Journal of Neuroscience Methods 187 (2010) 156–166

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Non-contact measurement of linear external dimensions of the mouse eye

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ARTICLE INFO

Article history:
Received 28 September 2009
Received in revised form 4 January 2010
Accepted 5 January 2010

Keywords:
Non-contact
Mouse
Eye
Laser micrometer
rd10
Resolution
Micrometer
Lens

ABSTRACT

Biometric analyses of quantitative traits in eyes of mice can reveal abnormalities related to refractive or ocular development. Due to the small size of the mouse eye, highly accurate and precise measurements are needed to detect meaningful differences. We sought a non-contact measuring technique to obtain highly accurate and precise linear dimensions of the mouse eye. Laser micrometry was validated with gauge block standards. Simple procedures to measure eye dimensions on three axes were devised. Mouse eyes from C57BL/6J and *rd10* on a C57BL/6J background were dissected and extraocular muscle and fat removed. External eye dimensions of axial length (anterior–posterior (A–P) axis) and equatorial diameter (superior–inferior (S–I) and nasal–temporal (N–T) axes) were obtained with a laser micrometer. Several approaches to prevent or ameliorate evaporation due to room air were employed. The resolution of the laser micrometer was less than 0.77 μm, and it provided accurate and precise non-contact measurements of eye dimensions on three axes. External dimensions of the eye strongly correlated with eye weight. The N–T and S–I dimensions of the eye correlated with each other most closely from among the 28 pair-wise combinations of the several parameters that were collected. The equatorial axis measurements correlated well from the right and left eye of each mouse. The A–P measurements did not correlate or correlated poorly in each pair of eyes. The instrument is well suited for the measurement of enucleated eyes and other structures from most commonly used species in experimental vision research and ophthalmology.

1. Introduction

The vertebrate eye is a precision optical sensor, with optically clear media bounded by smooth optical surfaces (Fernald, 2006; Pepose and Applegate, 2005). During development, the ocular dimensions of the eye can grow at different rates producing eyes of abnormal size, such as microphthalmia or macrophthalmia. In addition, the axial length of the eye is matched to the optical power of the eye in a process called emmetropia. The vertebrate eye can accurately grow to match the ocular power of the eye (Zhu et al., 2005). A mismatch of the length of the eye and optical power results in refractive errors such as hyperopia and myopia.

Measurements of the dimensions (linear distances) of the eye have helped to understand how the eye works and maintains clarity in the visible spectrum (Zhu et al., 2005; Wallman and Winawer, 2004). We were interested in measuring the linear dimensions of the mouse eye (axial length, and equatorial diameter). These linear measurements inform us about eye growth (Carson et al., 2004; Finlay, 2008; Martins et al., 2008; Zuber et al., 1999), emmetropization (Zhou et al., 2008), and genes that regulate fundamental visual pathways influencing the size of ocular structures (Collinson et al., 2001; Puk et al., 2009a,b; Schaeffel et al., 2004; Steele et al., 2000; Troilo and Wallman, 1991).

Opportunities to solve key biological problems have arisen by using genetically altered mice, especially in vision sciences (Everett et al., 1994; Jablonski et al., 2005; Kao, 2006; Kerscher et al., 1995; Le et al., 2006; Lyon et al., 2000; Peachey and Ball, 2003; Pierce, 2001; Schippert et al., 2007; Schweers and Dyer, 2005), but simple and rapid technologies to measure small phenotypic changes caused by subtle genotypic changes in mice need to keep pace. Determining ocular dimensions can pose a challenge in an eye of ~3.0 mm diameter, such as the mouse, since traditional devices for large eyes can have resolutions of about 0.05 mm, including, for example, A-scan ultrasound and calipers used with chickens (Irving et al., 1992). Additionally, optical modeling of the mouse eye has predicted that a 6 μm change in axial length would result in a 1 D shift in refractive error (Schmucker and Schaeffel, 2004b). This predicts the need for one hundred fold better precision than the human eye in which a 0.5 mm change in axial length results in 1 D of refractive shift (Atchison and Smith, 2000).

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doi:10.1016/j.jneumeth.2010.01.006

While several groups have measured mouse eye size (Glickstein and Millodot, 1970; Martins et al., 2008; Puk et al., 2006; Shupe et al., 2006; Zhou et al., 2001; Zhou and Williams, 1999a,b) and eye weight (Barathi et al., 2008; Shupe et al., 2006) and lens size and lens weight (Augusteyn, 1998; Shupe et al., 2006), there are difficulties in achieving extremely high levels of accuracy and precision with the rapidity and throughput needed for large mouse studies, including mutant screens, crossbreeding, and transgenic technologies. Similar eye measurement problems arise in mutant screens and breeding experiments with small fish, cf., zebrafish, or during mammalian embryonic development when the eye is quite small and rapidly growing.

