• Basic Research •

Digital measuring the ocular morphological parameters of guinea pig eye *in vivo* with Python

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Abstract

● **AIM:** To quantitatively measure ocular morphological parameters of guinea pig with Python technology.

● **METHODS:** Thirty-six eyeballs of eighteen 3-week-old guinea pigs were measured with keratometer and photographed to obtain the horizontal, coronal, and sagittal planes respectively. The corresponding photo pixels-actual length ratio was acquired by a proportional scale. The edge coordinates were identified artificially by ginput function. Circle and conic curve fitting were applied to fit the contour of the eyeball in the sagittal, coronal and horizontal view. The curvature, curvature radius, eccentricity, tilt angle, corneal diameter, and binocular separation angle were calculated according to the geometric principles. Next, the eyeballs were removed, canny edge detection was applied to identify the contour of eyeball *in vitro*. The results were compared between *in vivo* and *in vitro*.

● **RESULTS:** Regarding the corneal curvature and curvature radius on the horizontal and sagittal planes, no significant differences were observed among results *in vivo*, *in vitro*, and the keratometer. The horizontal and vertical binocular separation angles were 130.6°±6.39° and 129.8°±9.58° respectively. For the corneal curvature radius and eccentricity *in vivo*, significant differences were observed between horizontal and vertical planes.

● **CONCLUSION:** The Graphical interface window of Python makes up the deficiency of edge detection, which requires too much definition in Matlab. There are significant differences between guinea pig and human beings, such as exotropic eye position, oblique oval eyeball, and obvious discrepancy of binoculus. This study helps evaluate objectively the ocular morphological parameters of small experimental animals in emmetropization research.

● **KEYWORDS:** ocular morphological parameters; guinea pig; digital measurement; Python

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INTRODUCTION

During the sensitive period of emmetropization, there is increasing evidence that the biometry of the eye is influenced by the absence of visual experience[1-2]. The research on emmetropia requires high-quality experimental animal models. In contrast to another mammalian model such as mouse[3], the guinea pig is introduced as an ideal animal model: it is cooperative, inexpensive, and has relatively bigger eyeballs[4-5]. Previous studies suggested the ocular biological characteristics of guinea pig eyeball was not equivalent to smaller human eyes, there were significant differences of eyeballs between guinea pig and humans in histological anatomy, morphological structure, and optical pathway[6-9]. The knowledge of these differences is still insufficient due to the limitations of conventional measurement.

It is uneasy to measure the eyeballs of small animals like guinea pigs. The most commonly used keratometer is unable to obtain the guinea pig's curvature radius because the cornea is too much steep and beyond the measuring range. For this reason, McBrien and Norton[10] proposed to measure corneal curvature radius with a plus 8.0 D aspherical lens. Nevertheless, this approach required extensive experience and multiple cooperation in practical operation. The unfixed measuring distance inevitably led to unreliable and inaccurate results. In this regard, Schaeffel *et al*[11] devised a specialized keratometer to measure the eyeballs of small animals. However, it is still unsatisfactory not only additional expenditures, but also limited measuring parameters.

In previous research in guinea pig, we identified the contours with edge detection, and curve fitting the eyeball with Matlab

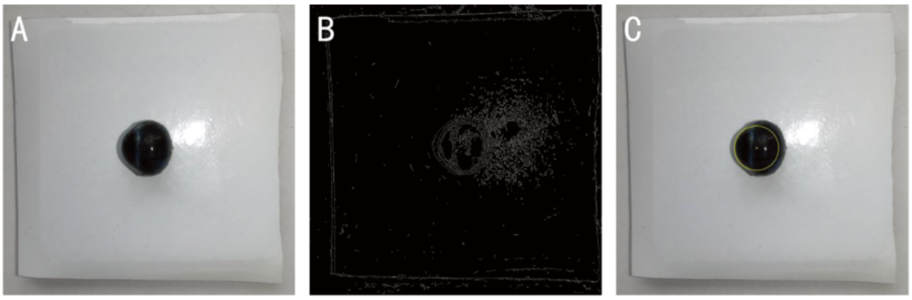

Figure 1 Non-contact measuring the curvature radius of guinea pig eyeballs *in vitro* by Matlab A: Guinea pig eyeball on the sagittal plane; B: Edge detection with canny algorithm to obtain corneal edge coordinates; C: After acquiring the target edge coordinate data and converting the pixels to actual distance, the radius of corneal curvature was obtained by circle fitting in the end.

