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Enhancement of Micro-Images Using Feature Linking Model for Cerebellum of *Alligator sinensis*

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ABSTRACT In order to realize the three-dimensional reconstruction of the cerebellum structure of *alligator sinensis*, it is necessary to observe the structure by using a microscope. In the process of slice making and micro-image shooting, individual operation differences lead to good or bad quality of micro-images. To solve this problem, the feature linking model (FLM) is used to enhance the micro-image of alligator cerebellum and improve the quality of micro-image. The transverse section and longitudinal section were prepared by using the tissue of cerebellum of *alligator sinensis* as a raw material, and the micro-images were obtained by the light microscopy. The different enhancement methods were used to process the transverse section micro-images, and the enhancement was evaluated subjectively by direct observation. It was found that histogram equalization (HEQ) and fuzzy set method (FSM) did not enhance the darker parts well, while enhanced the brighter parts excessively. And the enhancement with pulse coupled neural network (PCNN), spiking cortical model (SCM), and FLM are better. On the basis of subjective evaluation, three objective evaluation indexes (contrast, spatial frequency, and gradient) were used to compare and analyze the image quality improvement of PCNN, SCM, and FLM, and we found that FLM had the best enhancement on transverse section micro-images. Then, FLM was used to process longitudinal section micro-images, and the objective evaluation indexes before and after processing were compared. We found FLM also had a good enhancement on longitudinal section micro-images. The results show that FLM was effective in enhancing micro-images of the cerebellum of *alligator sinensis*, and FLM improved the quality of the longitudinal section micro-images more obviously. The proposed method can improve the quality of the microscopic image of the cerebellum of *alligator sinensis* so that higher quality images can be obtained, which is beneficial to improving the utilization rate of the material.

INDEX TERMS Feature linking model, micro-images, image enhancement, *alligator sinensis*, cerebellum.

I. INTRODUCTION

With the rapid development of computer technology, biological micro-imaging technology and equipment have been constantly updated [1], and the establishment of three-dimensional (3D) visualization models based on the anatomical structure of virtual human, animal and organ have become a hot topic of research [2]. *Alligator sinensis* [3] belongs to the alligator family alligator genus [4], is a unique crocodile in China, is one of the smallest crocodile species in the world. Known as ''living fossil,'' the alligator has many features of early dinosaur reptiles that can still be found today. Studying the structure of the cerebellum of *Alligator sinensis* and constructing the three-dimensional visualization model can grasp the three-dimensional structure more clearly, which is of great significance to the research and teaching of *Alligator sinensis*. At the same time, the 3D visualization model has certain reference value for people to study the rise and fall of ancient reptiles, paleogeology, and biological evolution.

In the process of brain slicing of the alligator [5], there is a gap in the quality of the slices due to the individual differences of the operators. At the same time, when using a microscope to observe and shoot micro images, the quality of micro-images will be affected by artificial

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factors [6] such as light, section placement and so on. The accuracy of cerebellum structure analysis and three-dimensional visualization model construction depends on whether the image collected by the microscope has a good visual effect. At present, there are some advances and achievements in the field of microscopic image processing [7]. Chang and Liu [8] which improved the image quality and obtained the image characteristics of the target quickly and accurately by means of grayscale transformation and histogram equalization of the original image of microscopic acquisition. Chen *et al*. [9] have mentioned low-contrast microscopic image enhancement based on multi-technology fusion. The low contrast microscopic image enhancement was realized combining with Sobel operator, LoG operator and improved contrast limited adaptive histogram equalization method. Kopriva *et al*. [10] proposed an offset-sparsity decomposition method for the enhancement of a color microscopic image of a stained specimen. According to the mean opinion score, estimated on the basis of the evaluations of five pathologists, images enhanced by the proposed method exhibit an average quality improvement of 16.60%. So far there are few studies on the field of microscopic image enhancement of the *Alligator sinensis* cerebellum. At the same time, the *Alligator sinensis* is a national protected animal, the acquisition of raw materials is not easy, and the production of slices is time-consuming and laborious.

To solve the above problems, a method based on feature linking model (FLM) [11] is proposed to enhance the cerebellum micro-images of *Alligator sinensis*. On the one hand, this method is used to enhance the conventional quality of micro-images. On the other hand, FLM is employed to process the image with poor slice quality and poor shooting effect, so that it can be used as an effective sample. This method helps to observe the microstructure more clearly and make effective use of some original useless samples. Hence, it is helpful to improve the utilization rate of the materials, obtain more effective images and speed up the research pace.

