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Application of Computer Vision in The Automatic Analysis of Feeding Behavior in *C. elegans**

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Abstract The nematode *Caenorhabditis elegans* has been widely used as a perfect model organism to study the relationship between genes and behavior. The pharyngeal microcircuit of the worm controls a complex feeding behavior. In order to study the molecular basis of this feeding behavior, it is necessary to identify subtle differences in feeding activity of the worm. However, most of the phenotype analyzing of feeding behavior is accomplished by human eyes. And it is a tough and poor efficiency job to analyze the fast pumping muscle of the worm. To help improving this problem, an automated system has been developed based on computer vision for the high-throughput analysis of the feeding behavior by virtue of a simple webcam. Our system enables the consistent and subtle analysis of *C. elegans* pumping recordings and the accuracy of pumping detection is up to 98%. Under this high accuracy, the time cost of the behavior analysis is cut down by 67% versus human manipulation.

Key words *C. elegans*, automated behavior analysis, pharyngeal pumping, template matching
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The investigation of cellular and molecular bases of behavior is an important goal in the study of neurobiology[1]. Like the *Caenorhabditis elegans*, even it has only 302 neurons, it could exhibit many sophisticated behaviors, such as pharyngeal feeding. With the identification of all the 302 neurons and the well-established genetic manipulation technique, *C. elegans* has been widely accepted as a classic model system to answer the hot question in neuroscience: how do genes control behaviors? The well-described rhythmic pharyngeal pumping behavior and the underlying pharynx circuit offers a window for the exploration of behavior of lives, especially with the rhythmicity of the feeding behavior and the homology of related genes with mammalians, the pharynx has been considered as an original heart-like organ to be studied[2].

Feeding behavior of *C. elegans* consists of two motions, pumps and isthmus peristalses. The two motions bring food into the pharyngeal lumen, grind it up, and pass it to the intestine[3]. As the importance and accessibility of feeding behavior, it has been developed as a phenotype assay to analyze the pumping rates of the worm. Sometimes more subtle information such as the interval time between two pumps and the duration time of each pump needs to be clear. According to the *Wormbook* [4], an easy routine for the measurement of pumping rate are performed by counting visible movements of the grinder by human eyes. However, most of the physiological rates of the animal are too fast to count by human eyes, which is also a tedious and low-efficient job. In order to solve this problem, Leon Avery *et al*[3] had developed a method measuring

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the electrical activity of the pharyngeal muscle during pumping, called the electropharyngeogram (EPG). The EPG is a powerful tool for feeding behavior study with high temporal resolution and reliable results, but the system is a bit complex with expensive instruments. As a large number of the feeding behavior assays only need a relatively simple analysis, it is not a suggested setting. Although there are several automatic behavior analysis systems as wormtracker[5–7] or MWT[8], none of them could be utilized for the feeding behavior. So the alternative way to EPG is to develop a simple and low-cost system by means of computer vision with intelligence and high efficiency for pharynx circuit study of *C. elegans*. Here, a mostly automatic pumping analysis system (autoPUMP) only based on a webcam and a desktop computer is bringing out. The autoPUMP system enables the consistent and subtle analysis of *C. elegans* pumping recordings and the accuracy of pumping detection is up to 98% . Meanwhile, time-consuming of analysis using autoPUMP has been reduced 67% from that through human eyes.

1 Framework outline

1.1 Hardware requirements

Comparing to EPG method, autoPUMP system has advantages of low-cost and easy assembling. As shown in Figure 1a, an ordinary microscope equipped with a webcam and a desktop computer with an image grabber card is required. In this paper, Micro-Manager is being used for data capture. Some automatic wormtracker for *C. elegans* behavior analysis[5] would need a motorized X-Y stage to keep the worm always in the field. Since locomotion analysis is not needed here, it is not necessary to have a motorized X-Y stage in autoPUMP system. Moreover, *C. elegans* shows minimal motion and could be kept in the middle of bacteria. So without the motorized stage, the autoPUMP system could accomplish taping the video of worm's pumping motion mostly by itself and analyze the video frames automatically. The whole system is programmed on base of ImageJ in Java and the video capture function could be easily performed by the cross-platform and open-source plugin of ImageJ.