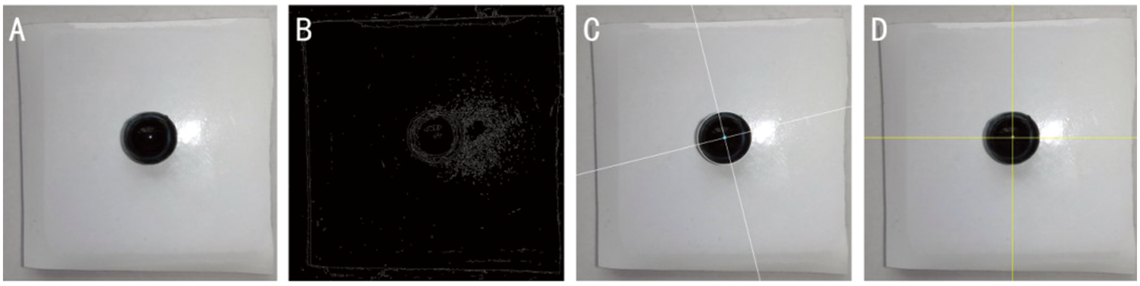

Figure 2 Non-contact measuring guinea pig eyeball *in vitro* by Matlab A: Guinea pig eyeball on coronal plane; B: Corneal edge detection with Canny algorithm; C: Conic curve fitting the corneal contour (Ax2+Bxy+Cy2+Dx+Ey+F=0). According to the geometric characteristics of ellipse, the center point, the longest axis, the shortest axis, and the tilted angle of the eyeballs are all available. D: Horizontal diameter and vertical diameter of the eyeballs could also be calculated after the curve fitting center point was determined.

Figure 3 Operators had to master complex Matlab codes to complete the calculating, and the technical threshold was high (A). Guinea pig (tricolor strain) often has black hair around the eyeballs, which makes the edge uneasy to identify (B). Extracting the target area, and removing the surrounding area were cumbersome, even if the corneal edge could be fully exposed by enhancing light (C).

software, the corneal curvature radius was obtained by means of numerical simulation[12-14] (Figure 1). The corneal eccentricity, the long and short axis of the eye and the eye axis were achieved in the same way (Figure 2). Such a measuring significantly improved the accuracy and reliability. Nevertheless, such method still suffered from some limitations: 1) The whole process was entirely implemented by code. It was quite difficult for the researchers who were unfamiliar with Matlab to complete the computational process (Figure 3A). 2) The eyeballs were required to be placed on a pure-white background in order to manifest clear edges. The eyeballs of many experimental animals like trichromatic guinea pigs, were covered with black hair. Thus, it was difficult to identify the corneal edge around the black hair (Figure 3B). 3) After edge detection, the non-target areas had to be removed manually, such process was time-consuming and laborious (Figure 3C). For the above reasons, we improved the method on the original basis: a graphical interface window with Python was developed. Such a software packed the whole calculation process with the built-in operational modules, which made the operators who were unfamiliar with calculation codes is also capable of completing the work. Meanwhile, we collected the edge coordinates artificially with ginput function, the results were compared with previous method. The results were as follows.

MATERIALS AND METHODS

Ethical Approval This research was approved by the Laboratory Animal Management and Ethics Committee of Shanghai Public Health Clinical Center (No.2022-A009-01). This research was carried out in strict accordance with the recommendations in the Regulations on the Administration of Experimental Animals issued by the Chinese Science and Technology Commission.

