

OCT in Fundus Examination of Mice and Research on Automatic Segmentation and Measurement of OCT Images

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Abstract: The reason why most ophthalmic medical research was carried out in mice is that the genes of mice and human are extremely similar. The retinal structure of a mature mouse has an important reference function for animals with ophthalmic diseases. Optical coherence tomography (OCT) is used to detect the delay of light echo time and obtain the layers of the detected tissue cells. The images of different layers of retina provided by OCT are closely related to histomorphology. The purpose of this paper is to study the application of OCT in fundus examination of mice and automatic segmentation and measurement of OCT images. First of all, due to the great difference in refractive structure between human and mouse eves, this paper established a mouse retina OCT imaging device to study the image presentation effect of different sampling methods, so as to achieve the accurate display of mouse eye image; secondly, using the automatic segmentation and measurement technology of OCT image, the edge is detected step by step and the effect of image presentation is enhanced, and the contour is successfully extracted and compared with manual segmentation Finally, we compared the data obtained from mouse retinal tissue section and the data detected by OCT image. The final results show that the data detected by OCT imaging equipment can completely replace the data obtained by mouse tissue section, and even the improved OCT imaging device can detect the data that cannot be obtained by tissue section. This method not only improves the time-consuming and laborious disadvantages of manual mouse tissue sectioning, but also effectively promotes the establishment of ophthalmic disease model through the high-quality images obtained by the computer automatic processing of OCT imaging device.

1. Introduction

The eye is a particularly important part of the human sensory system. For humans, eyes capture

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80% of the brain's memory and knowledge, so eyes are an important part of our daily work. In Ophthalmology, the eye is mainly divided into anterior segment and posterior segment. Among them, the anterior part of cornea, iris and lens, and the posterior part of retina, choroid and sclera. Fundus refers to the bottom of the posterior segment of the fundus, which is composed of fundus blood vessels, macula, part of retina and choroid [1]. The retina, also known as the inner membrane, is a translucent membrane located in the fundus of the eye. There is a depression in the center of the macula, called fovea [2]. The closer you get to the center, the denser the photoreceptors are, and the better the visual experience is. In order to avoid the formation of various eye diseases, most medical scholars regard mice with high degree of human retinal structure as experimental objects, so as to understand the tissue morphology of the retina, so as to better solve various diseases.

Optical coherence tomography [3] is a popular optical imaging technology, which can be used to measure the deepest depth of laser welding pool, the thickness and position of alumina ceramic pipe, the thickness of pearl, the degree of scalded skin damage, breast cancer and oral cancer lesions, periodontal lesions, etc. At the beginning of the development of OCT, foreign scholars were the "leading force" of OCT technology. They took the lead in making significant progress in a short period of time, leading the development upsurge of Western OCT technology. Although science and technology started late in China, the development is relatively slow, and the technology research for more than 20 years is later than that of western developed countries. However, the intersection of scientific and technological progress in our country makes the research direction of CCD camera, an important part of optical equipment, gradually matures. Many domestic scholars also begin to study in the field of biomedicine and have achieved good results. OCT images not only have a large amount of data, but also have low contrast of some interlaminar boundaries. Diseases often cause retinal morphological changes. Optical coherence tomography (OCT) and other technologies are rapidly popularized around the world to help doctors make better clinical diagnosis. As a new ophthalmic imaging technology, OCT imaging has the advantages of high resolution; non-invasive and low loss compared with other imaging technologies, and can directly see the subtle changes in the retina.

Automatic segmentation and measurement of OCT images [4] is the primary task of image analysis. The effect of the following tasks such as feature extraction and target recognition depend on the results of automatic segmentation and measurement of OCT images. OCT image automatic segmentation and measurement technology is difficult, deep and slow. Since the 1970s, it has been concerned by many scholars and researchers. Although researchers have proposed many methods for different problems, there is no broad-spectrum theory and method. Secondly, there is no standard theory to choose the appropriate segmentation algorithm, which brings many problems to the application of image segmentation technology. For example, the segmentation method chosen by researchers is not the optimal method, but the best one of several known algorithms. Therefore, the quality of automatic image segmentation is not high. In recent years, with the development of related imaging technology, more and more scholars participate in the research of computer-aided diagnosis, which greatly promotes the development of medical image processing technology.

