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*Published in:*  
Journal of microscopy-Oxford

*DOI:*  
[10.1111/j.1365-2818.1990.tb02980.x](https://doi.org/10.1111/j.1365-2818.1990.tb02980.x)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1990

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*  
STAVENGA, D. G., & LEERTOUWER, H. L. (1990). CURVATURE MEASUREMENT WITH REFLECTED-LIGHT MICROSCOPY AND ITS APPLICATION TO FLY FACET LENSES. *Journal of microscopy-Oxford*, 158(1), 87-93. DOI: 10.1111/j.1365-2818.1990.tb02980.x

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## Curvature measurement with reflected-light microscopy and its application to fly facet lenses

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**KEY WORDS.** Curvature, reflected-light microscopy, interference microscopy, Newton rings, Michelson, fly, facet lens.

### SUMMARY

Two methods for microscopically measuring the curvature of strongly curved surfaces are compared: one using a Michelson interferometer-type microscope and one using a novel reflectometrical method implemented in an epi-illumination microscope. The curvature values obtained with the two methods were very similar, but the latter proved to be by far the simplest.

Curvature measurements on the front surface of the facet lenses of various dipteran flies revealed that facet lens diameter and radius of curvature are linearly related over a wide range of facet lens sizes.

### INTRODUCTION

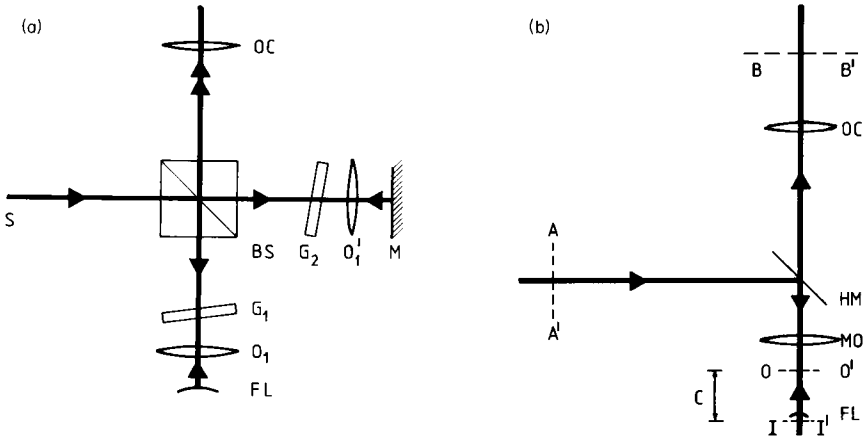
The power of a refractive surface is principally determined by its curvature. Measurement of the curvature of lens surfaces by optical methods is therefore standard practice. The measuring devices, called (optical) spherometers (lens surfaces commonly have a spherical shape), all are different types of reflected-light microscopes. Here, a simple spherometrical method is introduced, which can be executed with any standard microscope equipped with an epi-illumination attachment. This method was found to give quite accurate measurements of radius of curvature over a range of several millimetres down to several micrometres by comparing the results with those obtained with another, well-known method of measuring curvature, namely one using Newton interference rings. A reflected-light microinterferometer based on the Michelson interference principle was used and was found to work as well in the same range of curvatures, although in practice it appears not to be the easiest to use.

The immediate impetus for this work was a study of the optics of fly facet lenses. Quite surprisingly, the most important parameters, the radius of curvature of the front surface and facet lens diameter, appear to have an approximately constant ratio over the whole range of known lens sizes.

### METHODS

#### *Michelson interference microscopy*

A Linnik-type interference microscope was used (Fig. 1a), which applies the principle of the Michelson-interferometer, produced by Zeiss (Räntsch, 1945; see also



**Fig. 1.** Diagrams of two reflected-light microscopes for measuring curvature. (a) Zeiss-Michelson interference microscope. *S*, light source; *BS*, beam splitter; *G*<sub>1</sub>, *G*<sub>2</sub>, compensation plates; *O*<sub>1</sub>, *O*'<sub>1</sub>, identical objectives; *M*, mirror; *FL*, curved surface under investigation; *OC*, ocular. (b) Epi-illumination microscope. *AA'*, annulus pattern; *OO'*, its image projected by microscope objective *MO*; *II'*, image of *OO'* projected by curved surface *FL*; *BB'*, image of *II'* projected by compound microscope; *c*, distance between *OO'* and *II'*; *HM*, half-silvered mirror; *OC*, (photo-)ocular.