Experimental Animals Eighteen guinea pigs (English short hair stock, tricolor strain, 3 weeks of age, weight 110-150 g) were obtained from the Songlian Experimental Animal Farm, Songjiang District, Shanghai. Totally, there are thirty-six eyeballs. No corneal diseases were observed in the slit lamp.

Biometric Measurement of Corneal Curvature *in vivo* The radius of corneal curvature (CRC) was measured in alert guinea pigs with a keratometer (OM-4; Topcon, Tokyo, Japan) combined with a plus 8.0 D aspherical lens[10]. A set of stainless steel ball-bearings was used for calibration. The CRC for each animal was measured in triplicate.

Digital Measurement of the Cornea *in vivo*

Program and import images Python 3.9 was selected as the kernel compiler, we wrote codes in PyCharm Community Edition 2021.1.3 x64, Tkinter module was imported to build a Graphical User Interface (GUI) window, and packaged it into a executable exe-file with pyinstaller module to facilitate visual man-machine conversation.

This program was specially designed for the eye measurement of small animals. The function included importing pictures, calculating the function module of conversion factor, setting the option of line color and thickness, the module of temporary record, the module of obtaining the edge coordinates by manually taking the points and the module of edge detection, and finally, operator was able to export the generated picture ratio through the button of the export function, which is convenient for the calculation of a larger amount of data (Figure 4A).

All the guinea pigs were photographed on horizontal, coronal and sagittal plane, namely, from the top, front and side directions at a distance of 50 cm with a 13-megapixel camera without depth of field. All the corneal edges were ensured to be identified subjectively. One graduated scale was fixed on the same plane as the actual distance reference. The photos were inputted into the compiled program, then the images were opened with matplotlib module (*code: image=Image. open (path+pic, 'r')*). By doing so, each point in the image corresponded to a two-dimensional coordinate (x, y) (Figure 4B).

Method of actual length-photo pixels conversion Coordinates data in two-dimensional graphics was obtained with "ginput" function. The distance of any two points: (m_1, n_1) and (m_2, n_2), in the coordinate system could be computed according to the principle of geometry (formula: $Lmn=\sqrt{(m_1-m_2)^2+(n_1-n)^2}$).

In this way, the corresponding pixels of actual distance in the center area could be available, the actual distance between any two points in the picture was able to be obtained by conversion (Figure 4C). The actual distance conversion coefficient (Figure 4D) could be obtained by taking points on the graduated scale at 10 mm distance repeatedly and calculating the average value. This above method was the same as the method of actual length-photo pixels conversion in Matlab image in our previous research[12].

Acquisition of ocular edge data manually Ten data points were manually taken in the target contour (corneal edge) to obtain the coordinates. The data was saved in txt-file (Figure 4E, 4F).

Calculation of curvature radius by circle and conic fitting Assuming the center of the cornea is a circle, circle fitting was applied, the real curvature radius of the cornea by means of the conversion coefficient could be obtained, just like we reported before[12] (Figure 5A, 5C, 5E).

Calculation of Corneal Eccentricity by Conic Fitting The cornea surface was like a tilt ellipse curve. Such a curve was quite suitable to fit with the conic equation (Ax2+Bxy+Cy2+Dx+Ey+F=0). According to the principle of geometry and minimum squares, the eccentricity of the cornea on the horizontal, sagittal, and coronal planes could be calculated. The binocular separation angle, namely, the angle between the major axes of two ellipses, was further calculated (Figure 5B, 5D, 5F). The "cv2.fitEllipse" function was applied.

Calculation of corneal curvature radius and eccentricity *in vitro* with edge detection To confirm the reliability, the edge detection which reported before was also applied for comparison[12,15]: First, the guinea pig was sacrificed by excessive anesthesia. A point was marked by a glowing needle tip at the top of the corneoscleral limbus before eviscerating the eyeballs. Next, the eyeballs were taken out, and the bulbar conjunctiva was excised carefully. Afterward, the eyeballs were placed in the center of the platform and shot with a camera from top and side directions to get the images of the coronal and sagittal eyeball contours (Figure 6A).