In the following text, the experimental materials and the method of obtaining the microscopic images are introduced first. The brief introduction of the FLM algorithm and a common evaluation method of the effect of microscopic image enhancement are described next. Then, the enhancement ability of FLM and histogram equalization (HEQ) [12], fuzzy set method (FSM), pulse coupled neural network (PCNN) [13] and other common image enhancement algorithms to the cerebellum transverse microscopic image of the alligator is compared. After that, the optimal enhancement algorithm is applied to the longitudinal microscopic image, and the improved ability of the proposed method is observed. Finally, the paper concludes with a summary of this study.

II. MATERIALS AND METHODS

A. EXPERIMENTAL MATERIALS

Three new-hatching alligators (during 1 week since its birth), approximately 15 cm in length, were collected from Xuancheng Alligator Culturing Centre.

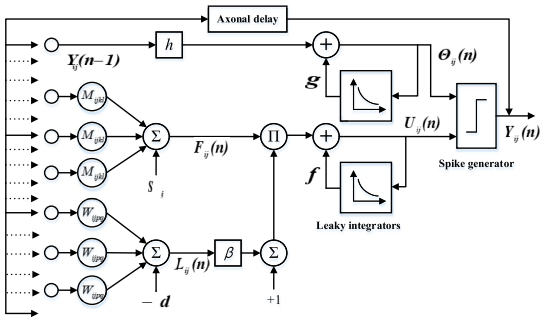


FIGURE 1. Schematic of the feature linking mode.

B. SLICE MAKING

These alligators were anesthetized with ether and quickly stripped the whole brain tissue from the cranial cavities. The brain weight was about 0.24g to 0.36 g. After fixing with Bouin's solution [14] for 48 hours, the fixed tissue samples were dehydrated with increasing gradients of ethanol, washed in xylene, and impregnated with molten paraffin [15]. The coronal and sagittal sections (thickness about 7μm) were serially sectioned using a Leica slicer [16]. Wax sheets were spread in clear water at 45°C and attached to clean glass slides, then placed in an oven at 37°C for drying.

C. NISSL STAINING

Each representative tissue section was first deparaffinized with two changes of xylene, 10 min each time. Slides were rehydrated with decreasing concentrations of ethanol and distilled water for 5 min each. Then, the sections were stained in 0.1% warmed cresyl violet solution [17] (warmed up in 37° bath) for 10 min, rinsed quickly in distilled water and differentiated in 95% ethanol for 30 min. Finally, sections were dehydrated with two changes of 100% ethanol for 5 min each, cleared two 5-min in xylene, and mounted with resin mounting medium.

D. OBSERVATION AND PHOTOMICROGRAPHY

All sections were observed and photographed with an Olympus BX61 microscope [18] and a Motic BA600-4 automatic scanning microscope.

E. FLM

As shown in Figure 1, the FLM consists of three parts: the membrane potential, the threshold, and the action potential. The dendrites receive post-synaptic action potentials by receptive wild synapses, and the resulting postsynaptic action potentials are transferred to adjacent neurons by local synapses located on the dendrites. If the membrane potential is above the threshold, the neuron will generate an action potential or pulse. In the FLM, both the membrane potential and the threshold are represented by leaky integrators.

Leaky integrators are the basic components of neural networks. The dynamic potential $v(t)$ of a neural oscillator

is represented by a leakage integrator:

$$\frac{dv(t)}{dt} = -av(t) + s \tag{1}$$

where t is time, s is input and a is leakage coefficient.

Most cortical neurons are bidirectionally connected: feeding synapses are feedforward and the linking synapses are feedback. The signal of the dendritic to the neuron is the sum of the feedback input and the connection input. Each neuron here is represented by (i, j), one of its adjacent neurons is represented by (k, l) or (p, q).

$$F_{ij}(n) = \sum\nolimits_{kl} M_{ijlk} Y_{ij}(n-1) + S_{ij} \tag{2}$$

$$L_{ij}(n) = \sum\nolimits_{pq} W_{ijpq} Y_{ij}(n-1) - d \tag{3}$$

where $F_{ij}(n)$ denotes a feeding input,$Y_{ij}(n-1)$ is the postsynaptic action potential, S_{ij} is the stimulus for the neuron, M_{ijlk} is a synaptic weight applied to feeding inputs,$L_{ij}(n)$ denotes a linking input,W_{ijpq} is a synaptic weight applied to the linking inputs, and d is a positive constant for the globally inhibitory.

