Registration of Regeneration in Planarians from Photographic Images

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Abstract. In this study, an approach to constructing a mathematical model for quantifying the dynamics of regeneration of planarian flatworms in biological experiments is considered, based on an analysis of a series of digital microscopic images. A method is proposed to describe the body shape of a planarian using a continuous morphological model, based on the concept of a medial representation of the worm's silhouette. The silhouette in this case is a polygon approximating the contours of the planarian's body. The medial representation of the figure includes a medial axis and a radial function that describes the width of the figure relative to the medial axis. We propose a set of morphological criteria for assessing the dynamics of regeneration based on a continuous morphological model and present the results of computational experiments. © 2021 Journal of Biomedical Photonics & Engineering.

Keywords: planarians; digital morphometry; medial representation; image skeleton.

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1 Introduction

The progress of modern biology is determined by the development of digital technologies for the creation and analysis of lifetime images of biological objects. A new science of digital biology is being formed as a synthesis of biology and mathematics, which reflects the process of transition of the description of biological objects "from a verbal one to a digital one". The results obtained in this new biological science can be verified by comparing the data generated by different software products, implementing mathematical models, hypotheses, methods and algorithms, on the same group of images.

An example of such a process is the development of a complex of methods for the dynamic morphometry of regeneration in planarian flatworms. Their flat body structure and ability of smooth ciliary movement allow researchers to obtain accurate lifetime images of the planarians that can be analysed by applying various criteria [1–2]. Lifetime photo capture of the regeneration process allows its registration in the same group of animals, which minimises the number of animals required in each experiment and increases the quality of the experiments themselves.

To minimise labour costs and human influence on the outcome, the tools for calculating the required criteria automatically are being developed [3–4]. The latter of these methods are based on the analysis of the planarian shape from images using continuous morphological models [5–6].

Planarians have a unique ability to regenerate: they are able to regrow any lost part of their body in a very short time. After removal of the head end of the body including the nerve ganglion, the regeneration process takes from 5 to 25 days. The special significance of this biological model of regeneration lies in the possibility of extrapolating the processes of cell proliferation and differentiation, and the processes of growth and morphogenesis, at the level of the whole organism [1]. This opens up prospects for the control of cellular processes at the macro level, which makes it possible to

obtain a complete quantitative description of the regeneration process *in situ*.

Owing to their ability to regenerate quickly, planarians are often used as a model test object. However, progress in this area has been limited by the laboriousness of the procedure for creating equivalent experimental groups. Therefore, the creation of a system for the automated determination of the dimensional characteristics of planarians is very urgent.

2 Materials and methods

2.1 Biological model

This study was carried out on planarians of the asexual race *Girardia tigrina*, of which a population has been established since 1970 at the Institute of Biophysics of the USSR Academy of Sciences, and the Institute of Cell Biophysics and the Institute of Theoretical and Experimental Biophysics of the Russian Academy of Sciences at the Pushchino Scientific Centre. The classic format of an experiment on planarian regeneration consists of cutting off the head end of the worm (in the region of the auricles). This operation was carried out with the aid of a Zeiss Stemi 2000 binocular microscope equipped with an AxioCam MRc video camera (1.2 MP) in non-sterile conditions. The process of restoration of the planarian body was observed by the method of lifetime digital morphometry [1–2].

The effect of the biologically active peptide CLV3 (amino acid composition: RTVPSGPDPLHH), a plant growth stimulator isolated from the roots of *Arabidopsis thaliana*, in solutions at concentrations of 10^{-9} M and 10^{-12} M, was studied [7].

Regenerating planarians moving randomly in the aquatic environment were photographed daily using a digital microscope. The body shape of a planarian is well described by a two-dimensional image in a photograph; therefore, a morphological approach to assessing the rate of regeneration based on an analysis of the size and shape of the worm's body is very effective.

