Lab 2 report

Trichotomatic: a pipeline to analyze moving objects in a moving environment

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Trichotomatic:

Analyzing moving objects in a moving environment

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Abstract (999 characters)

Trichoplax adherens (Trichoplax) is a marine organism from the Placozoa phylum, composed of two epithelial layers delimiting a space containing fibrous cells. Surpringly, Trichoplax displays properties such as wound healing and directed motion without the presence of a coordinating nervous system. Studying these properties is done using light microscopy techniques mainly because of their affordability and the ability to record over time. Such experiments produce large amount of data and need rigorus processing. In this report we introduce the **Trichotomatic** pipeline: a pipeline to analyse Trichoplax in a moving environement. We show how we implemented a preprocessing module in order to increase the contrast of bright field microscopy images. Furthermore, we show how we implemented a drift-detecting module to track the organism precisely over time. Finally we show how we implemented an intensity-based segmentation module and discuss the type of data the pipeline is capable of producing.

Keywords: Trichoplax adherens, image analysis, segmentation, tracking

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Introduction

First described in 1883 by the German zoologist Franz E. Schulze, *Trichoplax adherens* (Trichoplax), a flat crawling marine animal, is one of the three members of the Placozoan phylum [1]. The name comes from the Greek words θρίξ, *trich* = hair and πλάξ, *plax* = plate. Indeed, its epithelial surface is composed of ciliated 'hairy' cells (annex 2). Trichoplax displays the property of wound healing and seems to present collective cell movements[2]. These fascinating properties pose exciting questions, since they can be linked to cellular organization. Recently, Zhong et al. showed a thermotaxis behavior of Trichoplax without the evidence of a neural system[3]. Showing such property implies a tracking of the organism. In a more technical term this process implies to actualize coordinates for each given time points. This process requires a clear and precise analysis framework.

Experimentally, probing the movement of Trichoplax poses technical challenges:

- Firstly, Trichoplax strains stably expressing immunofluorescent markers are currently unavailable making most highly resolutive live imaging approaches impossible.

- Moreover, imaging a moving object implies that the object needs to stay in the field of view of the microscope. This leads to the need for a large field of view to limit camera movements and thus impacts the resolution.

Combining these difficulties, experiments are usually carried out using bright field microscope over time. These experiments yield temporal data that require segmentation and tracking. Segmentation is a classification problem where pixels in an image need to be categorized. From this classification one can compute properties of the segmented objects. In the case of tracking, we need to have information on the position in space and time of the objects. Such process cannot be performed by hand due to the large amount of data. However, segmentation and tracking are still not trivial to compute for a machine. In this report we demonstrate one approach to the problem and present its limitations. In the end we show how we developed **Trichotomatic,** a Python-based pipeline for the segmentation, tracking and quantification of Trichoplax movements and wound closure. We describe the different modules and present preliminary results for its usage. A summary of the pipeline can be found in Fig. 1.