The organizational structure of this paper is as follows: firstly, the OCT technology is introduced to understand the basic principle and development process of this technology; secondly, the existing problems and development of OCT in mouse fundus examination are described; secondly, in order to establish a reasonable OCT imaging device, MCNN-GS technology, data acquisition and amplification, OCT image noise reduction, contour extraction, edge OCT image automatic segmentation are introduced And measurement and so on. Finally, we compare the segmentation data obtained by different sampling methods, compare the measurement error between manual segmentation and computer automatic segmentation, and finally compare the detected data with the data obtained from the traditional method of mouse tissue slice, and conclude that the data detected by automatic segmentation measurement technology of OCT imaging device is more accurate than the traditional method. We also visited 26 animal research experts, and unanimously agreed that the automatic segmentation and measurement of OCT images solved the barrenness of manual detection and laid the foundation for future development.

2. Introduction of OCT Technology and Its Problems in Fundus Examination of Mice

2.1. Overview of OCT

OCT imaging [5] is based on interferometry and optical coherent reflection technology. OCT is a new optical imaging technology. In vivo, OCT can provide high-resolution images of 2-3 mm below the tissue surface with a resolution of 1-20 um. It was initially used for clinical diagnosis of Ophthalmology and heart disease. OCT combined with endoscopy can detect airway hyperplasia or early cancer that may be ignored by CT or bronchoscopy. In addition, because of the low invasiveness of endoscopic OCT, in vivo imaging can be used to track disease progression vertically and evaluate the effectiveness of treatment.

OCT is a medical imaging technology developed in the early 1990s for ophthalmology. It produces micron resolution 2D and 3D high-resolution images in the body without ionizing radiation. It works like ultrasound, but it uses low coherent near-infrared light instead of sound waves to produce high-resolution images of biological tissues. Compared with traditional imaging technologies (traditional endoscopy, fluorescence, confocal and multiphoton microscopy), OCT has higher sensitivity and spatial resolution, portability, low instrument cost and low operation cost.

2.2. Principle and Characteristics of OCT Imaging

Optical coherence tomography (OCT) is an imaging technology based on the principle of interferometry and optical coherent reflection [6]. It has the characteristics of high resolution, non-invasive and real-time in vivo. It can be used for biological tissue measurement and imaging. The principle of OCT imaging is to use a beam splitter to divide the light emitted by the light source into two beams. The two beams of reflected light interfere with each other, and the intensity of the light signal reflected from the tissue is different due to the different shape of the tissue. These optical signals are processed by computer. By comparing the reflected wave with the reference wave, the data of tissue reflectivity and distance can be obtained, and the tissue tomography can be obtained. According to the imaging principle, it is divided into time domain OCT and frequency domain Oct. The basic principle of time domain OCT is to superimpose, interfere and image the optical signal reflected from tissue and reference mirror. At present, OCT is widely used in frequency domain. Compared with time domain OCT, OCT has faster scanning speed and higher measurement sensitivity. Different from the time domain OCT, the frequency domain OCT fixed the reference arm and realized the signal interference by changing the frequency of the light source.

Another important feature of OCT imaging technology is that it can be used for non-destructive imaging. OCT imaging is similar to ultrasound in many ways, but it does not use sound. Instead, it uses a near-infrared frequency laser or scattering ordinary visible light as the signal source. In addition, OCT imaging technology has high imaging speed and can generate a large number of images in a short time for subsequent image processing.