After the transection, within 72 h the regrowth of a new head part of the body (regeneration blastema) becomes visible, which is not covered with pigment cells in the first 3–4 days after surgery (Fig. 1). This makes it possible to use the photocontrast between old (pigmented) and new (optically transparent) body parts of the planarian regenerant to determine the regrowth boundary during the interactive process of analysing the planarian image.

In order to measure and analyse the rate of the planarian recovery process, it is necessary to obtain a quantitative criterion that makes it possible to assess how much the parameters of experimental animals have changed over certain time intervals. Previously, the use of software products made it possible to accurately estimate the total size of the growing part of the planarian body and calculate the regeneration criterion as the ratio of the area of the blastema to the total area of the planarian body [1–2]. However, the problem of determining the shape parameters of the growing part of the body during regeneration remained unresolved. This indicator of shape change – that is, morphogenesis – is necessary to describe the growth and morphogenesis processes during regeneration completely. Solving this problem has been complicated by a number of objective factors [2, 5].

The quantitative registration of changes in the body shape of a planarian is complex for the following reasons:

- the body shape differs quite widely in individual worms, and therefore, the process of morphogenesis also has individual characteristics;
- when cutting a worm, it is not possible to ensure absolutely the same position of the cut in different individuals, which also adds individual characteristics to the regeneration process; and
- in the course of regeneration, the overall size of each worm decreases because the restoration of its body occurs through internal transformation of tissue rather than from external nutrition.

Large groups of worms are used in experiments to obtain representative estimates of the dynamics of planarian regeneration. Thus, the task is to assess the dynamics of shape change for a group of 20–30 individuals within 10–25 days, based on observing the regeneration process by registering images in the field of view of the microscope. For the prompt processing of the corresponding array of photographs and calculation of the blastema area, it is necessary to automate this process.

2.2 Method for assessing the morphogenesis of planarian regeneration

In this work, we propose an approach to assessing the dynamics of planarian regeneration, based on the analysis of the shape of flatworms from a sequence of photographs [2, 6, 7]. Within the framework of this

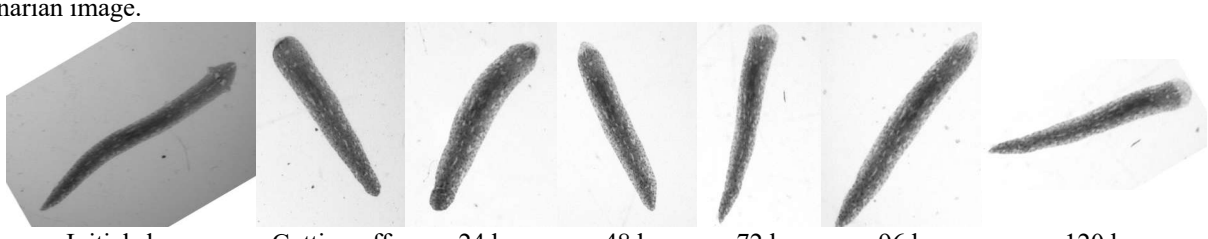
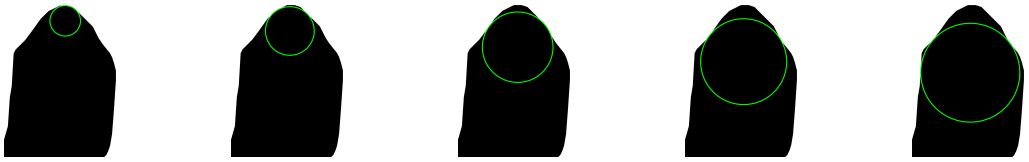


Fig. 1 Intact and regenerating planarians Girardia tigrina at different times after surgery.

24 h 48 h 72 h 120 h 144 h

Fig. 2 Dynamics of regrowth of the anterior end of the regenerative blastema with time elapsed since the head was severed. The size of the images in pixels is specified.



r = 8 px 13 px 18 px 22 px 26 px

Fig. 3 Inscription of circles touching with the border of the body into the blastema.

approach, we developed a criterion for quantifying the rate of change in the shape of planarians, as well as a numerical method for calculating this estimate, based on a sequence of images for the group of planarians we used in our experiment.