The membrane potential is essentially a leakage integrator, which can be expressed as:

$$U_{ij}(n) = fU_{ij}(n-1) + F_{ij}(n)(1 + \beta L_{ij}(n)) \tag{4}$$

By introducing formulas (2) and (3) into formulas (4), the expression of membrane potential can be obtained as follows:

$$U_{ij}(n) = fU_{ij}(n-1) + \left(\sum\nolimits_{kl} M_{ijkl} Y_{ij}(n-1) + S_{ij}\right) \times \left(1 + \beta \sum\nolimits_{pq} W_{ijpq} Y_{ij}(n-1) - d\right) \tag{5}$$

The threshold of a neuron is represented by a leaky integrator, the postsynaptic action potential is the input of threshold, the expression of the threshold is

$$\Theta_{ij}(n) = g\Theta_{ij}(n-1) + hY_{ij}(n-1) \tag{6}$$

where g is the threshold attenuation constant, h is a magnitude adjustment, and $Y_{ij}(n-1)$ is the postsynaptic action potential.

When the membrane potential of a neuron exceeds the threshold in the network iteration, the neuron produces a spike,

$$Y = \begin{cases} 1, & U_{ij}(n) > \Theta_{ij}(n) \\ 0, & otherwise \end{cases} \tag{7}$$

F. EVALUATION METHOD OF MICROSCOPIC IMAGE ENHANCEMENT

Since the image enhancement method is proposed, the image quality improvement requirement is different, which leads to the majority algorithm which is only suitable for specific types of images. Therefore, we need to evaluate the effect of image quality improvement through subjective and objective evaluation methods [19].

Subjective evaluation is that people score the enhanced image according to their personal visual experience [20]. This evaluation method has no uniformity. For the same image, different evaluators may get different results. Most evaluators draw conclusions according to their personal feelings, which is not suitable for quantitative analysis. In practical applications, subjective evaluation methods are often used as indicators of reference. The image is subjectively evaluated by a large number of evaluators, and the statistical results can be used to determine whether the objective evaluation index is reliable. However, the objective evaluation [21] is to quantitatively analyze the enhanced image based on the determined mathematical model, so as to effectively explain the quality of the image. This result is not affected by the subjective consciousness of the evaluator but is calculated based on the characteristics of the image, which is of great significance for measuring the degree of improvement in image quality. In order to compare the enhancement effect of different algorithms on the cerebellar microscopic images of *Alligator sinensis* more accurately, we use the average of a local contrast metric [22], spatial frequency [23], and average gradient [24] to objectively evaluate the degree of image quality improvement.

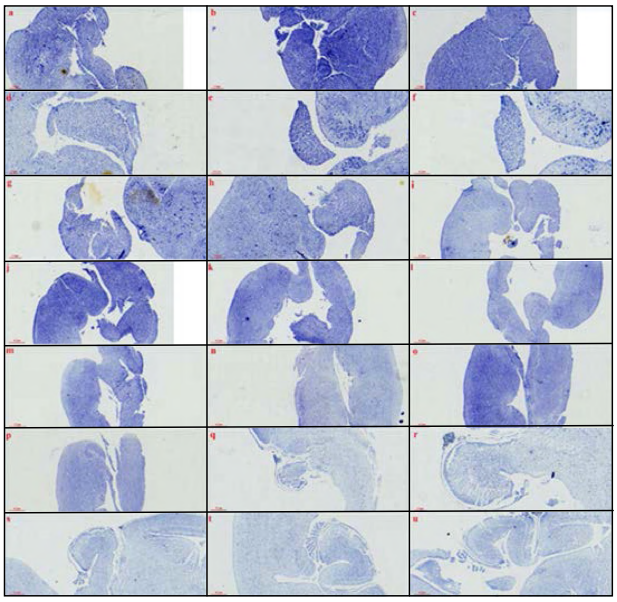


FIGURE 2. Primary micrograph of the cerebellum of the *Alligator sinensis*.