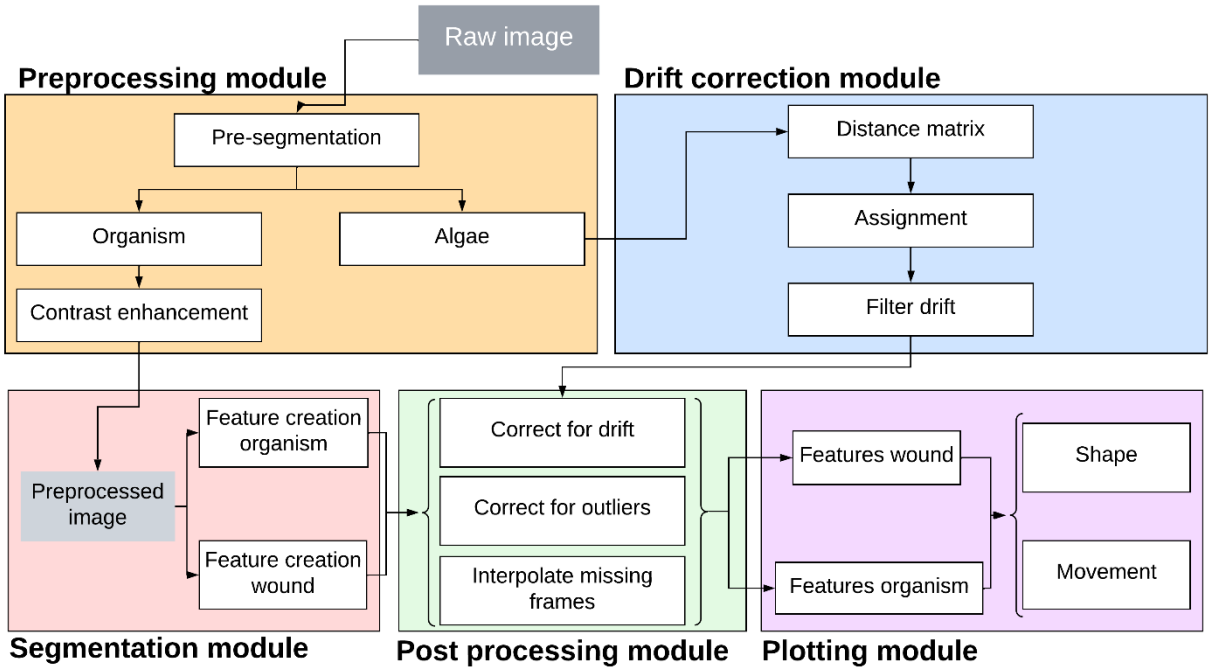

Figure 1: Graphical representation of the Trichotomatic analysis pipeline
A more detailed version can be seen in annex 1.

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Methods

Culture, manipulation and imaging of Trichoplax

Trichoplax of the strain H2 are maintained in artificial sea water (33g/liter commercial sea salt from Instant ocean in distilled H_2O) and fed with unicellular algae (*Rhodomonas salina*, *Nannochloropsis oculata* and *Dunaliella salina*). Individual animals are then placed between two coverslips in a drop of their culture media with algae. The images were acquired on a commercial Olympus bright field microscope using a magnification of 10x. The movies were recorded for a period of 15 minutes at a rate of 3 frames per second. The wounding was performed

using a high intensity laser for 1 second. The width of the created wound is approximately 200µm, corresponding to roughly 1/10 of the Trichoplax diameter.

Preprocessing module

An initial segmentation on the image is performed. The connected components are ranked by size and the largest component is used to create the preliminary organism mask. The other components are labeled as the algae mask. The algae that are not present on the organism mask are replaced by the average background intensity value, allowing for a global smoothening of the image. Finally, a contrast stretching is implemented.

Drift computation/correction module

The drift is computed using the algae mask. We compute the drift as the distance between an object at frame t and the same object at frame t+1. The algae are identified then paired between time points and finally a filter is applied to define a "drift". The assignment step consists of matching every point in a frame to its correspondence in the next frame. In order to match all the points between two consecutive frames we make the hypothesis that two algae close in space between two consecutive frames are the same. This hypothesis implies building a distance matrix and minimizing the sum of a distance function. A distance matrix computes a metric between each point in an image at frame t and every point in the next frame. The metric chosen for the pipeline is the 2D Euclidian distance, as described by eq 1.

$$d = \sqrt{(x_{(t+1)} - x_t) + (y_{(t+1)} - y_{(t))}} \qquad \text{(eq 1)}$$

A threshold has been set to avoid missalignements: computed distances above a certain value are attributed an extreme value to guide the assignement. The threshold value is set as a parameter and is estimated from the movement of the camera during an experiment. The assignment is done via the minimization of the cost function described by eq 2.

$$D^* = \underset{D \in \mathcal{D}}{\operatorname{argmin}} \sum_{(d_i, d_j) \in D} ||d_i - d_j|| \qquad \text{(eq 2)}$$

The assignment produces a list of optimal matches that is then used for determining drift. We compute a distance between the matched points (centroids) for the two different coordinates using eq 2 and 3:

$$dx = x_{(t+1)} - x_{(t)} \qquad \text{(eq 3)}$$

$$dy = y_{(t+1)} - y_{(t)} \qquad \text{(eq 4)}$$

We define the drift as being a movement of at least three pixels for more than four consecutive frames. This criterion was set using experimental data and can be adjusted as a parameter. A sliding window is performed in order to gather the frames that match the criteria. A dataframe is created containing information on the displacement vector: the direction of the displacement, the displaced coordinate and the amplitude.

Segmentation/ plotting module

The segmentation was performed using intensity-based methods. Two different methods have been implemented: Otsu algorithm and Chan-Vese algorithm [4], [5]. Feature extraction was preferably performed using Chan-Vese algorithm for better performances in edge detection. The feature extraction was performed using the scikit image package [6]. The pre-segmentation step during preprocessing was performed using the Otsu algorithm for faster

computation time. The plotting was performed in python using the matplotlib-pyplot and seaborn packages [7], [8].

Postprocessing module

The centroids of the segmented objects are corrected for the planes where drift was detected according to the drift dataframe. A sliding window on the area of the objects is implemented to remove outliers and segmentation errors. The values superior or inferior to a certain threshold to the mean of the sliding window are averaged. Finally, the wound properties are interpolated from the data using an univariate spline algorithm from scippy [9].

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Results

The pipeline matches and improves manual adjustments of the image

Trichotomatic aims at automating the analysis process of microscopy data. This objective poses a fundamental question that allowed to quantify the development of the pipeline: how much does the pipeline improves the analysis?

To address this question, we need to compare metrics to standard values. We constructed an image adjusting the contrast manually for such comparison. The contrast was adjusted using the Fiji software for each frame in order to increase details and the resulting performance of the segmentation [10]. We computed different metrics of the organism and compared the results. The results can be seen in Fig 2.