Canny edge detection algorithm was applied on three planes of the cornea *in vitro* (Figure 6B, 6C). After removing the non-target area, the edge coordinate data information was acquired through the "cv2.findContours" function. Cirvle and conic fitting were used and compared with the eyeball measurement data *in vivo*.

Statistical Analysis The results of the eyeballs *in vivo*, *in vitro*, and results by curvature meter were compared with ANOVA (Tukey HSD). The curvature radius and eccentricity *in vivo* and *in vitro* on horizontal and coronal planes were compared by paired *t*-test. $P<0.05$ was significant difference.

RESULTS

Numerical Simulation *in vivo*, *in vitro*, and Keratometer Measurement The results on horizontal and sagittal planes were shown in Table 1. No significant differences were observed among the results of three methods ($P>0.05$).

Table 1 Curvature and curvature radius with three methods on horizontal plane and sagittal plane mean±SD (range)

Parameters	*In vivo*	*In vitro*	Curvature meter	*F*	*P*	P_1	P_2	P_3
Curvature								
Horizontal plane	99.52±3.61 (91.82-105.8)	99.94±3.55 (90.0-105.8)	99.4±11.94 (57.0-120.0)	0.05	0.95	0.9	0.9	0.9
Sagittal plane	100.67±3.6 (91.96-107.48)	103.53±3.62 (97.62-109.39)	103.67±10.35 (70.0-125.0)	2.32	0.10	0.16	0.9	0.14
Curvature radius								
Horizontal plane	3.4±0.13 (3.19-3.68)	3.38±0.12 (3.19-3.75)	3.47±0.61 (2.8-5.92)	0.54	0.58	0.9	0.68	0.59
Sagittal plane	3.26±0.11 (3.09-3.46)	3.36±0.12 (3.14-3.67)	3.29±0.4 (2.7-4.82)	1.32	0.27	0.25	0.86	0.52

P_1: *In vivo vs in vitro*; P_2: *In vivo vs* curvature meter; P_3: *In vitro vs* curvature meter.

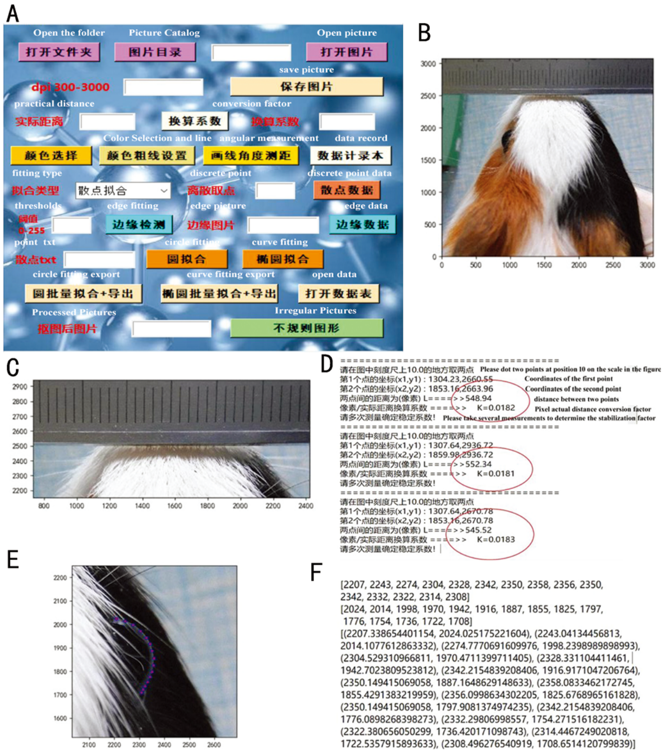

Figure 4 The program specially designed for the eye measurement of small animals A: The dedicated applet written by python 3.9 aimed to ocular measurement of guinea pig; B: Photo of guinea pig eyeball on the horizontal plane, with a ruler around for reference; C: Taking points repeatedly on the ruler to obtain the conversion coefficient; D: For instance, we took points repeatedly, then averaged the conversion coefficient. The pixel points measured at 10 mm distance for three times were: 548.94, 552.34, and 545.52 respectively. Therefore, the final conversion coefficients were 0.0182, 0.0181, and 0.0183 accordingly (the K in the red circle was the conversion coefficients). E: After enlarging the photo, we manually took 15 coordinate points at the corneal edge to obtain corneal edge coordinate data; F: After the 15 points were collected, the coordinates were saved in a txt-file for further calculation.