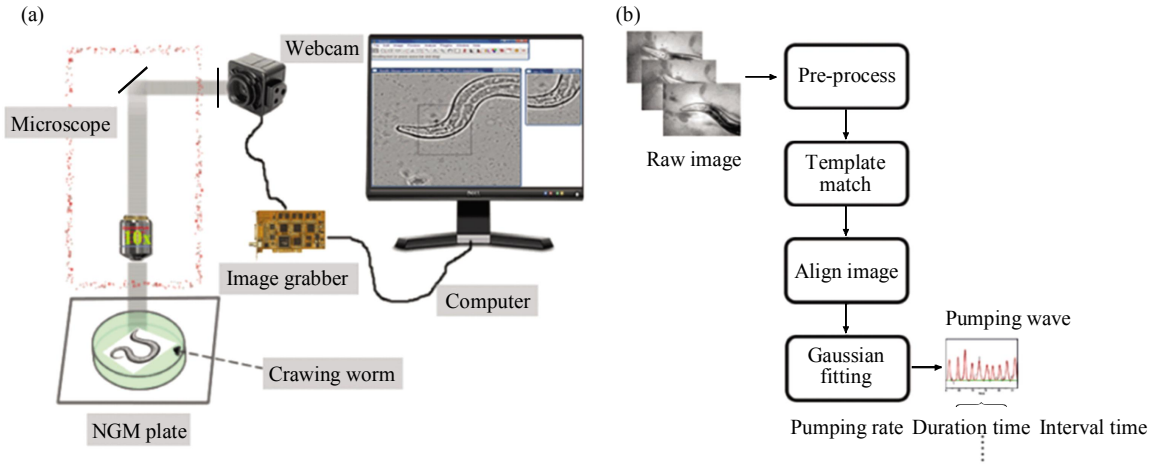

Fig. 1 Automated analysis system for feeding behavior

(a) A schematic drawing of the system based on a webcam. (b) The computer vision algorithm flow chart.

1.2 Pre-process: Fourier band-pass filter

As shown in Figure 2a, the original image captured from the webcam is not clear enough to identify the grinder area of worm's pharynx. The signal needs to be extracted from the data which has a different frequency against both the background and the rest parts of the worm. So a band-pass digital frequency filter to enhance the contrast of the grinder from the noisy background is used. The specific FFT filter is shown in Figure 2a. And because the FFT method is a traditional frequency digital filter, it is not spread out here.

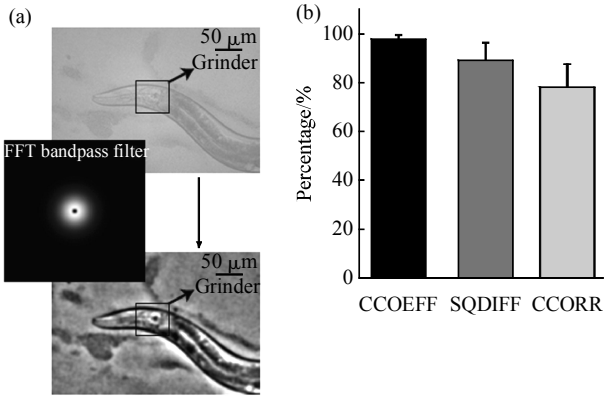

Fig. 2　Template match of *C. elegans* grinder

(a) Using a FFT band pass filter to make the grinder area of pharynx easier for matching. The above image is the original image captured from the webcam, the middle one shows the FFT filter been used and the lower image shows the result after the filter. (b) Comparing the matching accuracy of CCOEFF /SQDIFF /CCORR three different template match algorithms. The error bar is the standard error of the data. The total sample number used for statistic is 4300 frames.

1.3　Template matching

After processed with the band-pass FFT filter, the original image is enhanced against the background. Then the system is trying to find the grinder area where the pumping muscle located depending on template matching algorithm. So far, there are a lot of template matching methods in computer vision realm. According to the image character of the worms, three different algorithms named square difference matching method (SQDIFF), correlation matching methods (CCORR) and correlation coefficient matching methods (CCOEFF) are programmed, which are referred to a popular computer vision library called OpenCV[9]. The OpenCV is an open source computer vision library with its goal to provide a simple-to-use computer vision infrastructure that helps people build fairly sophisticated vision applications quickly[9].