Panels A to C show various properties extracted from the segmented object. We see that the results compare with the reference. This shows that the pipeline is able to match the performance of a “manually adjusted image”. Another metric we used was the absence of the wound as a feature of the image. To quantify this metric we plotted the area of the wound for the planes in which the wound was detected. We see gaps in the data both for the reference and the pipeline. However, we see that the movie processed by the pipeline was able to segment the algae in more planes (24% more planes). Moreover, we see that compared to the reference the pipeline shows smaller interval of missed planes, suggesting that that the missing planes correspond to individual frames rather than large interval.

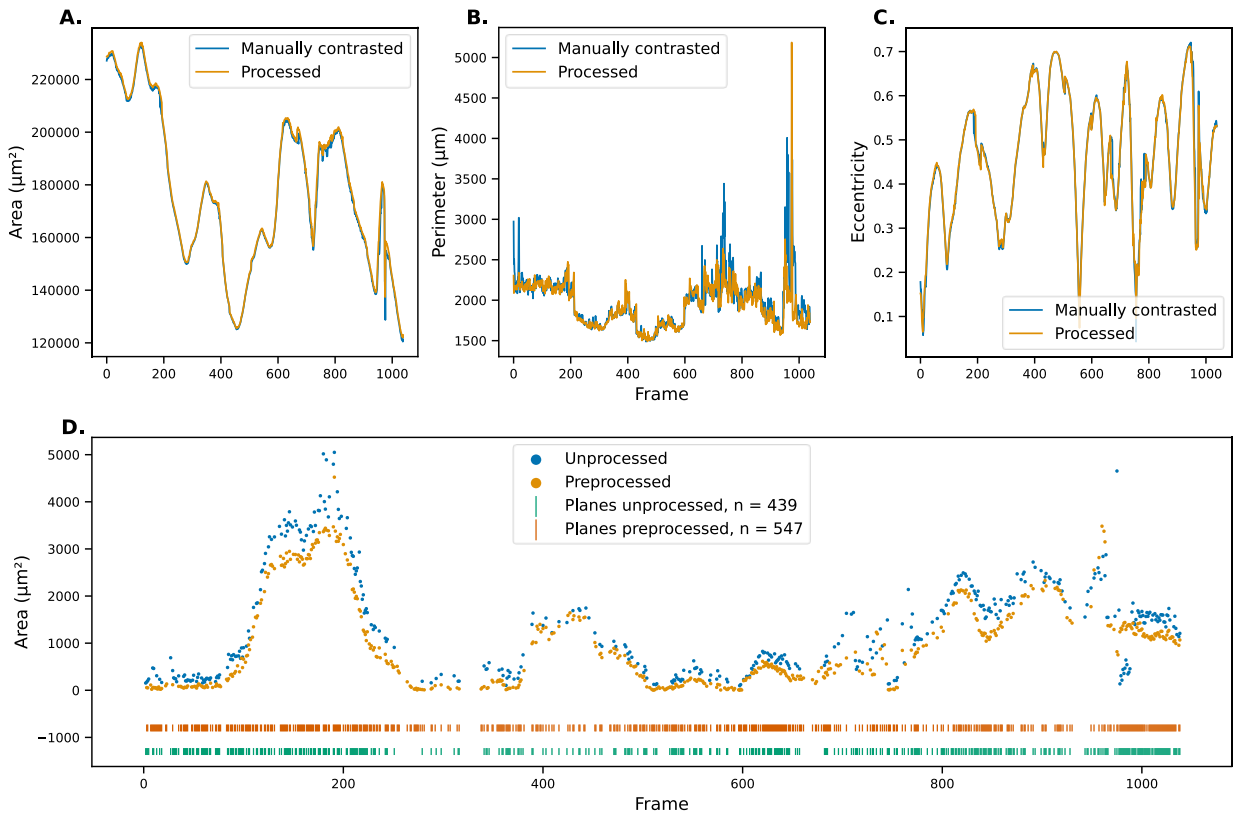

Figure 2: Trichotomatic matches manually improved data

A-C Different metrics extracted from the segmentation of Trichoplax using a manually contrasted movie (in FiJi, blue curve) and a preprocessed movie (in gold). The metrics are respectively: area, perimeter and eccentricity.

D. Area of the wound mask in the manually enhanced movie (blue) and a processed movie (orange). The straight colored lines represent a projection of every point on a line (green: manually enhanced movie, red: processed movie)

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Accounting and correcting for the movements of the camera

Following a moving organism means actualizing its position over time. However, following a moving organism in a moving environment means separating the two kinds of movement to account only for the organism movement. When acquiring an image, we place the image on the microscope referential. However, the organism moves in another referential: the liquid media. Indeed, the two referentials can be the same, but only if the field of view is static. In our experimental setup, the organism often moved out of the field of view, implying that the field of view had to be moved. In this configuration, our statement of the two referentials being identical becomes false. When the camera moves, a new referential is set. In order to correct for this, we designed a module to detect these camera movements on the image and correct for them.