2.3. Development Status of OCT Technology

OCT technology is mainly based on interferometry and optical coherent reflection technology. In 1991, the concept of OCT was proposed and applied OCT technology to ophthalmology. They successfully measured retina and coronary artery in vitro, which opened a new era of optical coherence tomography. Until 1995, after several years of improvement and improvement, OCT technology developed into a clinical and practical auxiliary treatment tool. Commercial instruments have been developed to determine its superiority in fundus and retinal imaging. It started the formal clinical application of Ophthalmology and became the advanced adjuvant treatment technology at that time. OCT has a high resolution that other imaging technologies can't match and has been developed for the imaging analysis and measurement of human tissues and organs. Since then, OCT has been more and more used in skin, respiratory and cardiovascular examination in medical field. Early OCT is a typical time-domain OCT system. It scans tissues in depth, then horizontally and point by point. It was not until 2003 that coherence tomography appeared in the frequency domain. Different from time domain OCT, frequency domain OCT only needs cross-section scanning, and can obtain depth imaging information through coherent spectrum and frequency domain transformation. This technology solves the problems of measurement sensitivity and scanning speed. The current research and application are based on frequency domain Oct. Because of its non-destructive, non-invasive and real-time imaging characteristics, OCT technology has attracted great attention of researchers at home and abroad. OCT technology has been applied to the detection and analysis of skin tissue, and the optical properties of skin have been studied.

2.4. Excellence of OCT

In the medical field, excellent medical imaging technology can help doctors to make correct and effective diagnosis of lesions. The commonly used medical image imaging technologies include ultrasound [7], confocal microscope [8], etc. From a practical point of view, the evaluation criteria of imaging mainly include:

(1) Resolution. The smaller the resolution value, the more pixels in the image, the more-fine spots can be observed, and the better the image quality. The resolution required for imaging varies with the characteristics of biological tissues.

(2) Detection depth. The deeper the detector is, the more internal information can be observed the more understanding of the internal structure will be.

(3) Security. Safety is an important part of imaging technology evaluation standards, which determines whether imaging technology can be applied in vivo.

2.5. OCT Classification

Optical coherence tomography (OCT) system is a system based on OCT technology. There are many types of October. In terms of detection and data processing methods, it can be divided into:

(1) Time domain OCT [9]. TD-OCT has priority, axial scanning is to obtain the depth information of the measured object through mechanical scanning. However, mechanical scanning cannot scan quickly for a long time, and the related equipment is easy to wear, resulting in poor imaging and low signal-to-noise ratio. Therefore, with the rapid development of technology, TD-OCT technology is gradually eliminated from the application market.

(2) Frequency domain OCT [10]. The biggest difference between TD-OCT and TD-OCT is that the mechanical z-axis scanning required by TD-OCT is removed. The depth scanning data is

obtained by Fourier transform of interferogram, which makes the imaging speed faster. At the same time, FD-OCT can measure the depth information of the object to be measured with only one optical probe. This transformation increases the possibility of real-time imaging of FD-OCT in vivo.

2.6. Problems of OCT in Fundus Examination of Mice

At present, only OCT can be used to detect mouse fundus, which can be divided into two categories: one is used in various clinical operations; the other is only used for animal experiments. The OCT equipment for clinical operation is specially designed according to the characteristics of the refractive system of human eyes, but there are significant differences between the refractive systems of human and mouse eyes. Therefore, it is difficult to meet the measurement of mouse retina data, and it is difficult to achieve accurate detection. It has been reported that the improved OCT equipment is more effective in the detection of eye drop in mice. It only adds the retinal contact lens with a diopter of 200D and a diameter of 7.34mm and can choose to add a retinal contact lens with a specified focal length.

Now there is OCT imaging devices to achieve arbitrary scan to present high-quality images, large-scale exploration of the fundus imaging. However, in view of the existence of retinal tomographic structure, the imaging results are not satisfactory. In addition, due to the presence of retinal blood vessels and various foreign bodies, the imaging is not clear. In addition, not all mouse fundus tissue structure is the same as that of the public mouse fundus tissue structure. It is difficult to trace the distant nerve fiber tissue layer only by surface scanning, and it is more difficult to clearly distinguish which part of the tissue belongs to. Therefore, the OCT imaging device can only observe the nearest membrane tissue. In this paper, we add a wide-angle optical component to help complete the mouse fundus imaging, and through image denoising and edge automatic detection technology to achieve separation measurement.