Françon, 1961). A parallel beam from the light source *S* is divided by the beam splitter *BS* into two beams which then travel through two identical paths except for the different mirrors *M* and *FL*, respectively; the two beams interfere and the resulting pattern is observed or photographed via ocular *OC*. *G*<sub>1</sub> and *G*<sub>2</sub> are two planparallel compensation plates, and *O*<sub>1</sub> and *O*'<sub>1</sub> are two identical microscope objectives. (Objectives of magnification 60 were used in Fig. 2.) The flat mirror *M* is selected from a set so that its reflectivity approximately matches that of the unknown mirror *FL*, being, for example, the curved front surface of a fly facet lens. The resulting interference pattern then consists of concentric light and dark rings. The pattern was analysed by photograph densitometry.

From the radius of the *n*th (dark) interference ring, *r*<sub>*n*</sub>, the required value of the radius of curvature *R* of the surface is calculated from

$$r_n^2 = (n + p)R\lambda = nR\lambda + pR\lambda, \tag{1}$$

where *p* is a constant ( $0 < p < 1$ ) and  $\lambda$  is the wavelength of the light. The phase factor  $p = 0$  when the two planes of the flat mirror *M* and that tangential to the vertex of the mirror *FL* (Fig. 1a) are conjugate. Only in this case does Eq. 1 reduce to the textbook case of the Newton rings.

The light source of the Zeiss interference microscope is a thallium lamp with a single line at 535 nm. It can be replaced with a white-light source for observing the 0th order.

### Microreflectometry

The radius of curvature of a curved surface can be determined with an incident light microscope set-up, shown diagrammatically in Fig. 1(b). The image of an annulus pattern (*AA'*), projected by the microscope objective (*MO*), serves as the object (*OO'*) for the surface of, for example, a fly facet lens (*FL*), which acts as a convex spherical mirror, thus creating an image (*II'*).

Following the convention of Longhurst (1981), the equation for reflection at a spherical surface is  $1/l + 1/l' = 2/r$ , with *l* and *l'* the object and image distance, respectively, and *r* the radius of curvature of the spherical mirror. In the conventional

incident light microscope the stage is moved while focusing, and hence the object  $OO'$  remains fixed, while the image  $II'$  (Fig. 1b) is moved until it is in focus. The object-image distance ( $OO'-II'$ ) is thus always constant ( $c$ ). In our experiment the object is in front of mirror FL, or the distance  $OO'-FL$ :  $l < 0$ . The image distance ( $FL-II'$ )  $l' > 0$  when  $r > 0$ , i.e. when the surface is convex, and  $l' < 0$  when  $r < 0$ , i.e. when the surface is concave; or, always  $-l + l' = c$ . Together with the transverse magnification  $m = -l'/l$  the radius of curvature of the spherical mirror is then readily derived to be

$$r = 2cm/(1 - m^2). \quad (2)$$

The distance  $c$  between object and image can be determined by reading the distance  $c/2$  between the two positions of the stage with a (slightly scratched) flat mirror in focus and with the image of the annulus in focus, respectively. The object size,  $OO'$ , is determined by measuring the size of its image in the specific case of the flat mirror. Together with the image size,  $II'$ , created by the spherical mirror, the value of the magnification is then calculated from  $m = II'/OO'$ . In the case of the very small facet lenses of flies  $m \ll 1$ ; then  $r$  follows simply from  $r = 2cm$ .

The values of  $OO'$  and  $II'$  were determined by photograph densitometry. Because the estimation of the distance  $c$  is quite critical, the method was calibrated with a ball-bearing. From the magnification  $M$  of the annulus pattern and the radius of the ball-bearing  $R$ , determined with a screw micrometer,  $r$  follows from:

$$r = R(m/M)(1 - M^2)/(1 - m^2). \quad (3)$$

A Zeiss Universal Photomicroscope with its epi-illumination attachment was used. For radii of curvature in the micrometre range, the (dry) objective Epiplan  $40 \times$  proved to be appropriate. Measurements on immersed facet lenses could be equally well performed with the water immersion objective Zeiss 40 W.