Unlike the template matching method based on histograms, the three algorithms being chosen here are realized by matching an actual image patch against an input image by sliding the patch over the input image using one of the formulas described as follows. $I_t(x', y')$ represents the template image and $I_s(x + x', y + y')$ represents the original image after band-pass filtering. The w presents the width of the template image and h presents the height of the template image.

$$\Delta D_{\mathrm{SQDIFF}}(x, y)=\sum_{x', y'}[I_t(x', y')-I_s(x+x', y+y')]^2$$

$$\Delta D_{\mathrm{CCORR}}(x, y)=\sum_{x', y'}[I_t(x', y')\cdot I_s(x+x', y+y')]^2$$

$$\Delta D_{\mathrm{CCOEFF}}(x, y)=\sum_{x', y'}[(I_t(x', y')-\frac{1}{(w\cdot h)\sum_{x', y'} I_t(x', y')}\cdot (I_s(x+x', y+y')-\frac{1}{(w\cdot h)\sum_{x', y'} I_t(x+x', y+y')})]^2$$

$\Delta D_{\mathrm{SQDIFF}}(x, y)$, $\Delta D_{\mathrm{CCORR}}(x, y)$ and $\Delta D_{\mathrm{CCOEFF}}(x, y)$ stands for the calculated difference between original image and the template image, using SQDIFF/CCORR/ CCOEFF respectively. The SQDIFF method employs the squared difference with a result at zero shows most perfect matching. The CCORR method multiplicative the differences so it is opposite to SQDIFF method. And the third method, CCOEFF, matches a template relative to its mean value against the image relative to its mean. So it means perfect matching when the answer equals 1, and if the answer equals –1 it means perfect mismatching, and if it is 0, it then means that there is no correlation.

The basic theory to automatic identification of the grinder in the behavior video frames is described as above. However, practically, from experiment to experiment, from frame to frame, the illumination situation is different. For this reason, normalized function is used to reduce the effects of illumination differences between the template and the image[9]. In each case, the normalization coefficient is:

$$Z(x, y)=\sqrt{\sum_{x', y'} I_t(x', y')^2\cdot \sum_{x', y'} I_s(x+x', y+y')^2}$$

And then the final result is:

$$\Delta D_{\mathrm{normalized\_SQDIFF}}=\frac{\Delta D_{\mathrm{SQDIFF}}(x, y)}{Z(x, y)}$$

$$\Delta D_{\mathrm{normalized\_CCORR}}=\frac{\Delta D_{\mathrm{CCORR}}(x, y)}{Z(x, y)}$$

$$\Delta D_{\mathrm{normalized\_CCOEFF}}=\frac{\Delta D_{\mathrm{CCOEFF}}(x, y)}{Z(x, y)}$$

The template matching accuracy for all the three algorithms is calculated as shown in Figure 2b. Though the CCOEFF method has the best accuracy and smallest SE (standard error), CCOEFF is selected

as the default method. As shown in Figure 2b, sometimes the other two CCORR and SQDIFF method can also accomplish a good accuracy.

During the template matching step, the very thing requiring human manipulation is to select the grinder area ROI at the first frame and then the system will do the rest part. Because most of the pumping analyzing movies were taped when the worm moved slightly, the twisting of the worm's body could be ignored. However, sometimes the animals were not calm. If that happens, one can just select more grinder area ROI among more frames which were in different angles. And it would keep the high accuracy of the template matching. As a matter of fact, all of the wrong matching results were referred to the twisting of the worm. And the smallest standard error shows a remind that CCOEFF method is the best anti-twisting for the worm's grinder matching.

1.4 Align image

The little creature is not well-behaved eating all the time. Although the worm stands still most time of the experiment, it would change its posture of eating from time to time. Based on this behavior character, the images have to be aligned after the template matching process. Sometimes registration of the image would be even more important for the rotation of the worm's body. For the sake of registration, the matched image ROI has been expanded to twice than the template. In this way, the clearly two lines of the body wall will be useful to the image alignment. The result of alignment is shown in Figure 3b.

1.5 Feature extraction

Use find-maxima algorithm to segment the grinder part from the image and then calculate the center of mass. Traditional segmenting algorithm does not work here, because the image is confused by the bacteria the worm swallowed. However, according to the grinder structure, it is shown as several black points in the image. In order to extract the center of mass of the grinder reliability, this local maxima feature is been taken advantage for the location calculation.

2 Quantification of feeding behavior

In previous context, how to set up the system and extract the grinder area has been illustrated. Once ROI image is ready, the math model of the feeding behavior should be defined in order to consistently describe the pumping rate and other quantification parameters.

2.1 The pharynx structure of *C. elegans*

The structure of the nematode is shown in Figure 3a according to Leon Avery's published paper[3]. The head of the worm consists of three parts: corpus, isthmus and terminal bulb. On account of the widely accepted assay[4], the back and forth motion of grinder, which located in the terminal bulb, for one cycle is called a pumping.