Prior work in vision research used biometrics instrumentation from four categories to measure eyes in many models:

(1) Calipers or micrometers (Barathi et al., 2008; Guggenheim et al., 2004; Irving et al., 1992; Prashar et al., 2009; Zhou and Williams, 1999b).
(2) Image analysis of histological sections or gross images of eyes with corresponding images of calibration standards (Kröger and Fernald, 1994; Schmucker and Schaeffel, 2004b; Zuber et al., 1999).
(3) MRI (Atchison et al., 2004; Chen et al., 2008; Goodall et al., 2009; Molokhia et al., 2009; Singh et al., 2006) or CT scan images (Berkowitz et al., 2004; Crow et al., 1982; Gumpenberger and Kolm, 2006; Tkatchenko et al., 2009).
(4) Interferometric techniques including OCT (Gumpenberger and Kolm, 2006), partial coherence interferometry (PCI) or optical low coherence interferometry (OLCI) (Schmucker and Schaeffel, 2004a; Liu et al., 2004; Molokhia et al., 2009; Nickla et al., 1998; Peachey and Ball, 2003; Wilson et al., 2006), or ultrasound-based (Guggenheim et al., 2004; Gumpenberger and Kolm, 2006; McFadden et al., 2006; Nickla et al., 1998).

While these techniques all work well for their intended purpose, in the different context of screening tiny mouse or fish eyes, they are slow and delicate.

Advances in manufacturing and industrial metrology now offer another method to measure small or fine structures with high accuracy and precision without contacting the object. For example, there are necessities to measure the diameter of wire or single optical fibers during production while the object is still extremely hot and moving quickly along a line of processing steps. The required measurements and production standards in this setting frequently require submicron resolution. An instrument that can perform such high-resolution measurements is the laser (aka, optical) micrometer (Fig. 1). We sought to determine the utility of the instrument in establishing external eye dimensions under experimental and control conditions. We tested the efficacy of the laser micrometer on one of the more challenging small eyes that is commonly used in vision research, the mouse eye, for which the previously mentioned four measurement techniques can be problematic. Because the instrument makes large numbers of measurements per second (e.g., about 2400 measurements per second) and because the instrument is designed to interface with a computer, sending data for storage in real-time, the instrument is ideal for obtaining measurements in a high throughput environment where many eyes are being studied nearly at once. The instrument is simple, fast, and easy to operate, lending itself to routine use in the laboratory by many different personnel.

In this study, we sought to validate the repeatability and reproducibility of laser micrometer-based mensuration of the mouse eye. We studied eye size and weight, and body weight in C57BL/6J and *rd10* mice ($Pde6b^{rd10/rd10}$ on C57BL/6J background) (Chang et al., 2002, 2007) that leads to blindness and retinal degeneration. Measurements of eye size using the laser micrometer were assessed for dependence and variability corresponding to eye and body weight.

2. Methods

2.1. Animal care

Protocols were approved by the Emory Institutional Animal Care and Use Committee and used in accordance with ARVO guidelines. Wild type C57BL/6J and *rd10* on the same C57BL/6J background, up to 800 days old, were housed at 23 °C in Emory University Division of Animal Resources facilities. They were provided standard

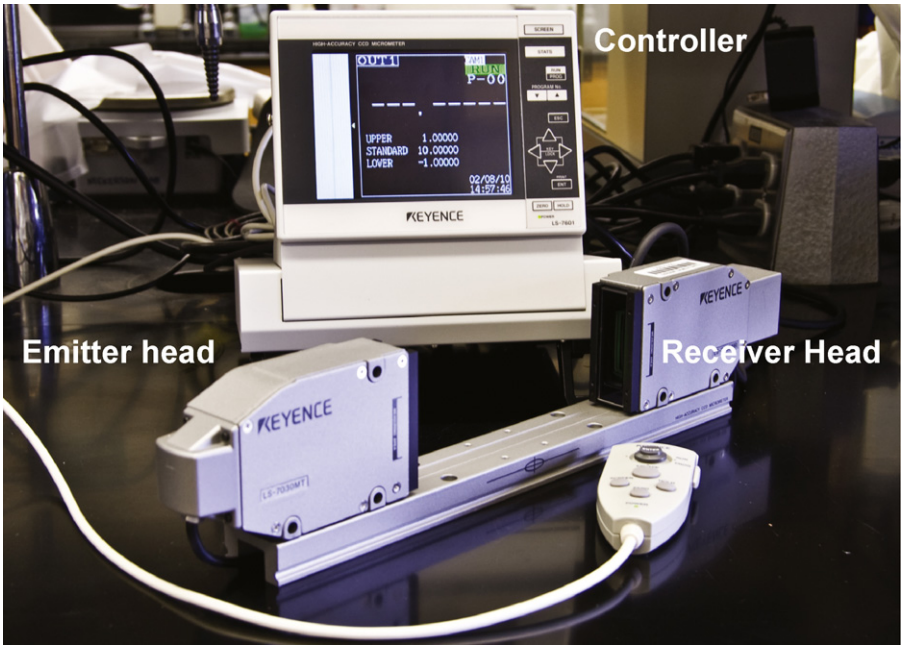

Fig. 1. Components of the laser micrometer. Components consist of an emitter head and a receiver head mounted on a rail, and a controller interfaced with a computer.