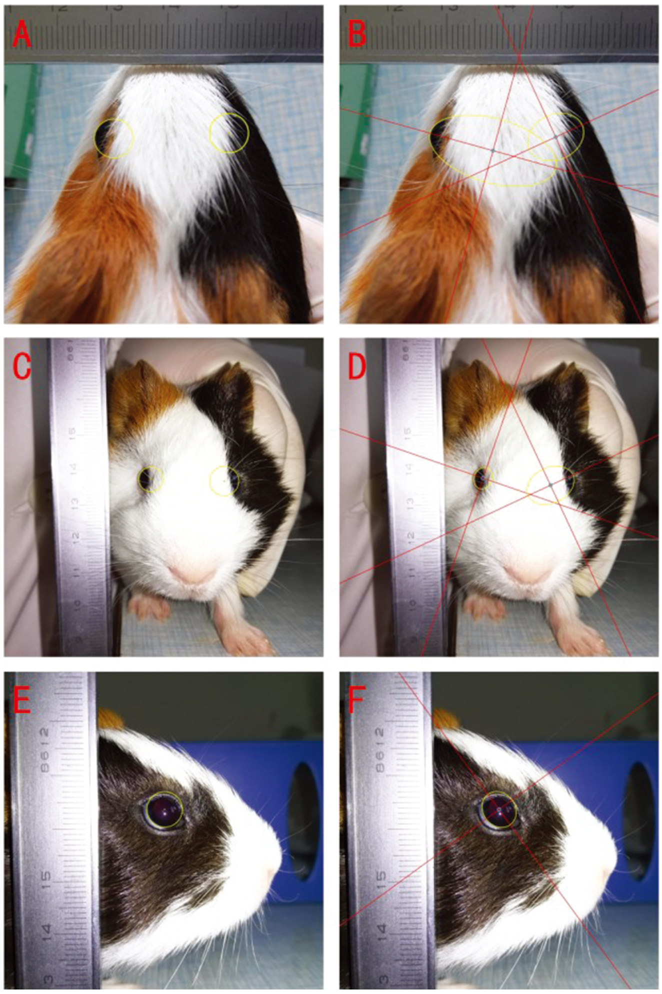

Figure 5 Numerical simulation techniques of the eyeball photo *in vivo* Calculating corneal curvature radius by circular circle fitting, binocular separation angle and eccentricity by conic fitting. A: Corneal curvature radius in horizontal view; B: Binocular separation angle and eccentricity in horizontal view; C: Corneal curvature radius in coronal view; D: Binocular separation angle and eccentricity in coronal view; E: Curvature radius in sagittal view; F: Binocular separation angle and eccentricity in sagittal view.

Corneal Eccentricity *in vivo* and *in vitro* The results on horizontal and sagittal planes were shown in Table 2. No significant differences were observed (P>0.05).

Measuring for Corneal Diameter and Eccentricity *in vivo* and *in vitro* In the coronal view, the difference of corneal diameter *in vivo* and *in vitro* was statistically significant (t=-2.13, P=0.03981; Figure 7A), and the eccentricity *in vivo* and *in vitro* was also statistically significant (t=2.5, P=0.01717; Figure 7B).