3. Automatic Segmentation and Detection of OCT Imaging Device

3.1. Overview of MCNN-GS Technology

In order to make the detected image have high resolution and clearly reflect the structure of the underlying tissue. The first mock exam is to use image normalization to pretreat the OCT image displayed on the fundus structure. On the other hand, we choose the edge and other parts of the retina part of the retina to divide the image blocks. Finally, we can detect the MCNN module in the light of the extracted content and describe the general outline characteristics. In addition, due to the imbalance between the samples, the data expansion command is carried out for each sample, which makes each category reach saturation state. During this period, we also need to preprocess the extracted image levels to improve the test effect. Using GTDB algorithm to divide the image simply, select the important part, that is, the part that needs measurement data, and use the MCNN model we set up before to optimize the content pixels, and automatically generate the corresponding icon of each image block. Finally, the icon is generated into directional pixel nodes, and the edge of fundus structure is observed according to the algorithm selected in this paper.

3.2. Data Acquisition and Amplification

Before testing, we need to extract the OCT image preprocessing training set of targets (retinal layer boundary) and background image blocks. In scanning, the 75×75 pixel image block extracts

the pixel's retinal layer boundary pixel as the center of the manual segmentation result. These extracted image blocks are taken as positive samples, and each boundary is classified and labeled. In addition, for the negative extraction samples, in order to distinguish the background pixels with different retinal positions, we randomly select three background pixels located in the upper, lower and inner boundary regions of the retina to extract image blocks of the same size. Similarly, three category tags are assigned to three background image blocks.

3.3. OCT Image Denoising

In the process of actual medical image collection, conversion and transmission, usually through the imaging equipment and external environment and other unstable factors, resulting in medical images and inevitable including noise, image noise significantly affects our image analysis and processing, the effect of image denoising is to improve the image signal-to-noise ratio, highlight the image features required, Therefore, noise reduction has become one of the most important tasks in medical image preprocessing.

OCT image noise mainly comes from the interference of light source and circuit, the nonlinearity of scanning and photoelectricity, and the uneven scattering of light by biological structure. It is also closely related to the imaging principle, imaging equipment and imaging parameter setting of OCT instrument. Generally speaking, OCT image mainly produces the following kinds of noise: speckle noise, light source and circuit noise, scanning noise.

Wavelet transform [11] and differential diffusion filter [12] are two mainstream image denoising methods, which have good effect in medical image denoising. In recent years, people gradually apply it to OCT images. In addition, the research shows that the wavelet and diffusion filter have a large amount of calculation and low efficiency. Therefore, in practical application, considering the performance of the algorithm, it is generally suitable to use simple filtering algorithm, such as median filtering. Secondly, median filter and adaptive filter are introduced.

(1) Median filter [13] is a filter based on statistical sorting. The basic principle is to replace the pixel value of the pixel in the image with the median value of each point in the neighborhood. If f(x, y) is the gray value of image pixel (x, y), the convolution template is $S_{x,y}$, the median filter can be defined as:

$$g(x, y) = median\{f(x, y)\}, (x, y) \in S_{x, y}$$

$$(1)$$

G(x, y) is the median value calculated in pixel (x, y). When n is odd or even, the median is sorted and two intermediate values respectively. For salt and pepper noise, median filter has better denoising effect on impulse noise and speckle noise, and the ambiguity is smaller than linear smoothing filter with the same size.

(2) Median filter has a good effect on salt and pepper noise. As long as the noise density is small, the noise reduction effect is good. According to experience, when the salt and pepper noise density is greater than 0.2, the median filtering effect is not ideal. In addition, due to the fixed window size, noise reduction and image detail protection cannot be considered at the same time. The adaptive median filter can deal with high density impulse noise and smooth the details when dealing with non-impulse noise. The filter will operate differently according to the preset conditions and the local neighborhood.

3.4. Automatic Contour Extraction Stage

The output image I of the preprocessing stage is taken as the input, and the fundus contour is detected by pipeline. Automatic segmentation and measurement of fundus contour

(1) Longitudinal scanning of A-line: Based on the output of binary image in the preprocessing stage, each A-line is traversed from bottom to top. When the gradient changes significantly, i.e. the first pixel with a gray value of 266 stops, the next A-line scan is continued until all A-lines are scanned, and the output image is marked as a.

