

## FOCUSING OF LIGHT BY CORNEAL LENSES IN A REFLECTING SUPERPOSITION EYE

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In recent articles Vogt (1975, 1977, 1980) and Land (1976, 1978) have put forward a theory of reflection optics as a method by which a superposition image is formed on the retina in macruran decapods, notably the crayfish. A feature of the reflecting superposition eyes investigated so far by Vogt & Land is that the corneal facets have negligible focusing properties, such focusing as exists being achieved by total internal reflection in the proximal part of the crystalline cone. The present work shows for the first time that the cornea of an Australian crayfish, *Cherax destructor*, which otherwise has all the required anatomical features of such a reflecting superposition eye (Fig. 1 A, B, C), possesses corneal facet lenses each of which focuses light from a distant object (forming a real inverted image) to a position behind the cornea approximately corresponding to that of the distal end of the rhabdom. Each corneal facet lens consists of two layers, the outer layer having a flat front surface and the inner layer having a convex back surface (see Fig. 3).

The optical measurements were made on freshly isolated pieces of cornea cleared of all adhering tissue and suspended from a coverslip on a drop of bovine serum albumen solution with a refractive index of either 1.411 or 1.365. Albumen does not affect the refractive index of the cornea (Vogt, 1974), and the two values of albumen refractive index were chosen to encompass the range of probable *in vivo* refractive-index values.

The back focal distance (B.F.D.) of individual corneal facet lenses (the distance between the back of the lens and the back focal point) was determined by focusing consecutively on the back surface of a lens and on the image of a distant object formed by the lens (object distance 50 mm, effectively infinity for a lens with a front focal length of about 400  $\mu\text{m}$ ).

The Zeiss compound microscope used had a calibrated micrometer gauge attached to the stage for accurate measurement of the focal distances. B.F.D. values were corrected for the apparent depth effect (Galbraith, 1955). The medium in front of the cornea was either Ringer solution or air, the refractive indexes of which closely approximate the natural environment of this crayfish, which lives in and beside ponds. Images of a figure '7' formed by several corneal facet lenses are shown in Fig. 2.

The B.F.D. for individual facets was found to be relatively constant over different regions of the eye in individual animals and from animal to animal of similar carapace size (35–45 mm long). The results are shown in Table 1. Although there is a localized position at which the sharpest image can be seen, a reasonable focus can be obtained over a depth of about 50  $\mu\text{m}$ . There is little difference between immersion in air/albumen and in Ringer/albumen as a consequence of the flat front surface of each facet (Kuiper, 1966).

Table 1. *Measured back focal distances of individual corneal facet lenses in different combinations of media*

(The values are the average of three measurements for each of six facets.)

Medium in front of cornea	Medium behind cornea	Range of B.F.D. values ( $\mu\text{m}$ )
(1) Air	Albumen, $n = 1.411$	420–550
(2) Ringer, $n = 1.338$	Albumen, $n = 1.411$	450–560
(3) Air	Albumen, $n = 1.365$	385–450
(4) Ringer, $n = 1.338$	Albumen, $n = 1.365$	410–465
(5) Photomicrograph measurement:- Distance, cornea – rhabdoms		450–500

The refractive index of albumen is similar to that measured for the distal region of the crystalline cones in other crustaceans (Land, 1976; Vogt, 1980), in which there was a gradation of values from  $n = 1.41$ , distally, to  $n = 1.38$ , near the rhabdoms. (These values may be a little high because of the fixation process carried out on the cones prior to measurement, and they remain to be determined accurately for *Cherax*.) With these values, an albumen solution of  $n = 1.411$  can be used *in vitro* to approximate to the *in vivo* situation in which the distal ends of the crystalline cones are in close proximity to the back surface of the cornea.

In both the *in vivo* and *in vitro* situations, the back surface curvature of the cornea is neutralized as a refracting mechanism because its measured refractive index ( $n = 1.417$ ) is similar to that of the distal ends of the cones and albumen. That an image is still formed (see Fig. 2) indicates that there is a refracting mechanism within the lens. This finding is discussed below. The ranges of B.F.D. values for the two combinations air/albumen and Ringer/albumen encompass the values of the distance between the back of the cornea and the distal tips of the rhabdoms measured from photomicrographs of longitudinal sections of the eye. It is also found from these photographs that there is little variation in this distance over the whole eye. This shows that the corneal lenses focus light onto the distal tips of the rhabdoms.

As the refractive index of the crystalline cone drops to 1.38 proximally, the actual B.F.D. in the eye may be slightly shorter than that measured in albumen with  $n = 1.41$ . To attain an estimate of the possible range of B.F.D. values, albumen of  $n = 1.365$  was used. From Table 1 it can be seen that there is only a small difference in the two sets of B.F.D. values and that there is still a good correlation with the measured photomicrograph values.