We name the camera movements “drift” for easier understanding. Correcting for drift implies to perform a set of operations on the image: the identification of an indicator of the drift, the measurement of this indicator and the filtering of the drift. In the case of Trichotomatic, we used as a drift indicator the algae, present on all frames. Since these are static objects, their coordinates are static as well. If put in a new referential, these coordinates change and can thus be detected. However, if a static object moves between frames its detection means knowing the coordinates of this specific point in the two frames. For one point this question is trivial, nonetheless for an image with a dozen of algae this becomes problematic. Each object in a frame needs to be matched to the same object in the following frame. The matching allows then to compute a distance between matched points in consecutive frames.

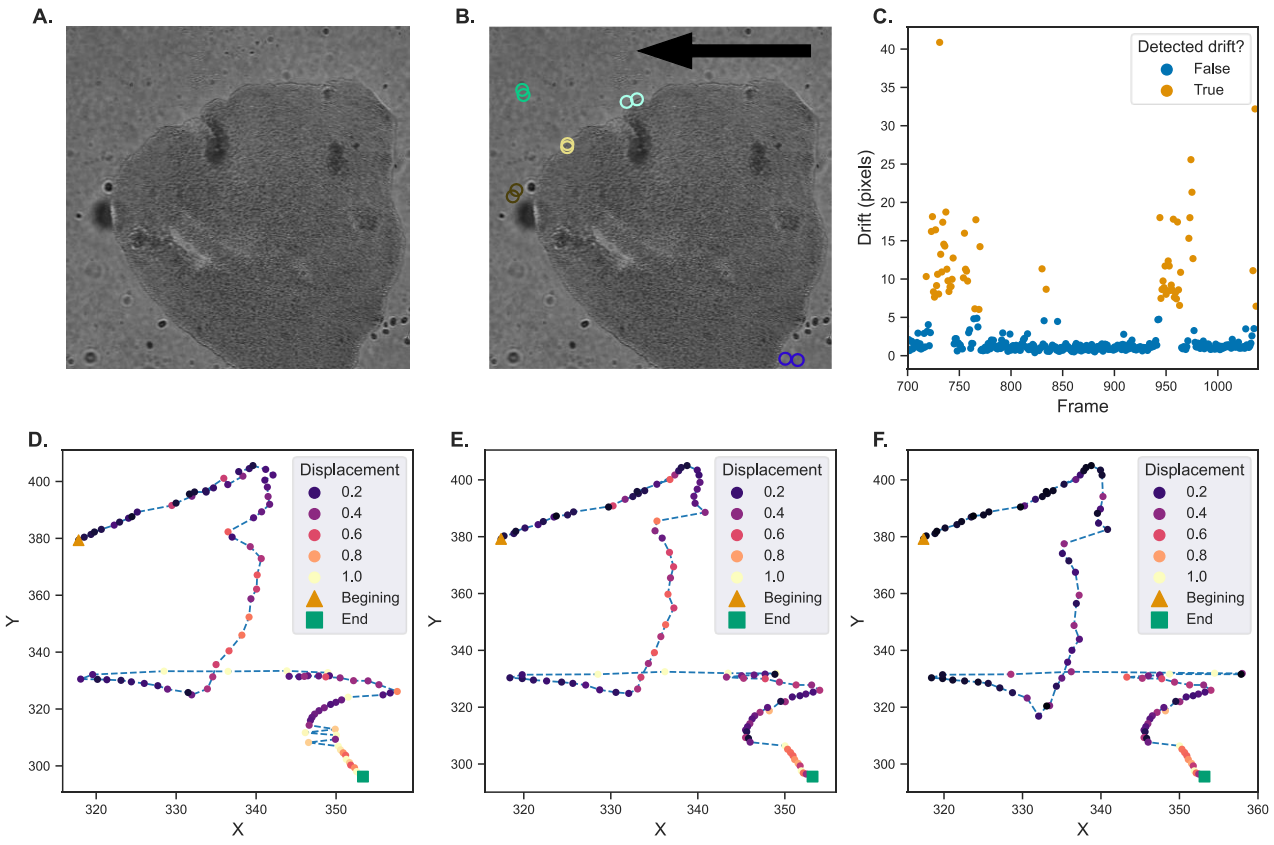

Figure 3: Correcting the camera movements

A-B. Illustration of the effect of the camera movements on the image. In B., the colored circles represent the algae used to compute the drift, colored according to their assignment. The black arrow represent the direction of the movement C. Criterion to declare a movement drift. D-F Trajectory of Trichoplax for respectively raw data, pipeline-corrected data and manually corrected data. The trajectories are colored according to the displacement between the frame and the previous frame as an euclidan distance.

