with triple front lens was tested by the star test and showed uniformly illuminated expanded star images above and below focus. It thus appeared to be satisfactory until tried with a large pinhole. When the condenser iris was opened beyond two-thirds of the objective aperture, a flare of light spread across the field of view. The reason is that the central parts of the lens are well corrected, but the aberration of the outer third is so great that it did not contribute to the star image, but scattered light over the whole field of view.

For those who wish to go even deeper into the performance of their lenses, it is also possible to use the star test to examine spherochromatism, the variation of spherical aberration with colour of illumination. The correction of spherical aberration in most objectives occurs by refraction of the rays at the diverging contact surfaces where the crown and flint elements are cemented together. The higher dispersive power of the flint results in stronger correcting action at the violet end of the spectrum than at the red. Thus, most achromatic objectives are spherically over-corrected in the blue-violet colours and under-corrected in red. This defect was particularly serious in old-fashioned objectives of the last century with triple cemented front components: it is reduced in single-front objectives and removed altogether in apochromats. Its effects can be easily seen by carrying out the star test with a variety of coloured filters in the illumination. For each colour, there may be a range of tubelength, correction collar setting or cover thickness for which the spherical aberration is satisfactorily corrected, and outside this range the image deteriorates. At the violet end of the spectrum, the acceptable tubelengths will be shorter than for the red. If the ranges of acceptable tubelength for violet and red do not overlap, there is no tubelength suitable for all colours.

The sensitivity of objectives to variations of cover thickness and tubelength varies enormously. The × 10 achromat is scarcely affected by any normal variations in cover thickness or tubelength. High aperture, short focus dry lenses, such as a 3 mm 0.9 NA, are extremely sensitive to cover thickness, but only moderately affected by tubelength, and so need correction collars for effective working. Conversely, 8 mm or 12 mm apochromats with apertures of 0.65 are scarcely affected by normal (No. 1 or No. 2) covers, but need critical adjustment of tube almost to a millimetre.

Finally, a negative (or black) star test is a sensitive way of detecting scattered light. A small opaque object on a transparent slide allows plenty of light to enter the objective. If no light is scattered, the image of the object will be completely black. This test will show up deteriorating balsam, dust and fingerprints, decomposing glass in apochromats, and shiny metal mounts. If a lens passes all these tests, the microscopist can be confident that he is not missing anything as a result of faults in his lens.

REFERENCES

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An Early Test Object

Of the little Greenish Grasshopper or Locust. This pretty Animal is a pleasant Object to look upon in our Glass, being of a bright green, and in the full Sunshine shews exactly like green Cloth of Silver; hath two horns and four leggs, two on each side. Her eyes are such little black atoms, that, unless to a very critical and smart eye, they are indiscernable; yet, if you advantageously place her and view her with a full light (transmitted through a Burning-glass, which article I sometimes use) you shall fairly see them to be as bigg as two small black round Beads, and drilled through also with innumerable perforations (as the eye of a fly) which will try the exquisiteness both of your Glass and Eye to behold.

Dr Henry Power in *Expermental Philosophy* 1664.

Aperture Does Not Count

Resolving power. We regret not to have the necessary authority to erase this word from the dictionary of the microscopist, since it appears to us to constitute an entire superfluity. To say of an objective that it has resolving power is, according to most authors, to attribute to it the power of isolating, so to say, one from another the finest details of structure on the surface of a transparent object such as striae, fibrillae, depressions, reliefs, *etc.*; but an objective that defines well in the complete sense of the word, ought it not to resolve perfectly?

From *Traité de Microscopie* by M. A. Zune, 1889.

Encouragement for Diatom Dotters

Let us say that in respect of diatoms a prejudgement is widespread amongst persons who are occupied with histology and comparative anatomy. It is that *examination of diatoms is a waste of time.* In our view the study of certain diatoms is a salutary exercise when one is starting in the art of microscopy; the eye is trained to notice minimal details and to see them as they are. One acquires the valuable ability of appreciating the worth of the instrument one works with, one arrives at the knowledge of the limits beyond which one falls into error.

From P. Francotte's *Manuel de Technique Microscopique*, 1878.