## RESULTS

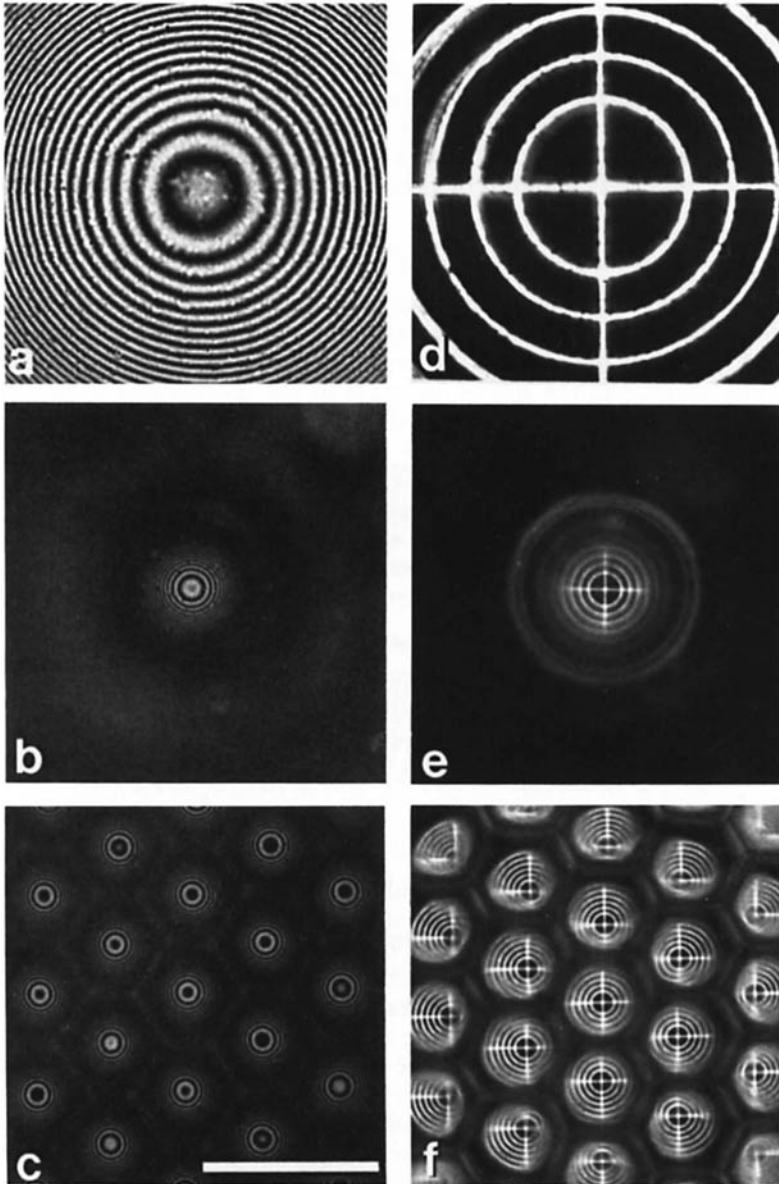
### *Michelson interference microscopy*

In order to appraise the practical usefulness of the various methods of curvature measurement a known standard was first studied. A steel ball with radius  $R = 497.0 \pm 0.5 \mu\text{m}$  (determined with a screw micrometer) was examined with the interference microscope. The interference pattern (Fig. 2a) displayed the classical light and dark rings. Densitometry of the photographs yielded the radii of the (dark) rings (Fig. 3). The results correspond well to the described linear function (Eq. 1). The slope of the linear fit,  $R\lambda$ , together with the value of the light wavelength  $\lambda = 535 \text{ nm}$ , yields the radius of curvature of the ball. However, the resultant value of  $R$  ( $513 \pm 5 \mu\text{m}$ ) does not fully conform with the directly measured value ( $497 \mu\text{m}$ ).

Closer inspection showed that the interference method is very sensitive to the slightest defocusing: a change in focus of a few micrometres yields a change of a few per cent in the value of the resulting radius of curvature. Only a focus setting at exactly the 0th order (observed with the white-light source as a dark achromatic spot at the top of the sphere) gave a value corresponding with the direct (micrometer) estimate. However, an error of a few per cent is quite acceptable for the strongly curved surfaces of the small glass bead and the facet lenses of Fig. 2(b) and (c), respectively.