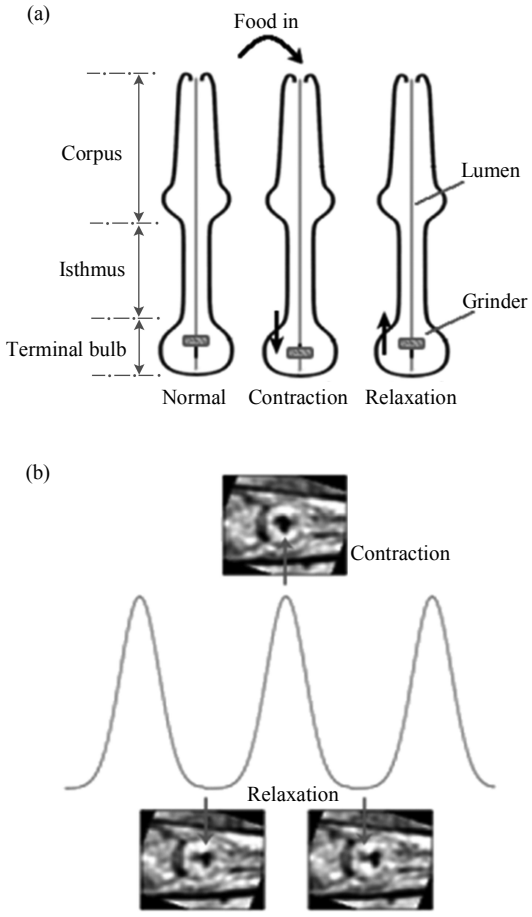

Fig. 3 Quantification of pumping rates

(a) The cartoon model of *C. elegans* pharynx. (This model is modified from Leon Avery's paper[3]). (b) The real extracted and aligned images including both contraction and relaxation.

2.2 T-model of the grinder for automated analysis

A T-model is used for the pumping rates counting for the moving grinder of the worm looks just like a letter "T" in the relaxed aligned images (Figure 3b). The position of the center of mass on X axis calculated from previous algorithm will be translated to a pumping wave (Figure 3b). And every peak of the

wave has been fitted with Gaussian function. When the grinder is holding at the relaxation status, it is considered as the valley of the Gaussian wave. The peak of the fitted pumping wave is corresponding to the contraction of the grinder as it shows in Figure 3b. The number of the peaks in a section of time can be used for the pumping rates calculation. Duration time is calculated as the width of 95% area of the Gaussian wave. Interval time is considered as the time between two adjacent pumps minus each pump's duration time.

2.3 Validation of the algorithm

Validation of the autoPUMP system compare to the result of human eyes is necessary to check the accuracy of the software. As a matter of fact, autoPUMP has 98% accuracy among 10 different wild-type worms (Table 1). And in the meantime, the time consumption for each worm is cut down to one fourth comparing to the human manipulations. The result shows the system is reliable for the automatic detection of pharyngeal pumps.

Table 1 Validation of the accuracy and time cost of autoPUMP at detecting the pharyngeal pumping rates of wild type worms

Pumping rates	N2	
	Precision/%	Time cost/s
Human eye	100	120 ± 20
autoPUMP	98	30 ± 2

The precision of pumps detection is compared to human eyes, and it is assumed that human is always right. Time cost is considered as seconds cost for each worm. n = 10 worms and in total 545 individual pumps were analyzed.

3 Experimental results

Tyramine is a neurotransmitter which is important for the pharyngeal function regulation [10]. Previous study showed that exogenous tyramine would inhibit the worm's pumping behavior[11]. However, the mechanism underlying this phenotype remains unclear. So an experiment was carried on and the results reveal the practicability of autoPUMP system.

3.1 Preparing of *C. elegans*

C. elegans strains were cultured following standard methods[12]. Hermaphrodite worms were fed and grown on a bacterial lawn and the age of experimental animals was synchronized by picking young adult stage animals to new plates ten minutes prior to experiments. Wild-type strains were obtained from the Caenorhabditis Genetics Centre.

3.2 Feeding behavior regulation by tyramine

The video of the experiment lasts fifteen minutes in total. The magnification of the microscope was 10× and the capturing speed was 30 frames per second. The fitted Gaussian pumping wave clearly shows that exogenous tyramine sharply inhibit the feeding behavior (Figure 4a). The pumping rate decreased about 75% (Figure 4a). It is not because of the change of pumping duration time, on the contrary the interval time between two pumps is prolonged as demonstrated in Figure 4c. Apparently subtle information about duration and interval time of the pumps cannot be obtained by human eyes; the autoPUMP can provide more consistent and precise result for the feeding behavior.