(2) Image subtraction: image subtraction refers to the process of subtracting the pixels at the same position of two input images to get the output image. It is mainly used to remove unnecessary superimposed patterns, motion detection and gradient images. Because the lumen tissue may contain lumen blood or bronchial mucus, these will affect the true lumen contour extraction. The main purpose of subtracting a-image from A-line scanning image is to eliminate these effects.

(3) Morphological operation: morphological operation can be used to fill small holes and connect adjacent objects in the body. In order to eliminate the whole and residual cavity shadow, the output image of the previous stage is etched and then expanded. The kernel size is 3. The number of operations is determined by previous experiments and can be adjusted appropriately. After many experiments, three operations are enough to remove the remaining holes and shadows without affecting the lumen size.

(4) Removal of guide wire area: in this study, the presence of guide wire in Oct equipment makes the polar coordinate system form a fixed angle black area, which is transformed into a rectangular area with fixed length and width after being transformed into polar coordinates. Due to the processing in step (1), the black area extends to the top of the image. This extra area is removed. First, a horizontal scan is performed at the top of the image to find all pixels that meet the requirements. These pixels form the width of the guide-line area. Perform a top-down A-line scan on each pixel until it reaches the edge of the lumen.

(5) Sobel edge detection [14]: the images processed in the whole process are binary images. After removing the guide wire area, the inner wall contour of the tube is taken as the boundary. The gray value of the upper part of the image is 266, and the lower part is 0. Sobel edge detection algorithm based on gradient can detect the contour of pipe wall well.

(6) Scale transformation: transform the detected contour image into polar coordinate display.

3.5. Automatic Edge OCT Image Segmentation

Edge segmentation [15] refers to the collection of pixels with step change or roof change in adjacent regions. In a broad sense, it can be divided into point-based edge segmentation, line-based edge segmentation and edge-based edge segmentation.

w ₁	<i>W</i> ₂	<i>W</i> ₃	9
W_4	W_5	W ₆	$R = w_1 z_1 + w_2 z_2 + L + w_9 z_9 = \sum_{i=1}^{n} w_i z_i$
<i>W</i> ₇	W_8	<i>W</i> ₉	

Table 1. Point detection template and calculation formula

Shown as Table 1, R is the central pixel value of the template. Point based edge segmentation is the process of using the specified template to detect discrete points in the area to be detected, and finally connecting the detected discrete points to a closed boundary.

On the basis of edge segmentation, the detection operator is usually used to detect all the nearby pixels, and then the gray change rate, quantization and calculation direction are determined. The amplitude and direction of the image edge are two important features. The amplitude transformation along the vertical direction of the edge is more intense, while the amplitude change along the horizontal direction of the edge is relatively gentle. In the step edge, the first derivative edge detection operator makes full use of the amplitude and direction of the edge. The function change degree of one-dimensional image is expressed by first-order differential derivative, and then the two-dimensional image is processed locally, and the significant change of its characteristics is represented by gradient. One of the mathematical tools to measure the change of function is gradient:

$$G(x, y) = \begin{bmatrix} f_x \\ f_y \end{bmatrix} = \begin{bmatrix} \frac{\partial f}{\partial x} \\ \frac{\partial f}{\partial y} \end{bmatrix}$$
(2)

When the directional derivative reaches the maximum value, it can be concluded that:

$$\theta(x, y) = \tan^{-1} \frac{f_y'}{f_x'}$$
(3)

The derivative gradient modulus is as follows:

$$\left|G(x,y)\right| = \sqrt{f_x' + f_y'} \tag{4}$$

Therefore, one of the operators suitable for edge detection is gradient mode, in which edge intensity is gradient mode and edge direction is gradient direction.

3.6. Automatic Segmentation and Measurement Process of OCT Image

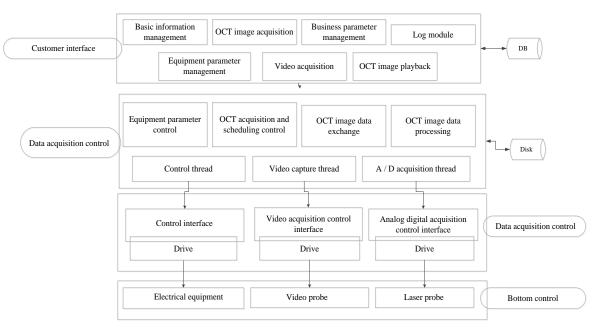


Figure 1. Overall architecture of OCT system

Figure 1 shows the overall design of the system. OCT system mainly includes bottom control, drive control, data acquisition logic control and customer interface. The work involved in this paper is mainly data acquisition logic control layer and user interface layer.