The initial data constituted photographic images of planarians, taken daily within 1–2 weeks after the planarians' heads were severed. Because it was impossible to fix the position of the planarians' bodies, the animals were presented in the photographs in arbitrarily bent positions.

The method we proposed was to construct a so-called morphological model of the planarian body based on the medial representation of its shape as a figure with a skeleton and radial function [8–9]. A skeleton is a set of medial axes in a figure, consisting of centres of all the circles inscribed in the figure. The radial function is defined at each point of the skeleton and is equal to the radius of the inscribed circle centred on that point.

The morphological model was designed to quantify the rate of regeneration. In the course of regeneration, the "regrowth of the head" of the planarian takes place. In particular, this process is expressed in a change in the shape of the head end of the worm. Fig. 2 shows how the shape of the head end changes. It is clearly seen that, over time, the change in shape is expressed in the elongation of the head, the formation of a pointed apex – apex of the blastema – as a result of which the head becomes more and more triangular. The morphological model was designed to assess the geometric characteristics of the planarian's body: the elongation of the head end and the "triangularity" of the head.

To register the degree of regeneration as a quantitative indicator, we needed to establish geometric criteria that would take into account the degree of sharpening, or increase in acuteness, of the angle of the blastema apex. This quantitative indicator had to be sensitive to the change in the angle during the regrowth of the worm's head.

3 Morphological model and criteria

3.1 Morphological model of planarian shape

The idea of constructing a morphological model is shown in Fig. 3. Circles with two or more points of contact with the boundary of the planarian's silhouette are inscribed in the contour of the worm's silhouette, and the points of contact lie within the area of the worm's head.

The inscribed circles can be of different sizes, determined by the radius r. In accordance with these dimensions, the circles are located at different depths relative to the apex of the blastema – the end point of the head. We used two functions as an indicator of head elongation: the area of the apex of the blastema $S(r)$ and the length of the apex of the blastema $L(r)$. The meaning of these values is illustrated in Fig. 4.

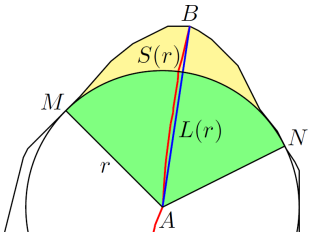
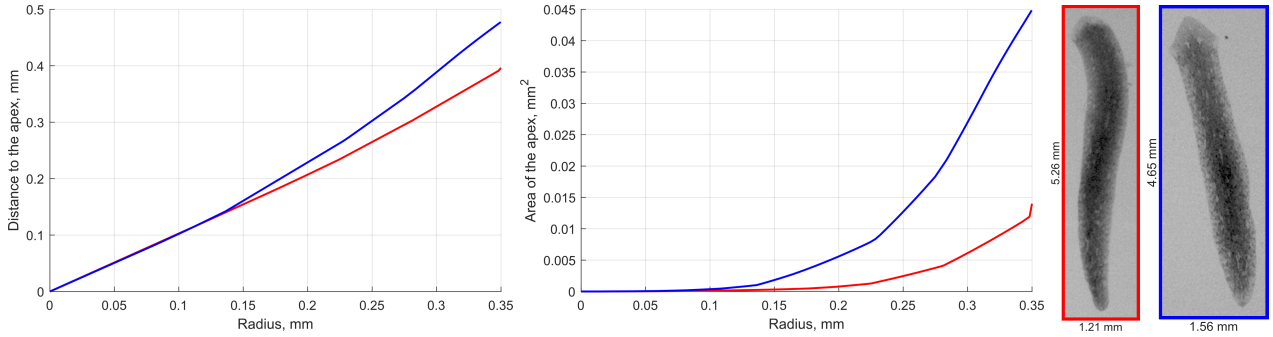


