IR Photorefraction and IR Photokeratometry - Measuring Refractive State and Corneal Curvature in Animals and Humans

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Questions & Answers from the Presentation

What is small eye artifact?

Because the light that is used to refract eyes is mostly reflected from the interface of vitreous and retina, rather than from the photoreceptor layer (which is on the posterior side of the retina), refractions are measured for a too short eye – too short by just about by the thickness of the retina. A shorter eye means more hyperopic. The effect is almost negligible in large eyes (like humans) but becomes increasingly prominent the smaller the eye. Because the retina has similar thickness across vertebrates, also in small eyes, there is a close link to eye size. In a mouse eye with 3 mm length and a retinal thickness of 300 µm, this could cause up to 30D more hyperopia in white light. However, we are using infrared light which penetrates deeper into the retina and this "small eye artifact" may be much smaller. First described by Glickstein and Millodot in Science 1970:

Glickstein M, Millodot M. Retinoscopy and eye size. Science. 1970 May 1;168(3931):605-6. doi: 10.1126/science.168.3931.605.

Small animals, eg. C57BL/6 mice, are very aggressive and are always moving their head. Do we need to measure it after anesthesia? Does anesthesia affect refractive measurement?

Different mouse strains are differently difficult to handle. I saw mice in China which behaved just as the researchers wanted and were therefore very easy to refract. In our lab, mice became more cooperative, the more often they were measured. Regarding anesthesia: Unfortunately, the optical quality of the eyes declines rapidly under anesthesia (not only in mice) and the lens may develop a cataract after a few minutes. Therefore, the best optics is found in alert mice (de la Cera et al 2006). Some researchers use cycloplegia (e.g. Tropicamide) since the measurements become more repeatable when the pupil is larger.

de la Cera EG, Rodríguez G, Llorente L, Schaeffel F, Marcos S. Optical aberrations in the mouse eye.Vision Res. 2006 Aug;46(16):2546-53. doi: 10.1016/j.visres.2006.01.011. Epub 2006 Mar 3. PMID: 16516259

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How can you measure the axial length of small animals, eg. C57BL/6?

A-scan ultrasound does not work in mice because the steep curvature of the cornea permits only limited contact with the probe. Second, the resolution of ultrasound is too small to detect changes in eye growth. Note that elongation of the eye by only 5-6 µm make already 1 diopter more myopia. Therefore, optical techniques must be used, such as: (1) optical low coherence inteferometry (2) optical coherence tomography (OCT). While previous versions of the Lenstar by Haag-Streit were very good in measuring small eyes, newer software versions may exclude the option (I did not check after 2012 when it worked very well). OCT is more expensive, and some labs build their own OCT.

How to adjust the parameters of the devices when measuring different kinds of animals? Adjust LED illumination or exposure? How do you make sure the parameters are appropriate for the animal, eg. guinea pig?

The difference between guinea pig and mouse refraction is that the pupil sizes are very different. This can be adjusted in the software. In other animals, no automated software was developed - but one can grab a video frame with the pupil and the light distribution visible. The brightness slope can then be measured offline by ImageJ (NIH Image) which is publicly available. A calibration with lenses (2 would be enough, for instance +-5D) would make the measurements reliable.

With an accuracy in humans of perhaps around 0.1D, is this corrected for the near-IR wavelength?

Human photorefractors are calibrated to achieve the best match between spectacle prescription and measured outcome. Therefore, effects of chromatic aberration have been corrected for.

What should be the ideal room illumination while performing refraction?

Not too bright but not complete darkness. We refract chicks and mice at room illuminances between about 1 and 10 lux – humans see still reasonably well at that brightness.

The photorefractometer gives quite a variable response (myopic to hyperopic) when we move an eye a little bit. How do we know we are measuring the right area?

It is good to turn the head of the animal until the first Purkinje image (the small white reflection of the IR LEDs on the cornea) is about centered in the pupil (pupil axis). While this does not match the fovea in primates (due to the angle kappa), it improves repeatability in mice and chicks which don't have a fovea.

Does the refractor work like a corneal topographer? If yes, do you need to determine the visual axis to calculate the distance from this point and the Led reflection onto the cornea?

We use a rather coarse approach to measure changes in corneal curvature during an experiment. While commercial corneal topographers generate an optical power map all over the pupil by measuring the curvature in all places, we measure only at one radial distance in the pupil. This is good enough to see

whether the corneal curvature has changed. It is important however to adjust the animal so that the circle of reflected IR LEDs on the cornea is centered in the pupil. It is well known that the cornea flattens out to the pupil periphery, so the corneal radius of curvature varies, causing more variable measurements of corneal curvature when not centered.

How to adjust the device to detect larger pupil sizes?

The software expects a certain upper and lower bound of pupil sizes which make the detection more stable. If you move from mice to guinea pig, this range has to be adjusted. This can easily be done in the software settings.

If we anesthetize with ketamine/xylazine, will this make a difference than using isoflurine?

I don't know. In general, animals become more hyperopic during anesthesia, but I don't know whether this may vary depending on the type of anesthesia.

Does restraining the mouse by an adaptor or something similar alter the results and data generated?

Excited animals tend to become more hyperopic. But I don't believe that this is a strong effect. It is still better than anesthesia.