As concluded above, most of the refracting power of the lens lies within the lens with the back curved surface contributing only slightly. Under the interferen-

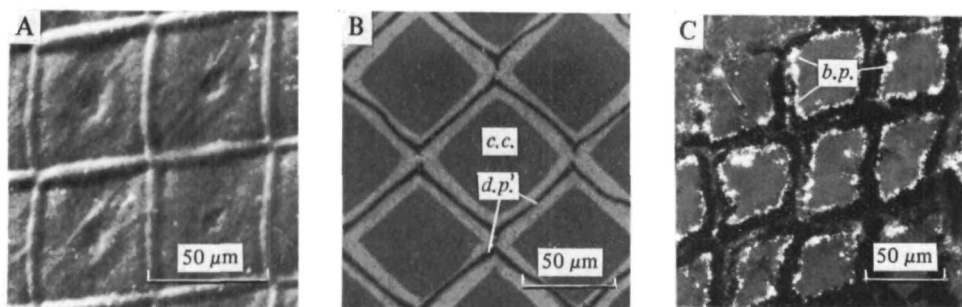


Fig.1

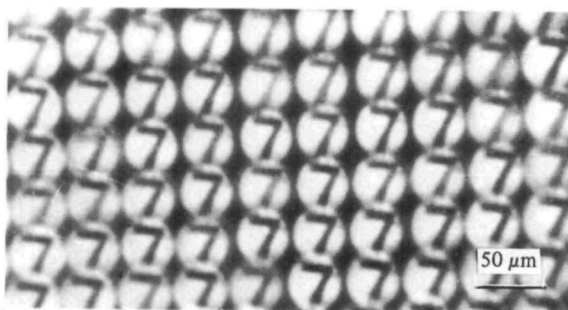


Fig.2

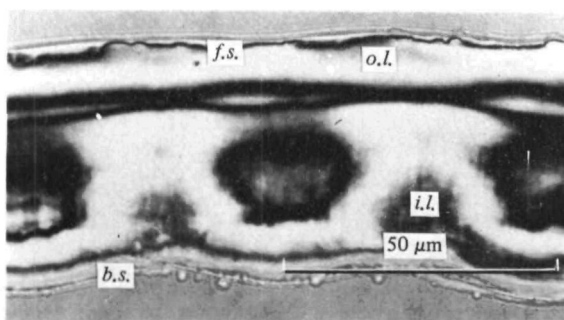


Fig. 3

Fig. 1 Anatomical features of a reflecting superposition eye found in *Cherax destructor*. (A) Scanning electron microscopy shows square corneal facets. (B) Light microscopy shows that the crystalline cones are square in cross-section. (C) Polarization microscopy shows birefringent pigment around the distal part of the cones, *cc.*, crystalline cones; *d.p.*, distal pigment; *b.p.*, birefringent pigment.

Fig. 2. Images seen through a cleaned piece of cornea of an object (the number '7') effectively at infinity.

Fig. 3. Interference microscopy of a longitudinal section through the cornea of *Cherax destructor* – the section is 26 μm thick. A higher refractive index in the centre of the inner layer of the cornea, indicated by the pattern of the interference fringes (which show regions of equal refractive index) creates the lens cylinder effect. The curved back surface can also be seen. *f.s.*, Front surface; *o.l.*, outer corneal layer; *i.l.*, inner corneal layer; *b.s.*, back surface.

Microscope, a decrease in refractive index from the centre of the lens ( $n=1.442$ ) to the edge ( $n=1.417$ ) can be measured. It is this gradient, creating a lens cylinder, that focuses the light (Iga, 1980). Interference fringes, demonstrating the existence of a refractive-index gradient, can be seen in Fig. 3.

In the reflecting superposition eyes described by Vogt and Land, parallel rays incident on an ommatidium are not focused by that ommatidium (except perhaps for the central ommatidium which tends to concentrate the light because of its taper), but are reflected by the multilayer mirrors around the crystalline cones, as a parallel beam, towards the position of the superposition image. The image is then a patch of light formed by the intersection of such beams from all the contributing ommatidia. With corneal facet lenses, the superposition image is now formed by the intersection of converging beams from the contributing ommatidia as a result of the focusing properties of the lenses. To make full use of this potentially finer-focused image with increased resolution would require the geometry of the 'aberration-free' superposition eye (outlined by Land, 1979) which would, in the case of *Cherax*, result in the points of focus of all the converging beams coinciding. However, this type of eye has a high resolution only over a limited range of angles and has certain disadvantages, especially its limited field of view (less than  $90^\circ$ ), which make it unsuitable as an all-purpose eye (Land, 1979). Preliminary anatomical work on *Cherax* also suggests that two of the main 'design features' mentioned by Land are not present: the surface containing the crystalline cones is not a sphere centred on the receptor layer, and the receptor layer is not flat. However, the depth of focus of the facet lenses is sufficiently large ( $\Delta z = \pm 65 \mu\text{m}$ ,  $f = 400 \mu\text{m}$ ,  $\lambda = 500 \text{nm}$ ; Born & Wolf, 1975) that the precise geometry of the 'aberration-free' eye may not be essential to the formation of a good superposition image in the eye of *Cherax*.

A second possible advantage in having corneal facet lenses arises when the animal is in the light-adapted state; during light adaptation, screening pigments move into the clear zone, so that each receptor probably receives light through only one facet. Each lens then focuses light onto a single receptor (as in an apposition eye), whereas in a reflecting superposition eye without lenses, no such focusing takes place.

It thus appears that the corneal lenses in this system are an additional mechanism to enable a finer-focused image to be formed, despite the apparent divergence of the eye of *Cherax* from the geometry necessary for a perfect superposition eye.

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