The overall process of the system: the user interface layer triggers and starts the relevant processing threads of the logic control layer through interface events. The motion control thread, video acquisition thread and analog-to-digital acquisition thread in the logic control layer call the relevant interfaces of the drive control layer respectively. The driver control layer realizes the underlying control by calling the SDK provided by the underlying control card. The user interface mainly included: case information display, OCT image rotation and scaling, pseudo color display, image smoothing, brightness and contrast adjustment, image contrast and other functions. The main interface displays the image display of a frame of data, and the basic information and remarks of patients and related processors are displayed at the bottom of the picture. In the upper right corner is the video display area. If the saved trachea video image exists, it will be played back automatically; otherwise, it will be displayed as blank. Videos and images will be mapped according to the agreed naming format.

4. Results and Discussions

4.1. Comparison of Segmentation Results under Different Sampling Methods

In order to test the segmentation results of different sampling methods, we choose two sampling methods to test. However, because the data collected by the data block is relatively large, the image block will grow rapidly due to the aimless increase of data. Therefore, we carry out experiments in the OCT data storage location. Among them, SM2 and SM1 are data incremental method and non-data incremental method respectively.

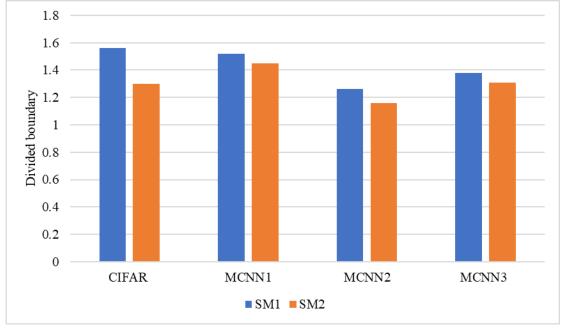


Figure 2. MUE index comparison of segmentation results of different sampling methods on dataset

Shown as Figure 2, the results of boundary segmentation under different sampling methods are still different. Without the data incremental method, the segmentation value in CIFAR network is as

high as 1.56, which is higher than that under all networks. However, after using the data increasing method, the value obtained is as low as 1.30, which increases by 0.26 on the basis of it. It can be seen that even though the contour with perfect segmentation contains some omissions, SM1 can effectively alleviate the segmentation effect, optimize the image and enhance the beauty.

4.2. Manual Segmentation vs Computer Automatic Segmentation

In the process of manual segmentation measurement and computer automatic segmentation measurement, the former will have human error, it is difficult to achieve accurate measurement, and artificial intelligence machine operation greatly improves the occurrence of such problems, so the error obtained from the two kinds of segmentation measurement is analyzed.

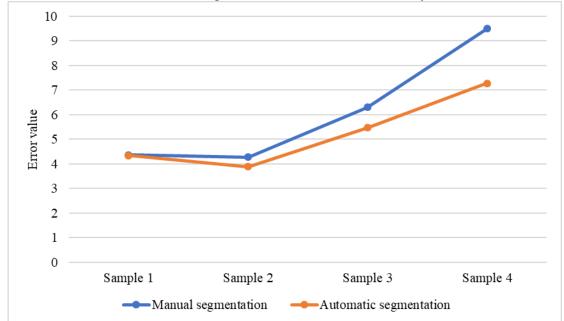


Figure 3. Error analysis of manual segmentation and automatic segmentation

Shown as Figure 3, it is difficult to recognize some edges of the image by human eyes, which results in errors. Therefore, it is difficult to measure accurately with the computer automatic segmentation. After four experiments, the error of automatic segmentation is much lower than that of manual segmentation.