A visual example of drift can be seen in fig 3 A and B. The final problem of computing such drift is distinguishing a real drift movement from the set of point distances between two consecutive images. To solve this problem, we implemented a thresholding criterion where ‘drift’ is defined as a movement that is continuous across multiple frames and has a certain amplitude.

Finally, these drift measurements were used to correct the trajectory. We replaced every moving point to the same referential but considering its displacement. An example of correction can be seen in fig 3 D. To visualize the correction, we colored the trajectory of the organism according to its displacement between the

considered frame and the previous one. In the case of a camera movement this displacement is high as a new referential is set. We see that the pipeline changes the displacement value of certain points as they were replaced into the correct referential. In order to estimate the accuracy of the correction we computed drift on an image by sampling algae by hand and computing their coordinates. This manually annotated data constitute a ground truth in order to validate the pipeline. We see that the pipeline doesn't match the ground truth correction thus implying an error on the correction.

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Error measurement and limitations of the pipeline

How to trust a result coming from a pipeline? Following the previous result, we observe that the pipeline doesn't match the ground truth dataset. In this section we detail the approach we took concerning error estimation and current limitations of the pipeline. We focus here on the error of the drift measurement and correction. We chose to focus on this error as it impacts a lot of downstream operations in the pipeline.

We chose to estimate the error using the ground truth dataset. We computed a cumulative error by computing the difference between the computed value and the corresponding ground truth value then adding over all planes. We also computed an absolute value of the error in order to have an average error. The output of these operations can be seen in fig 4.