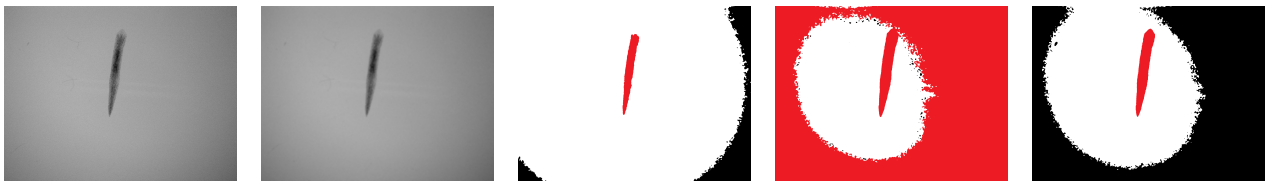
Fig. 4 Scheme of the morphological model.

To determine the value of the function describing the area of the blastema apex at point A, we calculated the tangency points of the circle centred on A inscribed in the boundary polygon. In Fig. 4, these are points M and N. Using points A, M and N, as well as the vertex of the blastema B and the vertices of the boundary polygon, we calculated the area of the polygon $AMBN$, and that of the



(a) (b) (c) (d)

Fig. 5 Dynamics of the length and area functions of the blastema apex $L(r)$ (a) and $S(r)$ (b) respectively and the source images (c, d). The colour of the image frames corresponds to the colour of the chart lines.



(a) (b) (c) (d) (e)

Fig. 6 General sequence of operations for constructing a morphological model of the length and area of the blastema apex. See the list above for a description of each stage.

circular sector AMN, shown in the Fig. 4 in green. The area of the apex of the blastema $S(r)$, shown in yellow in the Fig. 4, is equal to the difference between these two areas. The length of the apex of the blastema $L(r)$ is equal to the distance between points A and B and is shown in blue. The main axis (locus of the inscribed circles' centres) is shown in red.

The functions of the length and area of the blastema apex $L(r)$ and $S(r)$ are monotonic; examples of their plots are shown in Fig. 5. Here, the functions are constructed for two images of planarians. The image in the red frame was taken on the second day of regeneration, and the image in the blue frame was taken on the ninth day. The left diagram shows the plots of the blastema apex length, and the right diagram shows the area of the blastema apex. It can be seen clearly from the diagrams that the blue curves describing the later stage of regeneration dominate the red curves belonging to the earlier stage. This is quite consistent with our visual observation of the blastema apex's elongation and the resulting increase in its length and area.

The general sequence of how we segmented (selected) each planarian image – i.e., built a binary silhouette of the planarian – is as follows and is shown in Fig. 6:

a) obtaining a grayscale image containing the individual planarian;

b) smoothing this image with a Gaussian filter with standard deviation $\sigma = 3$;

c) applying threshold binarization to the image so that 10% of the pixels turn black and determining the base of the connected component of the planarian – the maximum connected component that does not touch the frame boundaries (red area);

d) determining the limit value of the threshold t_{max} by increasing the threshold for binarization until the connected component of the planarian touches the frame borders; and

e) applying binarization to the image with a threshold of $t_{max} - 4$ and selecting of a connected component containing the base.

The general sequence of how we built the morphological model of a planarian is as follows and shown in Fig. 7:

a) approximating the binary silhouette of the planarian (Fig. 7a) by a polygon (Fig. 7b);

b) building the skeleton and radial function of the polygon (Fig. 7c);

c) pruning the skeleton – i.e., cutting off insignificant branches – according to the algorithms from Ref. [8] with maximal possible deviation of 20 pixels from the original silhouette (red line in Fig. 7d);

d) searching for potential apexes of the blastema – i.e., constructing the rays tangent to the end vertices of the pruned skeleton (dashed lines in Fig. 7d) and determining contour points with the most distant projections on these rays (blue points);

e) completing the pruned skeleton to the main axis of the planarian by paths to the potential apexes in the non-pruned skeleton and determining the path lying in the head by the greater average radius $\frac{\int r(l)dl}{\int dl}$ (Fig. 7e); and

f) determining the reference circle by moving along the pruned skeleton from the terminal vertex in the head (where its radial function is r_{cut}) to a distance of $0.2 * r_{cut}$.