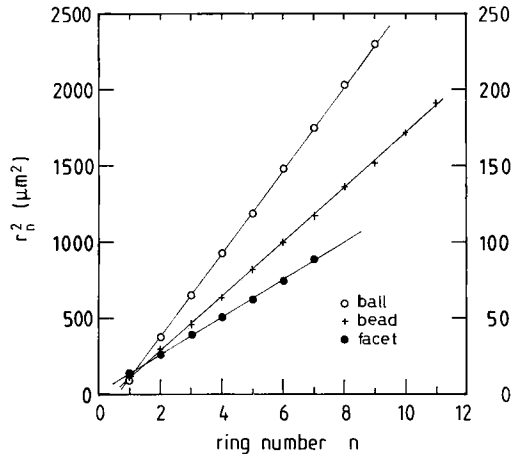
The radius of curvature of the glass bead estimated from a photomicrograph of the cross-section at the equator of the bead was  $33.2 \pm 0.8 \mu\text{m}$ , and the value calculated from the evaluated interference patterns (Fig. 2b) was  $33.5 \pm 0.9 \mu\text{m}$  (Fig. 3).

The cornea, i.e. the assembly of facet lenses, of a fly's eye observed under the interference microscope displays interference patterns in each facet (Fig. 2c).



**Fig. 2.** (a)–(c). Interference patterns observed with an objective of magnification = 60 in the interference microscope from a steel ball (a), a glass bead (b), and the front surfaces of the facet lenses in the eye of a blowfly, *Calliphora erythrocephala* (c). (d)–(f). Images of an annulus pattern projected by the steel ball (d), glass bead (e) and fly facet lenses (f). Scale bar = 50  $\mu\text{m}$ .

Evaluation establishes that the neighbouring facet lenses have almost identically shaped front surfaces. The resulting radius of curvature is  $23.0 \pm 1.1 \mu\text{m}$  (Fig. 3). The uncertainty originates from the errors in the evaluation procedure of the photographs, and also from the impossibility of viewing the apex of all facets simultaneously in the focal plane, because of the shape of the eye.

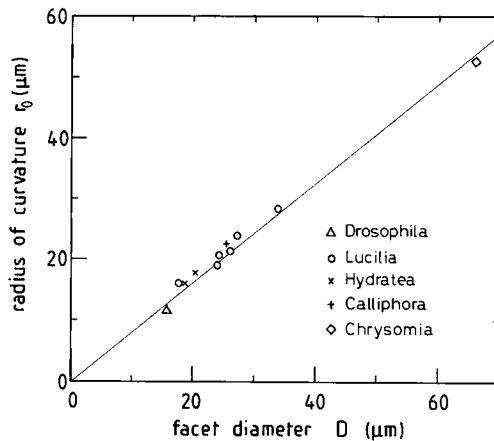


**Fig. 3.** The square of the radii of the dark interference rings, obtained from the ball, bead and one of the facet lenses of Fig. 2(a)–(c), plotted as a function of the ring number. The left hand coordinate values apply to the steel ball only, whereas the right hand values apply to the bead and the facet lens. The slope of the linear function equals the radius of curvature of the surface multiplied by the wavelength  $\lambda$  (Eq. 1).

*Microreflectometry*

The same objects (i.e. steel ball, glass bead and fly eye) were investigated with the microreflectometrical method. Figure 2(d)–(f) shows the images of an annulus pattern observed in the three cases, respectively. Using the value of the radius of curvature of the ball ( $497 \mu\text{m}$ ), the value for the bead and the facet lenses, resulting from Eq. 2, were  $33.2 \pm 0.8 \mu\text{m}$  and  $22.7 \pm 1.3 \mu\text{m}$ , respectively, in good agreement with the above results from the interference experiments.

This technique was applied to a number of fly species in a series of comparative measurements. The flies were immobilized with wax and mounted on a rotatable stage, thus allowing *in vivo* measurements from various areas of the eyes with differently sized facets. The radius of curvature of the front surface of fly facet lenses,  $r_0$ , was



**Fig. 4.** Radius of curvature of the front surface of the facet lenses determined with the microreflectometrical method for a number of fly species. The facet lens radius of curvature,  $r_0$ , appears to be linearly related to the facet lens diameter,  $D$ . The errors are approximately the size of the symbols.

found to increase linearly with the diameter,  $D$ , of the facet lens (Fig. 4);  $D$  is the diameter of the inscribed circle in the usually hexagonal to square facet. The measured values approximate to the linear function  $r_0 = 0.82 D$  over the full range of diameter values (15–65  $\mu\text{m}$ ).