III. RESULTS AND DISCUSSION

A. ORIGINAL MICROSCOPIC IMAGE

The microscopic images of the cerebellum of the alligator were obtained by using motic BA600-4 automatic scanning microscope, and then 16 transverse and 5 longitudinal sections of the cerebellum microscopic images (derived from the same alligator) were selected according to the sequence of the slices. Fig. 2 (a) - (p) are the micrograph of the transverse section of the *Alligator sinensis* cerebellum, and Fig. 2 (q) - (u) are the micrograph of the longitudinal sections of the *Alligator sinensis* cerebellum.

The central nervous system of new-hatching *Alligator sinensis* is about 4.5cm in length, which can be divided into

telencephalon, diencephalon, mesencephalon, cerebellum, medulla oblongata and spinal cord from front to back. The mesencephalon and the medulla oblongata are usually called the brainstem. The cerebellum of the new-hatching *Alligator sinensis* is located at the back of the mesencephalon and the dorsal front of the medulla oblongata, oval in shape, and the transverse diameter is longer than the longitudinal diameter. On the transverse section, it can be observed that the caudal end of the cerebellum is embedded in the upper end of the brainstem and encloses the fourth ventricle (Fig. 2a). Going to the head, the cerebellum gradually widens on the back of the fourth ventricle (Fig. 2b, c). Further to the head, the cerebellum continues to widen and the middle of the cerebellum is embedded in the fourth ventricle (Fig. 2d). And the fourth ventricle gradually becomes larger, and the median and lateral sulcus of the fourth ventricle is clearly visible (Fig. 2e-l). Anteriorly, the cerebellum is separated from the brainstem, and the fourth ventricle gradually becomes smaller and is only closed by the brainstem (Fig. 2m-p). On the longitudinal section, the cerebellum grows larger and larger as it moves toward the center (Fig. 2q-t). It can be seen that the cerebellum and brainstem from the fourth ventricle and the fourth ventricle are closed through the choroid plexus, and the closure is not complete (Fig. 2t-u).

In the process of experimental operation and microscopic image acquisition, poor image quality is mainly caused by human factors or irresistible error factors. For example, some of the transverse section and longitudinal section images in Fig. 2 have quality problems such as inconspicuous edges and unclear structures. By improving the quality of microscopic images, it will lay the foundation for the three-dimensional reconstruction of the *Alligator sinensis* brain.

B. IMAGE ENHANCEMENT OF TRANSVERSE SECTION

HEQ, FSM, PCNN, spiking cortical model (SCM), and FLM was used to improve the quality of the transverse section of the cerebellum of *Alligator sinensis*. In order to compare the enhancement effects of different algorithms more intuitively, we randomly selected four original transverse sectional microscopic images to show the results before and after enhancement, which was convenient for subjective evaluation of different enhancement algorithms and analyze the processing effects under different image enhancement algorithms.

As we can see from Fig. 3, when microscopic images are enhanced by HEQ and FSM algorithms, their contours become clearer, but some of the structures inside the images become blurred and not easy to observe. In other words, when HEQ and FSM are used to enhance the microscopic images, they did not enhance the darker part well but over enhanced the brighter part. This feature is more prominent in the FSM algorithm. The effect of the FSM algorithm is to make the dark areas darker and the bright areas brighter. The three methods of PCNN, SCM and FLM all make the original transverse surface microscopic images have been enhanced to a certain extent, but it is not accurate to evaluate and