Binocular Separation Angle of the Eyeballs *in vivo* According to the conic fitting principle, the tilted angle and the

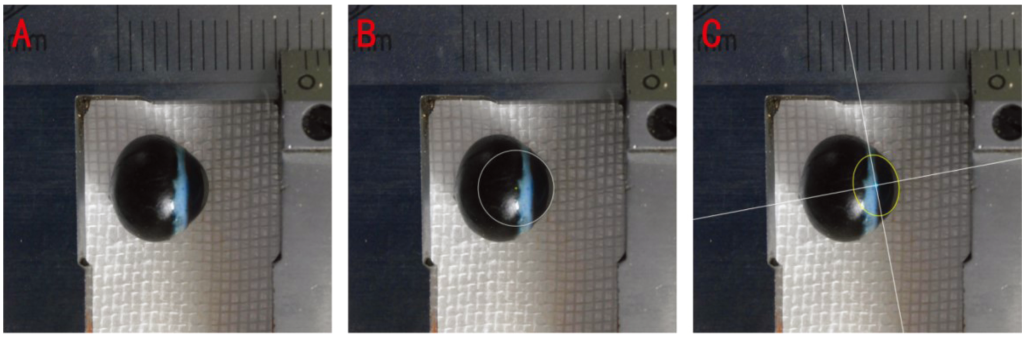

Figure 6 The eyeballs were eviscerated, then the static eyeballs *in vitro* were analyzed as reference A: Eyeball pictures on sagittal plane; B: Curvature radius after circle curve fitting; C: Eccentricity and tile angle after conic curve fitting.

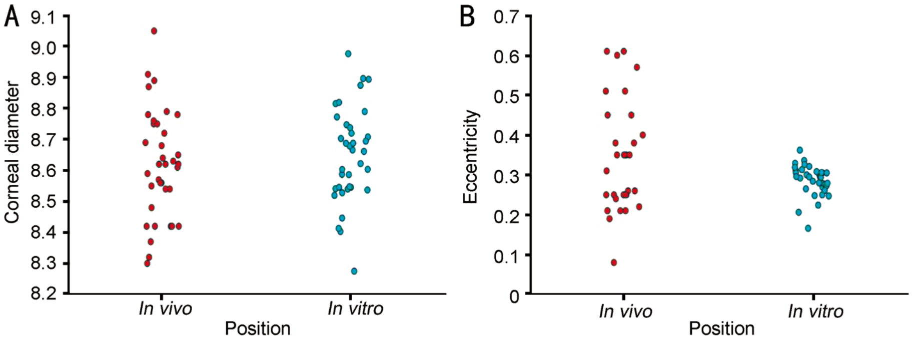

Figure 7 Corneal diameter and eccentricity *in vivo* and *in vitro* A: Comparison of corneal diameters *in vivo* and *in vitro* in the coronal view (*in vivo*: 8.62±0.17, *in vitro*: 8.65±0.15 mm); B: Comparison of corneal eccentricity *in vivo* and *in vitro* in coronal view (*in vivo*: 0.36±0.16, *in vitro*: 0.29±0.04).

Table 2 Corneal eccentricity *in vivo* and *in vitro* on horizontal and sagittal planes mean±SD (range)

Corneal eccentricity	*In vivo*	*In vitro*	*t*	*P*
Horizontal plane	0.67±0.15 (0.38-0.9)	0.66±0.1 (0.49-0.87)	0.48	0.63
Sagittal plane	0.68±0.16 (0.37-0.91)	0.65±0.13 (0.31-0.85)	0.66	0.85

Table 3 Tilted angle and binocular separation angle between eyes on horizontal and vertical planes mean±SD (range)

Planes	Tilt angle of right eyeball	Tilt angle of left eyeball	Separation angle of the eyeballs
Horizontal plane	29.4±3.6 (25.3-34.9)	160.0±5.6 (151.7-171.4)	130.6±6.39 (120.0-142.2)
Vertical plane	156.7±8.12 (143.1-167.7)	26.9±7.3 (17.3-38.9)	129.8±9.58 (108.5-145.9)

binocular separation angle between guinea pigs' eyes (°) were shown in Table 3.

Comparison of Binocular Corneal Curvature and Eccentricity The comparison of binocular curvature radius and eccentricity *in vivo* in horizontal and vertical views were shown in Figure 8. Significant difference was found with paired *t*-test ($P<0.05$).