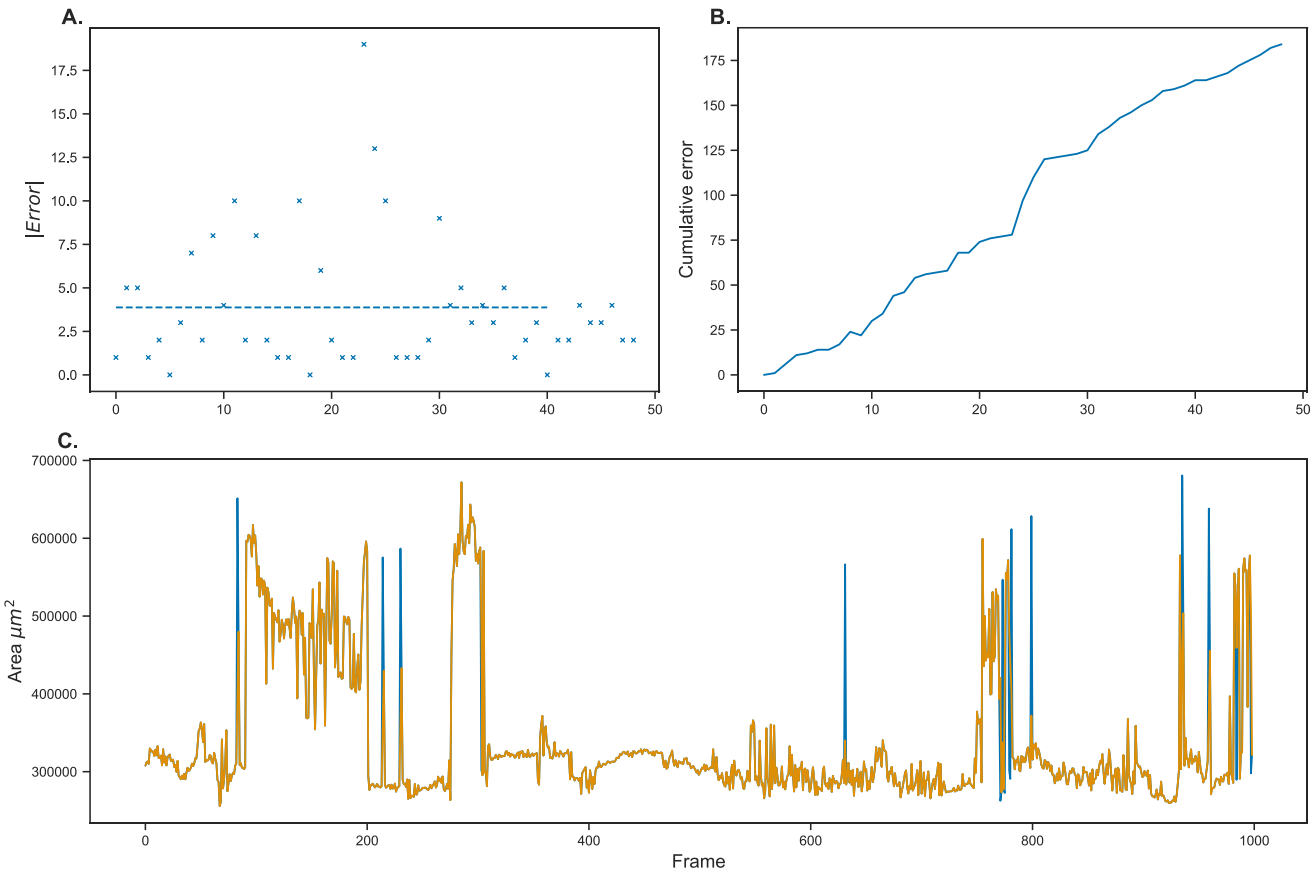

Figure 4: Computing the pipeline error and limitations

A. Distribution of the absolute value of the error on the drift computation. The dotted line represents the average. B. Cumulative error for the entire drift computed in the movie. C. Area over time of Trichoplax. In blue the data output from the segmentation and in gold the data that have been post processed.

From panel A we see the cumulative error. We see that this error reaches the value of 130 pixels for all the planes considered. This value corresponds to about 25 % of the image size. When looking at panel B we see that the mean error is relatively low at around 4 pixels. However, we observe the presence of outliers in the data (points at 10 pixels in error) for some planes.

Finally, we looked at the robustness of the pipeline to estimate its limitations. Panel C shows the area of Trichoplax coming from an experiment that was not used to develop the pipeline. We see that the post-processing and the quality of the data are relatively poor. This high error might be due to a combination of factors in the pipeline.

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Toward quantification of the Trichoplax movements.

We saw during this report the general performances of the analysis pipeline and its current limitations. In this section we discuss the possibilities of quantification to understand the process of wound closure in Trichoplax.

From the different modules of **Trichotomatic** we can extract multiple information. How to extract such information? We implemented a segmentation module which allows us to gather a mask: a region defined as the organism for every time frame. This mask can then be used to compute properties and features of the object. In this report we showed that we can compute Trichoplax shape descriptors: area, perimeter and eccentricity. In the case of wound closure these properties can be correlated between the wound and the organism as shown in Figure 5, panels B and C. We observe a periodicity in the wound area over time that can be related to the process of closure.

Furthermore, **Trichotomatic** is able to give another type of descriptor: movement. As we are able to track over time the organism and the wound we can compute movement descriptors. We see in Fig 5, panel A and B the trajectory represented. In panel A, the trajectory is colored by frame number showing the movement over time in the 2 coordinates. In panel B we colored the trajectory according to the ratio wound area/organism area. We observe that upon changes of direction the color intensifies indicating a higher wound/organism ratio. This information could be related to other type of experiments in order to propose a definite model of wound closure and movement.