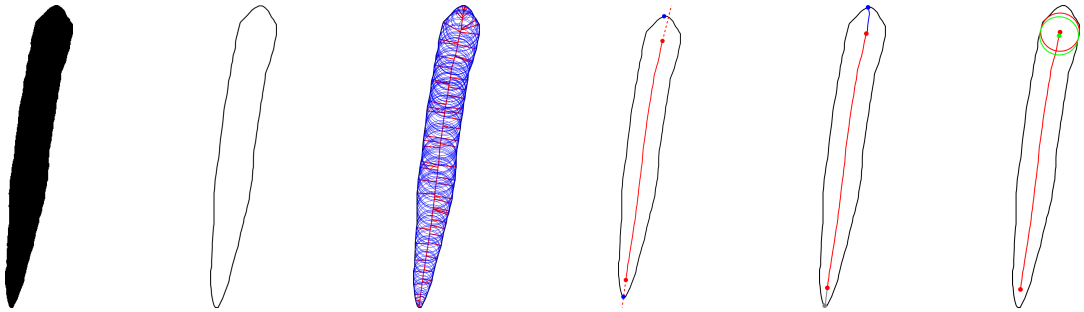


Fig. 7 General sequence of operations for constructing a morphological model of the length and area of the blastema apex. See the list above for a description of each stage.

The length and area functions of the blastema apex $L(r)$ and $S(r)$ were calculated using a continuous morphological model. Further, these functions were normalized by taking into account the radius of the reference circle $\bar{r}$: $\tilde{L}(\rho) = \frac{L(\rho\bar{r})}{\bar{r}}$, $\tilde{S}(\rho) = \frac{S(\rho\bar{r})}{\bar{r}^2}$. Note that after normalization, the arguments and the values of these functions become dimensionless quantities (arbitrary units). The constructed morphological model was further used to determine the first point with the given radius on the main axis, following from the apex. Since each edge of the skeleton consists of points equidistant from two sites (edges or concave vertices) of the boundary, this model allowed us to directly find tangency points M and N, as shown in Fig. 4, and to estimate the length and area of the blastema apex from the change in the radial function along the axis. The procedures described above were implemented in C++, MATLAB was used to analyse the results.

3.2 Morphological evaluation of a group of planarians

The functions $L(r)$ and $S(r)$ were calculated sequentially at discrete points of the axis, selected with a certain step. Thus, the functions of the length and area of the blastema apex were calculated for each planarian from the group. Group functions could be obtained as averages over a group of m planarians:

$$\hat{L}(\rho) = \frac{\sum_1^m \tilde{L}_i(\rho)}{m};$$
$$\hat{S}(\rho) = \frac{\sum_1^m \tilde{S}_i(\rho)}{m}, \quad (1)$$

where $\tilde{L}_i(\rho)$ and $\tilde{S}_i(\rho)$, $i = 1, \ldots, m$ are the normalized length and area of the blastema apex of the i-th planarian from the group. Planarians were photographed in the resolution of 97 pixels per mm (2413 ppi), the sizes of individuals varied in the range of 2 to 6.7 mm (length) and in the range of 0.9 to 3.6 mm^2 (area). The diagram in Fig. 8 illustrates some of the experimental results.