DISCUSSION

In the current study, two main morphometric parameters of the eye, the corneal curvature and curvature radius on the horizontal and sagittal planes, no significant differences were observed among results *in vivo*, *in vitro*, and the keratometer. The horizontal and vertical binocular separation angles were 130.6°±6.39° and 129.8°±9.58° respectively. For the corneal curvature radius and eccentricity *in vivo*, significant differences were observed between horizontal and vertical planes. The ocular differences between guinea pig and human beings were significant, such as exotropic eye position, oblique oval eyeball, and obvious discrepancy of binoculus.

Measuring Method Comparison between Python and Matlab Matlab is the combination of matrix&laboratory, which means matrix factory (matrix laboratory). Matlab provides a powerful digital operation function and mainly faces the high-tech computing environment of scientific computing, visualization, and interactive programming[16]. In our previous work[12-15], we proved edge detection and curve fitting technology with Matlab could accurately evaluate the ocular morphological parameters both in guinea pigs and humans. However, the calculation process is too much complicated. Operators had to master complex codes. In contrast, Python provides an efficient and advanced data structure, as well as simple and effective object-oriented programming[17-20]. The advantages including simplicity, readability, and extensibility make the articles on scientific

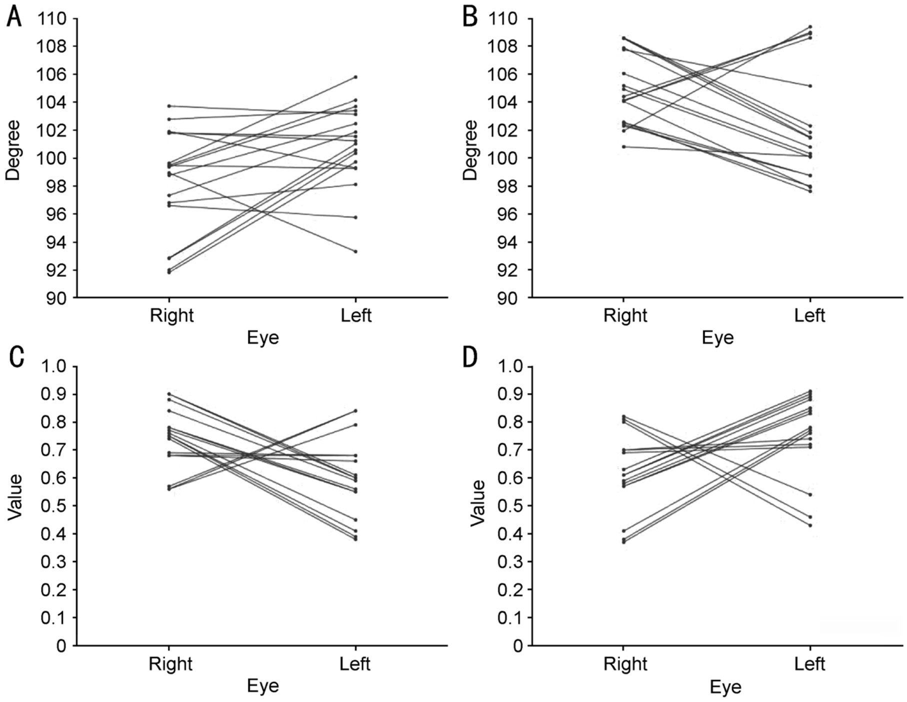

Figure 8 Binocular comparison of measurements *in vivo* A: Horizontal curvature radius (t=-2.66, P=0.01651); B: Vertical curvature radius (t=2.35, P=0.03115); C: Horizontal eccentricity (t=2.90, P=0.00987); D: Vertical eccentricity (t=-2.63, P=0.01759).

computing with Python are increasing in recent years. Python integrates multiple geometric operation libraries, and also provides the same operation functions from Matlab, therefore, it has been widely applied in scientific research. This current research used mainstream GUI (user graphical interface) to write a complete set of computing programs. Such an applet made the operators who were unfamiliar with computing codes were also able to complete the same research without difficulty. The computational efficiency was obviously improved by this approach[21].