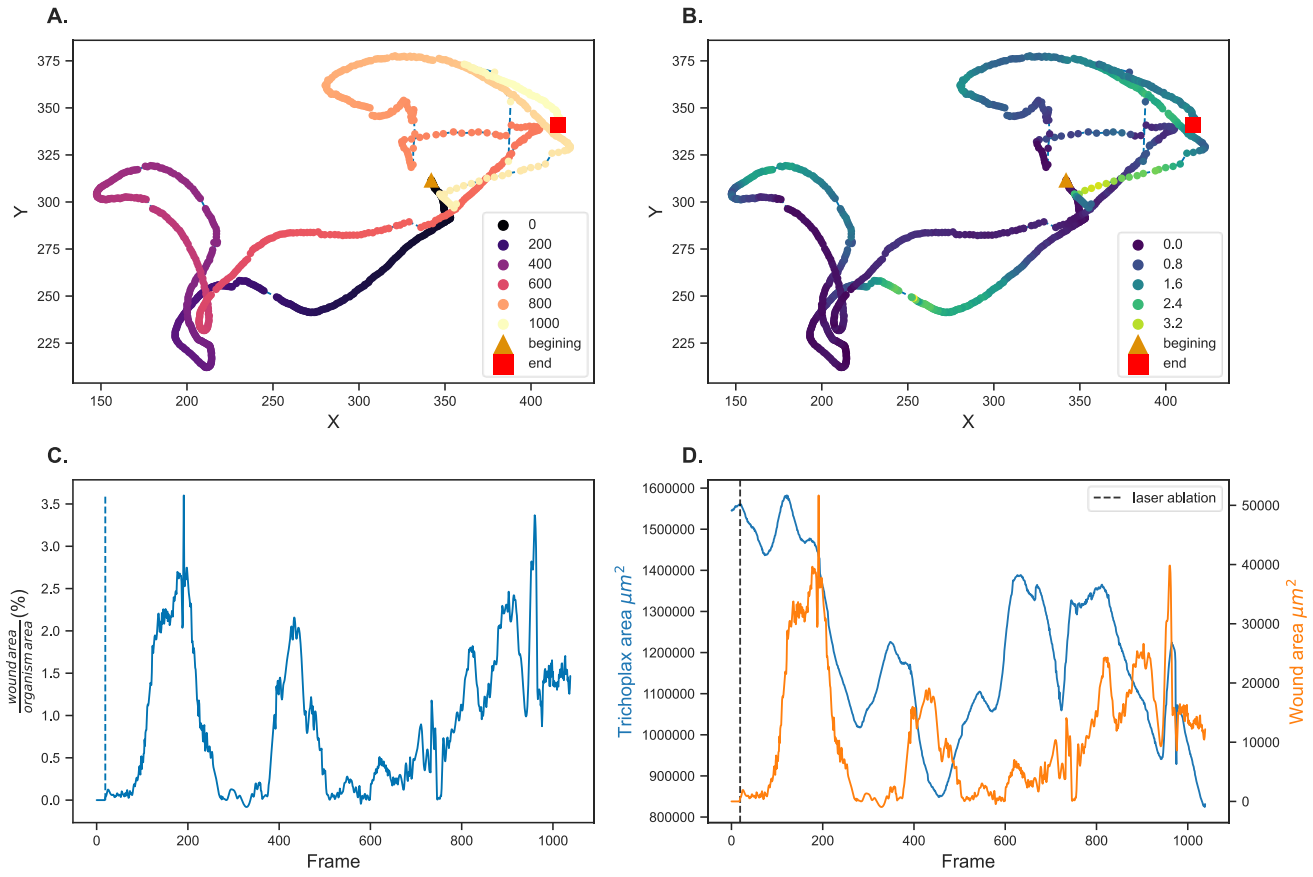

Figure 5: Towards quantification using Trichotomatic

A. Trajectory of T.adherens colored by frame number. B. Trajectory of T.adherens colored by the ratio wound area over organism area. C. Ratio wound area over organism area over time. The dotted line represent the wounding frame. D. Evolution of the area of the wound (orange) and the organism (blue) over time (in µm²).

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Discussion

Throughout this report we showed how we developed the **Trichotomatic** pipeline and its usage. We showed that **Trichotomatic** has a variety of application and is very flexible in the kind of information it provides. Furthermore, as **Trichotomatic** is an open source project it can be easily tailored to fit different kinds of experiments and purposes.

The pipeline was designed to be easy to use for experimentalist while remaining flexible to various biological questions. In this report we presented the major building blocks. A preprocessing step to increase the contrast of the image and thus maximize the performance of the segmentation is followed by a module to detect the camera movements and correct the trajectory.

Furthermore, we discussed how we computed an error for the drift computation and we discussed the current limitations of the pipeline. Mainly, we hypothesize that the major reason for the high error observed comes from the matching of objects between consecutive frames. This failure might be linked to a failure of segmentation. Further optimization of the segmentation parameters could improve the general performance of the

pipeline. In addition, machine learning approaches could be implemented in the pipeline in order to obtain more reliable results in the segmentation.

Finally, we showed plausible applications of the pipeline on Trichoplax experiments. The segmentation can enable to obtain shape descriptors for Trichoplax such as area, perimeter, eccentricity. These parameters could also be applied to the wound in the organism and could provide insights on the underlying wound closure mechanism. Movement descriptors, such as trajectory, can also be extracted. All of this framework could also be linked with different kinds of experiments at different scales in order to address larger biological questions and go beyond the current pipeline limitations.

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Code availability

The complete pipeline is implemented within the placozoa package developped during the 2022 Centuri Hackathon. The code used to produce the figures can be found on Github. The package is under an opensource license BY-NC.