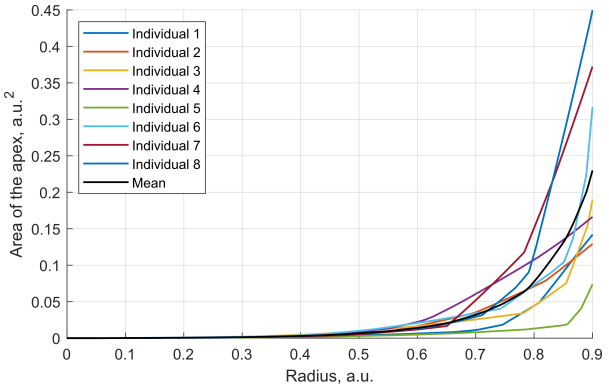
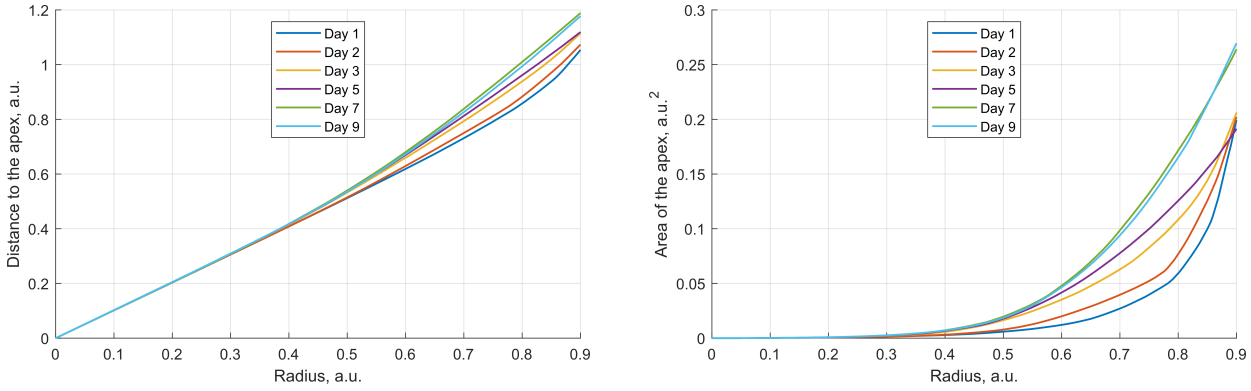


Fig. 8 Diagram showing the dependence of the area of the blastema apex on the radius of the inscribed circle r, for different individuals from the same group.

As can be seen from the diagram, these curves for individual planarians vary significantly. However, the average curves calculated from them, which characterize a group of planarians, made it possible to assess the main quantitative regularities of the regeneration process. This can be seen in the two diagrams in Fig. 9, which show integral curves for the same group of 20 planarians, plotted from images in the first 9 days after regeneration. The curves show the growth of the planarians' heads and provide quantitative estimates of this growth.