4.3. Feasibility Analysis of OCT Imaging System

In this paper, matlab statistical software is used to record, and the data obtained from OCT imaging device with the help of optical microscope are visually analyzed, and the regression analysis of correlation coefficient is carried out. We take the data obtained from the image presented in OCT imaging device as the abscissa, and the data measured by optical microscope for mouse tissue section as the ordinate, we can obtain the scatter plot shown as Figure 4 below.

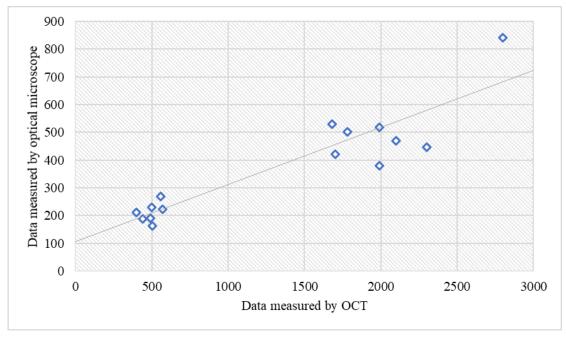
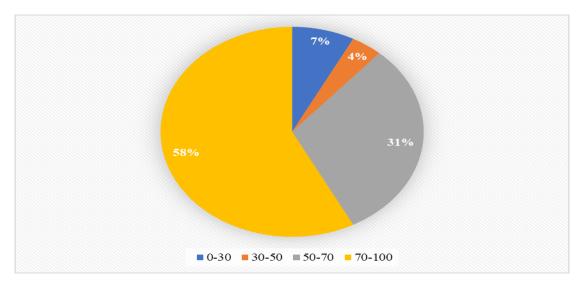


Figure 4. Scatter plot of OCT measurements and overlay measurements

Shown as Figure 4, in the scatter diagram, we get the quadratic value of the correlation coefficient is 0.892, and the quadratic power is obviously close to 1. Thus, the correlation between the two groups of data is very high. According to the results of several measurements by different groups, the feasibility of the OCT imaging system in the detection of mouse fundus is very high.



4.4. Distribution of Experts' Scores

Figure 5. Expert score distribution

Shown as Figure 5, we interviewed 26 experts who have contributed to animal medicine research and asked them to score the automatic segmentation measurement of OCT images with a full score of 100. We can clearly see that 58% of the students scored 70-100, and only 11% of them scored

0-50. Some experts pointed out that the computer automatic segmentation measurement process can reduce the error caused by manual operation, and is more inclined to the data detection of objective factors than the data measured by subjective factors, which reduces the number of mouse experiments and improves the accurate value, which is worthy of promotion.

5. Conclusion

Optical coherence tomography (OCT) is a new medical imaging technology based on the principle of optical coherence measurement and optical coherence reflection. It is widely used in the treatment of various early-onset diseases in medicine and can be used as an evaluation method to observe the therapeutic effect and disease development. At present, only OCT can be used to detect the fundus of mice. In view of the great difference between human and mouse fundus structures, it is difficult to complete the detection and imaging. In this paper, MCNN-GS technology is used to extract the boundary features of the retinal layer. The collected data are magnified and segmented to show the different layers of the retinal fiber membrane of the mouse fundus, which can accurately measure its thickness and the data that cannot be detected by tissue section. In this paper, we take two different sampling methods, compare the manual segmentation measurement with the computer automatic segmentation measurement, compare the data measured by OCT imaging device with the data measured by tissue section, and comprehensively evaluate the effectiveness of the device in terms of expert scoring. The final results show that the quality of the extracted image can be effectively optimized without increasing the data; the data measured by computer automatic segmentation is more accurate, avoiding the interference of human factors; the data detected by automatic segmentation of OCT image is highly correlated with the data measured by tissue slices, and OCT imaging system can detect more data, such as the thickness of nerve fibers; expert score The general score was between 70 and 100. It can be seen that the application of OCT in the fundus examination of mice is very feasible. The data obtained from the automatic segmentation of OCT images also reduces the workload of the staff, and the measurement results are more accurate. It plays an important role in the establishment of animal fundus retina model in the future, and the cure rate of eye diseases will be improved year by year.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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