#### DISCUSSION

The radius of curvature of spherically shaped surfaces can be determined with several spherometrical methods (Kessler, 1927). The most straightforward optical spherometer microscope is that using auto-collimation (see, e.g., Longhurst, 1981), so that for a curved reflecting surface there exist two positions of the surface which give an image of cross-wires coincident with the cross-wires themselves; the distance between the two positions of the surface equals its radius of curvature. Sophisticated, automated spherometer microscopes for routine applications are now commercially available. However, these are specialized instruments and the auto-collimation method does not readily allow curvature measurements when deviations from a spherical shape occur. Deviations from sphericity can be quite well determined using the two methods described in the present paper (see, e.g., Landwehr, 1959).

Accidentally, an ophthalmological instrument for determining deviations from sphericity of the human cornea, the keratoscope, also called Placido's disc (Knoll, 1969), is rather similar to the microreflectometer presented here. In keratoscopy, a pattern like that of Fig. 2(d) is imaged by the corneal surface, and deviations from the normal shape are thus readily observed.

Radius of curvature measurements by interference microscopy have been described by Krug *et al.* (1964). They evaluated the radii of the interference rings and subsequently calculated the radius of curvature of the spherical surface from the formula:  $R = (r_m^2 - r_n^2)/\lambda(m - n)$ . Taking different combinations of ring numbers  $m$  and  $n$ , Krug *et al.* (1964, p. 223) found quite different values for  $R$ . However, re-evaluating their exemplary case with Eq. 1 yields a very satisfactory linear fit. Our results thus show that instead of factoring out the phase parameter  $p$  (Eq. 1) it is a much better approach to make a direct, linear fit to the squared values of the interference rings radii. The interference method thus is, contrary to what is stated by previous authors, quite valid for curvature measurements.

For the interference measurements at the facet lenses it was necessary to decapitate the flies, because the slightest movements proved to be catastrophic. Also, the need to select the proper reflectivity of the reference mirror and carefully adjust the two beams for coincidence of the optical pathways does not make the interference procedure the method of choice, regardless of the visual attractiveness of the patterns produced. Furthermore, the interference method requires a very special instrument. This is not the case with the microreflectometrical method, which is simple to execute on any curved reflective surface and certainly on the facet lenses of live flies. Because the accuracy of the two compared methods is approximately the same, the microreflectometrical method is clearly advantageous.

One limitation of the microreflectometrical method is that the formula, used in calculating the radius of curvature, assumes the applicability of Gaussian, paraxial optics. Experiments on glass beads with radii in the range of 15–30  $\mu\text{m}$  showed that this is valid for the inner annuli of the pattern images, but that the outer annuli gave systematically larger values for the radius of curvature, though the deviations were no more than a few per cent from that obtained from the inner annuli. An almost identical increase in radius of curvature was calculated from the outer annuli in the facet lens images, indicating that fly facet lenses are, very approximately, perfect spheres over their total exposed surface.

After Hooke's first microscopical reflections on fly eyes, showing his workroom windows (Hooke, 1665), the radius of curvature of the front surface and the diameter of fly facet lenses have been shown to have an approximately constant ratio, for all investigated dipteran flies. If it is assumed that the power of a facet lens predominantly resides in its front surface (see Stavenga *et al.*, 1989), it follows that the focal distance of the facet lens is roughly proportional to the radius of curvature of the front surface. Consequently, the focal distance and facet lens diameter then have a constant ratio; i.e. fly facet lenses have a constant F-number. In a separate study on blowflies, *Calliphora erythrocephala* and *Chrysomya megacephala*, the effective F-number was between 2.1/2.4 (Stavenga *et al.*, 1989). Presumably, the F-number is set to an optimal value for visual acuity (see van Hateren, 1985).

The machinery underlying the construction of the constant optical properties of the facet lenses is completely unknown. Curvature measurements by reflectometry during lens growth may be a simple, but valuable method for gaining further understanding of the factors determining the parameters of the facet lens, being the initial optical element in the pathway of visual information processing by the fly visual system.

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