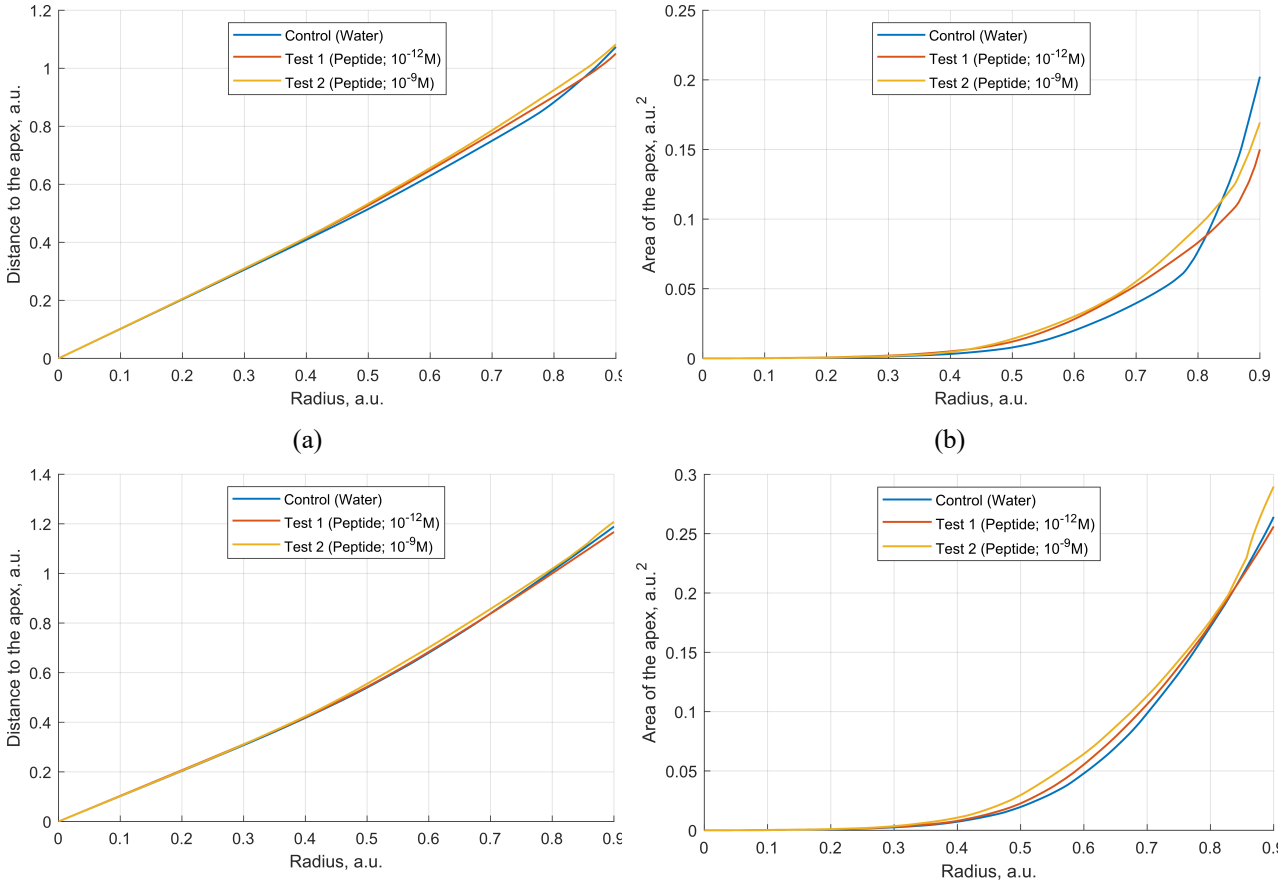
In Fig. 10, the diagrams show the results of our experiments to assess the rate of regeneration of planarians placed in various media. Groups of 20 planarians each were studied in pure water (control group) and in plant growth stimulant pCLV3 peptide solutions at concentrations of 10^{-9} M and 10^{-12} M (test groups). All graphs are based on images taken on the third day of the regeneration process. These diagrams confirm that it is fundamentally possible to assess the dynamics of planarian morphogenesis, which are dependent on various external physical and chemical factors. Our approach makes it possible to obtain reliable quantitative estimates of this dependence.

The graphs are consistent with the hypothesis that the plant growth stimulant pCLV3 peptide also stimulates the regeneration of lost tissue in planarians [10].



(a) (b)

Fig. 9 Integral curves of the length (a) and area (b) of the blastema apex for the same control (without peptide) group of 20 planarians, plotted from images in the first 9 days of regeneration.



(a) (b)

(c) (d)

Fig. 10 Integral curves of the length (a, c) and the area (b, d) of the blastema apex for the control group and two experimental groups of 20 planarians each, plotted from images on the 2nd (a, b) and 5th (c, d) days of regeneration.