Method Comparison between Edge Detection and Manual Data Acquisition Edge detection comprises a set of mathematical approaches that identifying points in a digital image at which the brightness changed strikingly[22-24]. Image edge detection is mainly used to enhance the contour edges, details, and gray level jump parts to form a complete object boundary so as to separate objects from the image or detect the area representing the same object surface[25-26]. In our previous work, the points at which image brightness changed sharply were organized into a set of curved line segments termed edges[12-15]. However, it was obviously more convenient to collect points in a manual way at a comparably fuzzy region, like guinea pig's eyeballs around black hair.

Our current study indicated no statistically significant differences were observed between the results *in vitro* and *in vivo* about the corneal curvature radius and eccentricity on the horizontal and coronal plane, despite such an identifying approach was subjective, and the manually acquired data points in number were less than points from edge detection. Such a method resolved the original defects that the contour edge information could only be obtained through executed experimental animal eyeballs. Obviously, this current method *in vivo* had better application in the research of eye development.

In addition, in the sagittal view relative to guinea pig, namely, on the coronal plane of the eyeball, significant differences were found between the results *in vitro* and *in vivo* about the diameter and eccentricity of the eyeball. One reasonable explanation was, the eyelids of the guinea pig blocked part of the corneal edge, and only the exposed contour could be identified. The reliability of the results will inevitably be affected. Apparently, it was necessary to fully expose the eyeball when we require to evaluate the corneal edge on coronal plane.

Ocular Biometric Differences between Guinea Pig and Human

Binocular visual separated angle The guinea pig, as one kind of small herbivorous rodent, the eyeballs are distributed on both sides of the head. After conic curve fitting, the horizontal binocular visual separation angle was 130.6°±6.39°, and the vertical binocular visual separation angle was 129.8°±9.58°. These results indicated that the eyeballs of guinea pigs, in the natural state, deviated forward and upward, as shown in Figure 5B, 5D. This deviation corresponded to the sharp head. This result suggested the results must be significantly deviated from the central area of the eyeball of guinea pigs, when the measuring instrument, which was designed for human, was place directly in front of the eyeball. Obviously, in our research, the data obtained by curve fitting was close to the true corneal curvature radius better. How to accurately measure the guinea pig eyeball, just like a tilted and oblique elliptic curve in three spatial dimensions, remains to be further studied.

Differences between oblique elliptical eyeballs of guinea pigs and human eyes In our study, as shown in Figure 5F, in the sagittal view photo of guinea pigs, namely, on the coronal plane of eyeball, the cornea was observed like a tilted ellipse. Obviously, such an oblique ellipse was closely linked to the eyelids direction and the orbits shape of guinea pigs. After measurement, the eccentricity of the cornea in the coronal view *in vivo* was 0.36±0.16. Obviously, the routine method used in human, namely, measuring corneal astigmatism in horizontal and vertical view, was not suitable to guinea pigs[22,25,27]. Until now, there is still not a convenient and effective instrument to measure the refraction of guinea pig eyes. Most of studies relied on artificial skiascopy[28]. It is uneasy to measure such an oblique astigmatism like guinea pig eyes, moreover, the axial position is hard to be determined accurately. How to design a feasible way to obtain refraction, further, the relationship between corneal oblique astigmatism and refraction of guinea pigs, both of these needs a further investigation.

Binocular difference of guinea pigs Guinea pig, as one kind of rodent, has a quite limited growth cycle, which is much shorter than that of human beings[29]. This current study indicated the binocular difference about corneal curvature and eccentricity were more significant than that of human eyes (Figure 8). Such a result noted it was necessary to fully consider the remarkable discrepancy when the small animals like guinea pigs were used as experimental animals for eye development.

In conclusion, the Graphical interface window with Python is proved more convenient than the original computing method from Matlab. It improves the deficiency of edge detection which requires too much definition. It is worth mentioning that this method is not only for guinea pigs, but can also be applied to other experimental animals, *e.g.*, rabbits and mice. This research helps to accurately access the ocular morphological parameters of experimental animals in the emmetropization research.

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