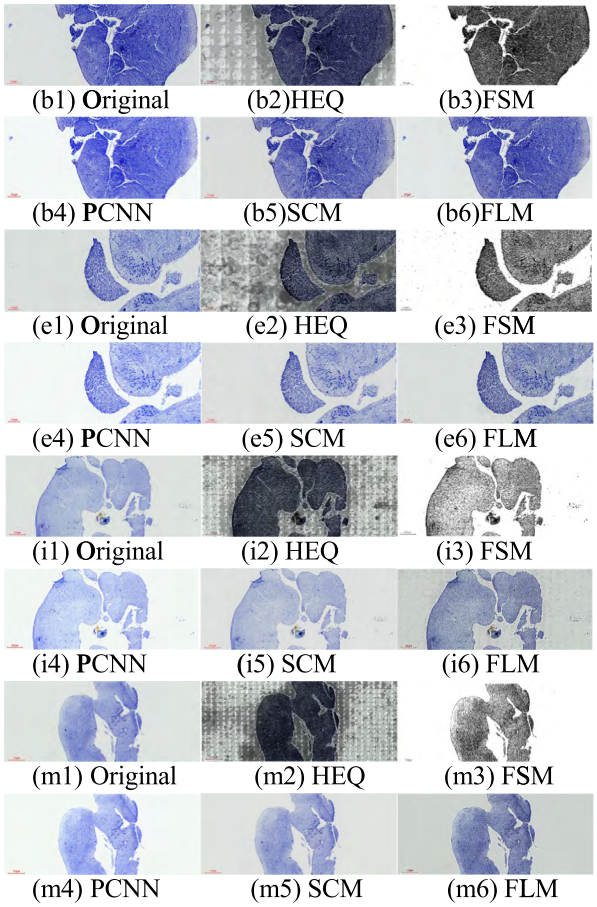


FIGURE 3. Enhanced results of different algorithms.

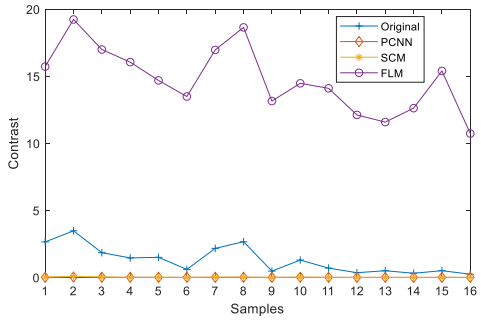
compare the advantages and disadvantages of three methods intuitively.

Considering the inability to visually distinguish the enhancements of the three algorithms with PCNN, SCM, and FLM, we use the average of a local contrast metric, spatial frequency, and average gradient to objectively assess the extent of image quality improvement. The contrast, spatial frequency, and gradient of the three algorithms are calculated respectively and then compared with those of the original microscopic images. The result is shown in Fig. 4.

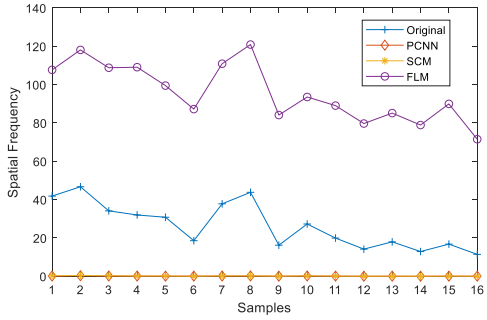
As shown in Figure 4, the FLM is superior to the PCNN and SCM methods in terms of contrast, spatial frequency, and gradient. At the same time, compared with the image quantization index before and after FLM enhancement, it can be seen that the original image has a significant improvement with contrast, spatial frequency, and gradient, which significantly improves the quality of the microscopic images. In other words, FLM has a good enhancement effect on the microscopic images of the cerebellum transverse section of the alligator and can improve the quality of the microscopic images.

C. IMAGE ENHANCEMENT OF LONGITUDINAL SECTION

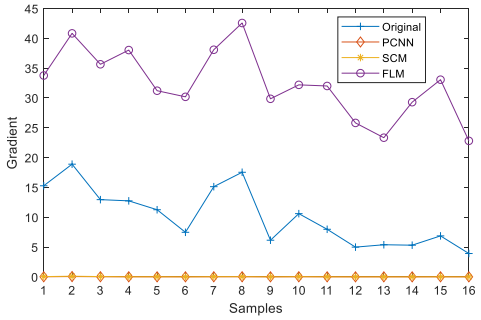
Several different algorithms are used to enhance the micro-images of the transverse surface of the cerebellum



(a) Average of a local contrast metric of different algorithms



(b) Spatial frequency of different algorithms



(c) Average gradient of different algorithms

FIGURE 4. Objective evaluation indicators of different algorithms.

TABLE 1. Objective evaluation indicators before and after FLM.

Longitudinal section	Contrast		Spatial Frequency		Gradient	
	Original	FLM	Original	FLM	Original	FLM
Sample 1	0.3348	**10.8353**	13.6738	**75.0099**	4.8617	**24.7394**
Sample 2	0.9962	**12.1967**	23.9779	**86.2787**	9.7482	**32.5217**
Sample 3	0.4368	**13.8409**	15.4619	**84.8526**	7.3663	**36.4623**
Sample 4	0.5154	**14.0465**	17.3823	**89.0857**	7.5553	**35.2551**
Sample 5	0.5542	**14.7717**	17.8645	**91.2223**	8.3805	**38.1683**

of the alligator, which proves that the enhancement effect of FLM is the best. Then FLM was used to enhance the longitudinal section micro-images, and whether FLM was effective in enhancing the quality of the longitudinal section micro-images was analyzed. Contrast, spatial frequency and gradient of five *Alligator sinensis* cerebellar longitudinal micro-images before and after FLM processing were calculated respectively. The results are shown in Table 1.