4 Conclusion

The results of this study demonstrated that it is fundamentally possible to quantify the rate of regeneration in flatworms from a series of images. The method we developed for obtaining such estimates is based on the morphological analysis of images: segmentation of the body of a planarian, approximation of the silhouette by a polygon with the highest possible accuracy for a digital image, obtaining a medial representation of the silhouette and calculating curves based on it, and showing the dynamics of changes in the worms' body shapes for different periods of time elapsed since the beginning of the regeneration process. The quantitative estimates we obtained allowed a clear and meaningful interpretation, which in turn made it possible to assess their reliability. The method we proposed was

based on the construction of a continuous morphological model, which made it possible to ensure high computational efficiency of this solution and convenient, meaningful visualisation. The approach described here is being implemented by means of corresponding software and has found application in practical research. Further development of non-contact, non-invasive approaches to assessing the processes of morphogenesis of planarians can be possible using such optical methods as hyperspectral technique [11] and Raman spectroscopy [12], which are now widely used to analyse the state of biological tissues.

Disclosures

All authors declare that there is no conflict of interests in this paper.

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References

1. K. P. Tiras, V. I. Khachko, "Criteria and stages of regeneration in planarians," Ontogenez 21(6), 620–624 (1990) [in Russian].
2. K. P. Tiras, O. N. Petrova, S. N. Myakisheva, A. A. Deyev, and K. B. Aslanidi, "Minimizing of morphometric errors in planarian regeneration," Fundamental Research 2(7), 1412–1416 (2015) [in Russian].
3. S. Flygare, M. Campbell, R. M. Ross, B. Moore, and M. Yandell, "ImagePlane: an automated image analysis pipeline for high-throughput screens using the planarian Schmidtea mediterranea," Journal of Computational Biology 20(8), 583–592 (2013).
4. N. Macleod, "On the Use of Machine Learning in Morphometric Analysis," Biological Shape Analysis: Proceedings of the 4th International Symposium, World Scientific Publishing, 134–171 (2017).
5. L. M. Mestetskiy, K. P. Tiras, "Morphological assessment of the dynamics of regeneration of planarians from photographic images," Mathematical Methods for Pattern Recognition 18(1), 120–121 (2017) [in Russian].
6. K. P. Tiras, L. M. Mestetskiy, "Normalization of planarian images by the fat curve method in biological research," in 17th International Conference on Mathematical Methods for Pattern Recognition, Svetlogorsk, 19–25 September 2015, 182–183 (2015) [in Russian].
7. K. Tiras, U. Vorobeva, K. Novikov, V. Voeikov, V. Emelyanenko, G. Davidova, and A. Skripnikov, "Peptide regulation of plant and animal morphogenesis: general mechanisms and specificity of action," IOP Conference Series: Materials Science and Engineering 487, 012020 (2019).
8. L. M. Mestetskiy, Continuous morphology of binary images: figures, skeletons, circulars, Moscow, Fizmatlit (2009) [in Russian]. ISBN: 978-5-9221-1050-1.
9. R. Gonzalez, R. Woods, Digital Image Processing, 4th ed., Pearson, India (2018).
10. X. Wang, M. G. Mitchum, B. Gao, C. Li, H. Diab, T. J. Baum, R. S. Hussey, and E. L. Davis, "A parasitism gene from a plant-parasitic nematode with function similar to CLAVATA3/ESR (CLE) of Arabidopsis thaliana," Molecular Plant Pathology 6(2), 187–191 (2005).
11. V. P. Sherendak, I. A. Bratchenko, O. O. Myakinin, P. N. Volkhin, Y. A. Khristoforova, A. A. Moryatov, A. S. Machikhin, V. E. Pozhar, S. G. Kozlov, and V. P. Zakharov, "Hyperspectral in vivo analysis of normal skin chromophores and visualization of oncological pathologies," Computer Optics 43(4), 661–670 (2019).
12. L. Bratchenko, I. Bratchenko, Y. Khristoforova, D. Artemyev, Y. Konovalova, P. Lebedev, and V. Zakharov, "Raman spectroscopy of human skin for kidney failure detection," Journal of Biophotonics 14(6), e202000360 (2020).