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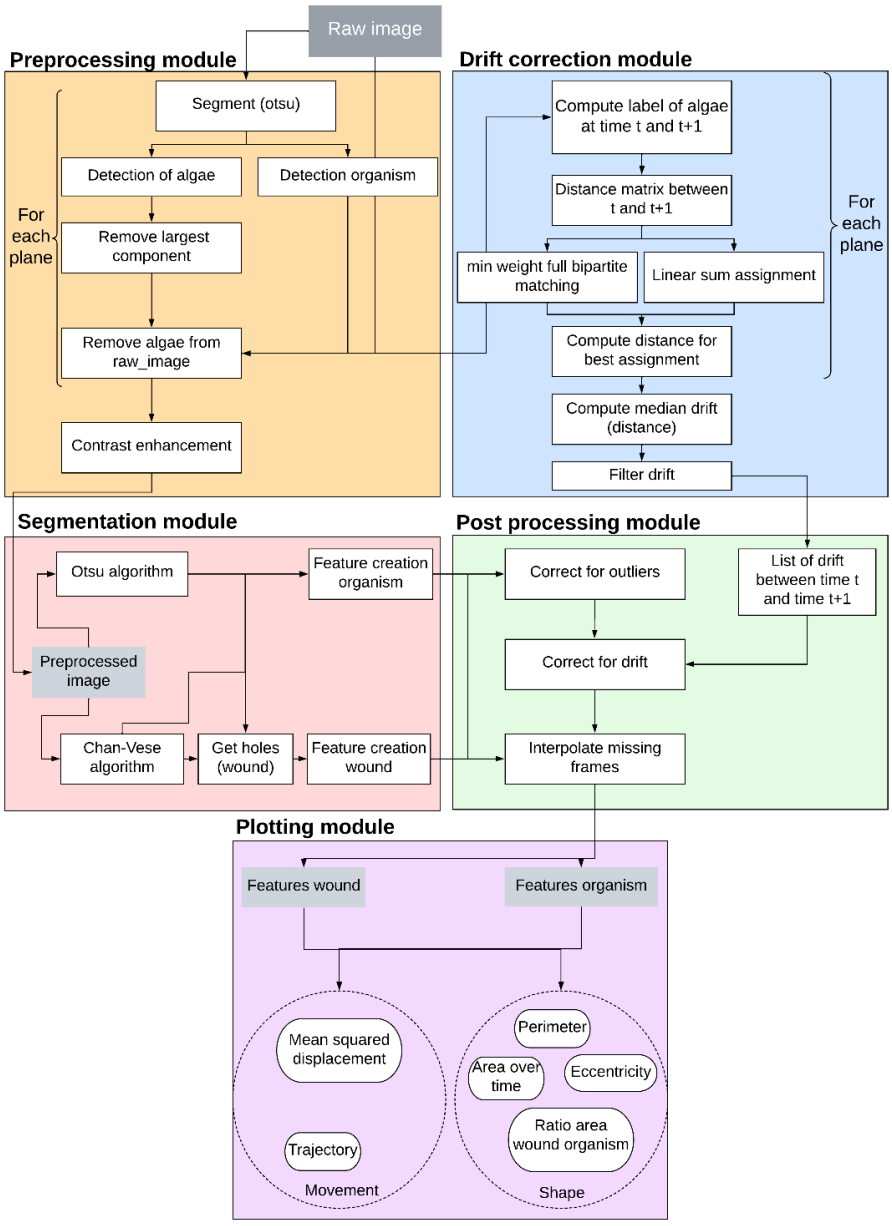
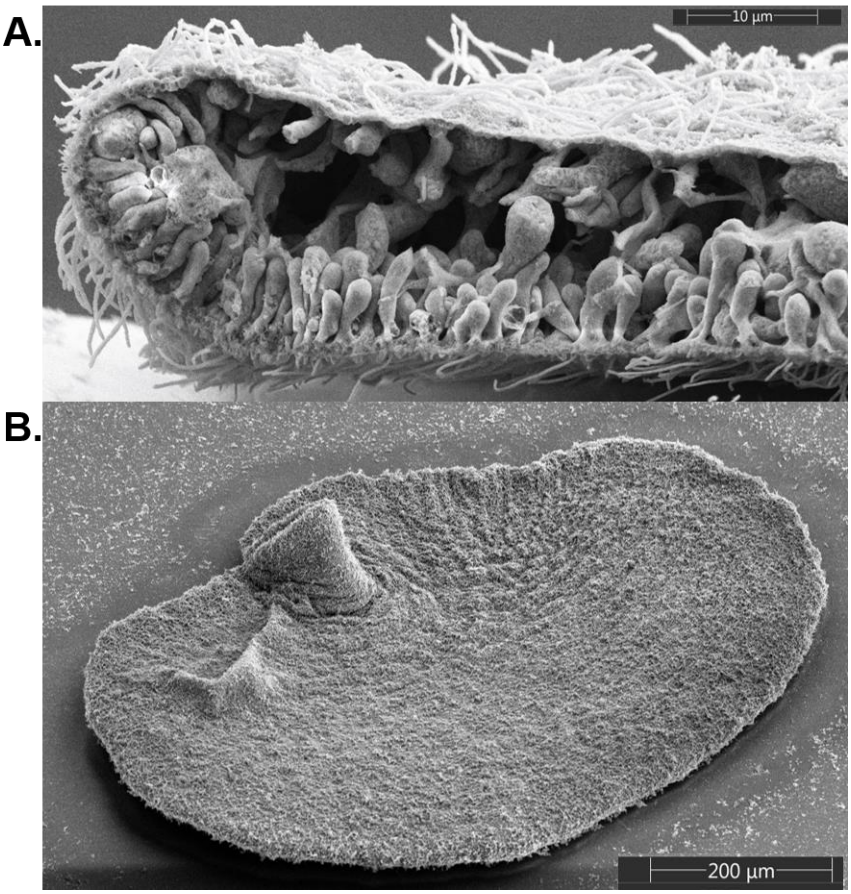
Annexes



Annex 1: Complete representation of the Trichotomatic pipeline



Annex 2: Scanning electron microscopy(SEM) images of Trichoplax adherens.

A. A fractured SEM image, showing Trichoplax adherens internal organization B. SEM image showing the overall aspect of Trichoplax adherens. Credits: N.Brouilly and A.Pasini, BioRxiv,2019