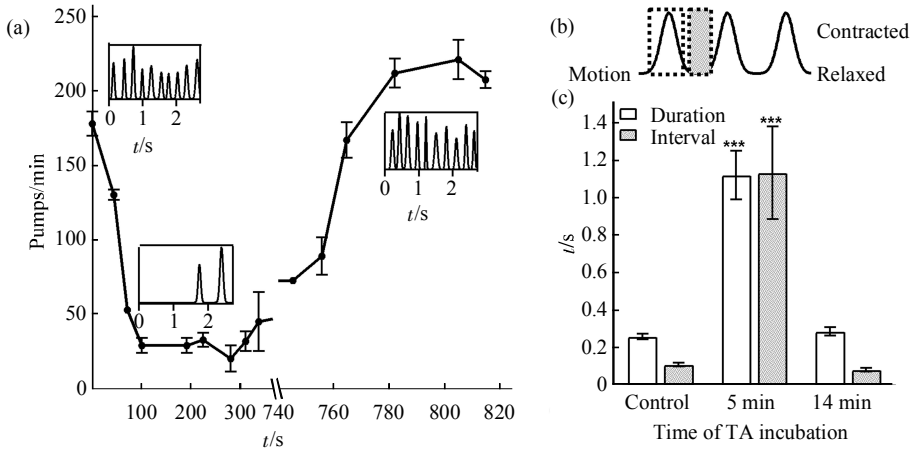

Fig. 4 Pumping wave changes with the treatment of TA

(a) The effect of tyramine incubation on pumping rates. The black lines indicated the changes of pumping rate after tyramine application and the waves in black boxes represent the pumps during the indicated periods. (b) A schematic for Gaussian fitted pumping wave. The transparent box with dashed outline and the dotted filling box with dashed outline are corresponded to the pumping duration and interval, respectively. (c) Tyramine incubation increases both the pumping duration and the pumping interval.

4 Discussions

An alternative way has been developed to quantitatively analyze the feeding behavior of the worm by a simple webcam. The method is accurate, reliable, repeatable and easy to manipulate. With the help of this system, the statistical analysis of pumping rate would not be tedious and time-consuming. The temporal resolution of the system could reach milliseconds and the spatial resolution could reach micrometers. And autoPUMP can get sufficient information under this temporal resolution and spatial resolution. By comparing manually corrected and automatic annotations autoPUMP is able to show high levels of accuracy, with respect to both detection of discrete signals and their correct annotation. Then analysis of the data recorded from wild type worms incubation with pumping antagonist tyramine validate findings as previous reported [11]. The result of the tyramine treatment experiment for wild-type worms demonstrates that the autoPUMP system is reliable with standard behavior assay of *C. elegans*. Although it could be used as a well-working system to facilitate the worm's phenotype analysis, the automatic computer vision algorithm still needs to be improved. For instance, sometimes biologists have to analyze the pumping rate while the worm is seeking for food. The little worm will twist a lot during this behavior. Current template match algorithm in autoPUMP could not cover the twisted situation.

In this paper, it has been demonstrated that the automatic pumping analysis system provides a new tool for the identification and quantitative description of pharyngeal phenotypes. Combined with *in vivo* calcium imaging and optogenetics, the autoPUMP system will be a promising and valuable tool for dissecting the neural circuits of feeding regulation.

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计算机视觉在线虫进食行为自动分析中的应用 *

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摘要 秀丽隐杆线虫被广泛地用作研究基因与行为关系的绝佳模式生物．线虫的咽部神经元回路控制着复杂的进食行为．为了研究进食行为的分子机制，有必要对线虫进食行为表型分析鉴定．然而，目前为止，几乎所有的线虫进食行为表型鉴定都是通过人眼来判断．因为其泵入食物的肌肉运动频率高，该行为的分析是很困难而且效率低下的．为解决这个问题，我们设计了基于计算机视觉技术的自动化成像系统来高通量分析线虫进食行为表型．此成像系统对进食表型的检测准确率达到 98% 以上，并使得连续可靠地分析其表型细微变化成为可能．同时，在保证高准确率的前提下单位时间内分析数据的效率比人工分析提高了 3 倍．

关键词 秀丽隐杆线虫，行为自动分析， 咽部抽动，模板匹配

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