TABLE 2. The degree of change in the average value of objective evaluation indicators after FLM processing.

Microscopic images		Transverse section	Longitudinal section
Contrast	Original	1.29	0.57
	FLM	14.74	13.14
	Increased multiple	11.43	**23.05**
Spatial Frequency	Original	26.41	17.67
	FLM	95.89	85.29
	Increased multiple	3.63	**4.83**
Gradient	Original	10.15	7.58
	FLM	32.42	33.43
	Increased multiple	3.19	**4.41**

From Table 1, it can be seen that the contrast, spatial frequency, and gradient, which can objectively and quantitatively evaluate the quality of the images, have been improved to some extent after the processing of the longitudinal cerebellar micro-images of *Alligator sinensis* by FLM. That is to say, the FLM algorithm is also effective for improving the quality of the longitudinal micro-images.

In order to further grasp the degree of elevation of the FLM algorithm to the transverse and longitudinal section microscopic images of the cerebellum of the alligator, we analyze the degree of change of the average of the relevant objective evaluation indexes of all transverse and longitudinal section to approximate quantitatively evaluate the image enhancement capabilities by using FLM

Comparing the three parameters of the contrast, spatial frequency and average gradient of the transverse microscopic images and the longitudinal section microscopic images, the contrast and spatial frequency of the cross-sectional microscopic images are higher than that of the longitudinal section microscopic images, and the gradient of the microscopic image is higher than that of the transverse surface microscopic image after using FLM. In other words, after the treatment of FLM, the overall image quality of the cross-sectional microscopic image is higher than that of the longitudinal section microscopic image, and the edge information of the longitudinal tangent microscopic image is better than that of the transverse surface microscopic image, that is, the longitudinal section microscopic image with FLM processing has a clearer edge contour information. At the same time, we also noticed that for the microscopic images of the cerebellum of the *Alligator sinensis*, the three parameters increase with the longitudinal section microscopic images after FLM treatment were 23.05, 4.83 and 4.41, the degree of increase is greater than the cross-sectional microscopic images, that is, the enhancement of the longitudinal section micro-images by FLM algorithm is higher than that of the transverse section micro-images.

IV. CONCLUSION

Microsections of the cerebellum of *Alligator sinensis* were made from new-hatching *Alligator sinensis* samples,

and microscopic images of the cerebellum were obtained by the automatic scanning microscope. Different methods of image enhancement were used to process the cross-sectional micro-images of the *Alligator sinensis* cerebrum, and the enhancement effects of different methods were subjectively evaluated through artificial observation. It was found that the HEQ and FSM algorithms had poor visual effects when the micro-image was treated, and the visual effects were better when the three methods of PCNN, SCM, and FLM were used. On the basis of subjective evaluation, the image quality improvement effect of three methods of PCNN, SCM, and FLM is evaluated by contrast, spatial frequency and gradient objective index, and it is found that FLM has the best enhancement effect on cross-sectional microscopic images. Then the objective evaluation indexes before and after FLM algorithm processing longitudinal section micro-image are analyzed, and it is found that FLM also has a good effect on improving the quality of longitudinal section micro-images. The results show that the use of FLM can effectively enhance the microscopic images of the cerebellum of the *Alligator sinensis*, and the FLM to improve the quality of longitudinal section microscopic images is higher.

At present, the number of samples used in this paper is relatively small, and the staining of the slices is single. In the future, the types of slice staining and the number of samples of the micrograph of the Chinese crocodile cerebellum will be increased to verify the effectiveness of FLM in large-scale multi-type samples. At the same time, in order to better construct the three-dimensional visualization model of the brain structure of the alligator, the enhancement methods for microscopic images of the telencephalon, mesencephalon and medulla oblongata of the alligator will be further studied.

V. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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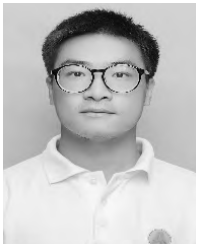

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