Quantitative Tracking of Heart Nuclei Dynamics Over Time in Heart Tube Development April 12, 2024

Group Members: Najat ElFarra, Sarvath Sharma, Sidy Ndiongue, Nour Ghanem

ABSTRACT

An essential component of embryonic heart development is the formation of a primitive heart tube which occurs in both vertebrates and invertebrates. It has been shown that cardioblasts migrate in a medial-lateral position as they form the lengthened heart tube. To investigate what factors could be causing the heart tube lengthening, *Drosophila melanogaster* embryos were imaged using a fluorescent marker for the cardioblast nuclei using time-lapse microscopy. A pipeline was then created to segment the images, distance transform and watershed for image analysis. The anterior-posterior position as well as the change in compactness, area and roundness were measured over time. While there was no statistical significance in the AP migration pattern in cardiac progenitor nuclei, the study showed a directional trend in the posterior direction. In addition, the study showed a statistically significant increase in the overall area of the nuclei in 2/4 of the datasets, further signifying the importance of exploring area and posterior migration in heart tube formation in Drosophila embryos.

INTRODUCTION

Heart development in the *Drosophila* embryo begins with the formation of a primitive heart tube around stage 14 of embryonic development [1]. Two major cell types are involved in the formation of the fly heart: cardiac progenitors (cardioblasts) and pericardial cells, with cardioblasts playing an important role in heart tube formation while the pericardial cells accompany them at the trailing edge [2]. During development, two columns of cardioblasts, one at each side, migrate from opposite sides of the embryo in a coordinated manner, meeting at the medial line [3]. It is known that improper heart tube formation may lead to congenital heart defects in newborns [4]. Given the role of cardioblast migration in heart tube formation, it is important to better understand how these cells are migrating.

During cardioblast migration, cells take a series of forward and backward steps, exhibiting oscillatory behavior. The amplitude and duration of the forward step is larger, leading to a net forward movement medially for both columns of cells [3]. However, it is not well understood how the tube elongates as the cells move medial-laterally and form a linear structure. We hypothesize that as the cells migrate medially, they are also moving along the anterior-posterior (AP) axis, effectively lengthening the tube in the AP direction. It is also possible that the cells are instead increasing in area or changing circularity to lengthen the linear tube.

METHODS

The fly line used was midE19:GFP (kept at room temperature) which fluorescently labels the cardioblast nuclei. The embryos were obtained around stage 14, and mounted dorsal side down for imaging, covered in halocarbon oil. Using a 60x oil immersion lens on a spinning disk confocal microscope, Z-stacks were acquired every 15 seconds (until heart tube closure) with a step size of 0.75 µm and maximum intensity projection images were used in the analysis.

Our dataset consisted of multi-frame TIFF files containing images of the nuclei movement over time. The images went through a pipeline which consisted of three steps: image segmentation, object tracking and statistical analysis. The output of each step was used in the subsequent one. Parameters for segmentation and object tracking were fine tuned for each dataset.

For the image segmentation pipeline the following operations were applied as shown in Figure 1: gaussian filtering, contrast stretching, local thresholding, binary opening, small object removal, distance transform and peak finding to get watershed seeds followed by watershed segmentation.

Two methods were implemented for nuclei tracking, one based on nearest neighbor search, and another based on optical flow. Both methods use the watershed seed locations at the first frame as a starting point. Properties of the cell containing

the position are extracted, if no cell is found contain the position, then the tracking stops. The result of the tracking is a data structure shown in **Error! Reference source not found.**.

Figure 1: Segmentation steps visualized for the first frame of dataset n1.
To improve the visibility of the seeds image a 3x3 dilation filter was applied.

The nearest neighbor method works by finding the closest point to the current position in the next frame within a threshold distance (around the size of a cell). If no point is found within a distance, then the tracking for that point stops. This method assumes that the cells are moving slow enough between different frames. To accelerate the nearest neighbor search, a KDTree implemented in SciPy [5] was used, this reduced the search dependence from $O(n)$ to an average of $O(log\ n)$.

The optical flow-based method calculates the optical flow for each frames using the iterative Lucas-Kanade (iLK) solver [6] implemented in scikit-image [7]. The seed locations at the first frame are propagated using optical flow vectors. For successive frames, interpolation of these motion vectors was required since the points are no longer at integer coordinates. This was accomplished by applying bi-linear interpolation on flow vector components using the *RegularGridInterpolator* function in Scipy [5].

Nuclei ↓								
	...	(x80, y80)	(x82, y82)	(x82, y82)	(x83, y83)	(x84, y84)	(x85, y86)	
	...	(x80, y80)	(x81, y81)	(x82, y82)	(x83, y83)	(x84, y84)	(x85, y85)	(x86, y86)
	...	(x80, y80)	(x81, y81)	(x82, y82)	(x83, y83)	(x84, y84)		

Time →

Figure 2: Data structure for storing nuclei information over time. Each cell contains the position and geometrical information.

To reduce noise on the tracked paths, the coordinates were filtered as follows:

$$X_{smooth} = X * H, \qquad Y_{smooth} = Y * H, \qquad H = [1\ 1\ 1\ 1\ 1\ 1\ 1\ 1\ 1]/9$$

Where H is the averaging filter, X and Y represent the sequence of the x and y coordinates for a tracked path and $*$ is the convolution operator (strictly this is a correlation since H is symmetric). The X and Y arrays were padded at each end with the first and last elements.

Figure 3 shows the results of the different tracking methods with and without the smoothing process described above. The optical flow-based tracking is superior as the tracked paths look more natural and have less noise. In some datasets, the nearest neighbor had cells tracked for a duration. However, this is because the nearest neighbor algorithm picked a different cell, this can be seen in the sharp discontinuities in the detected path.

The primary issue with the tracking methods discussed is that the segmentation is inconsistent across frames due to segmented nuclei disappearing, this results in the tracking for that cell stopping. Using optical flow-based tracking, the minimum number of consistently tracked nuclei was used across all datasets (which amount to 7 nuclei).

For analyzing the results provided by the tracking methods, we aim to determine the change in position as time increases in the following directions: medial, lateral, anterior, and posterior. For the medial and lateral movements, we use a user provided medial line image for the given dataset to find the coordinates for comparing how much further the nuclei traveled and in what direction with respect to that medial line. For the anterior and posterior movements, we identify that a negative change in the y -coordinate (moving up) is an anterior movement and a positive change in the y- coordinate (moving down) is a posterior movement.

Figure 3: Optical flow and tracking results with and without smoothing shown for frame 220 of dataset n1.

RESULTS

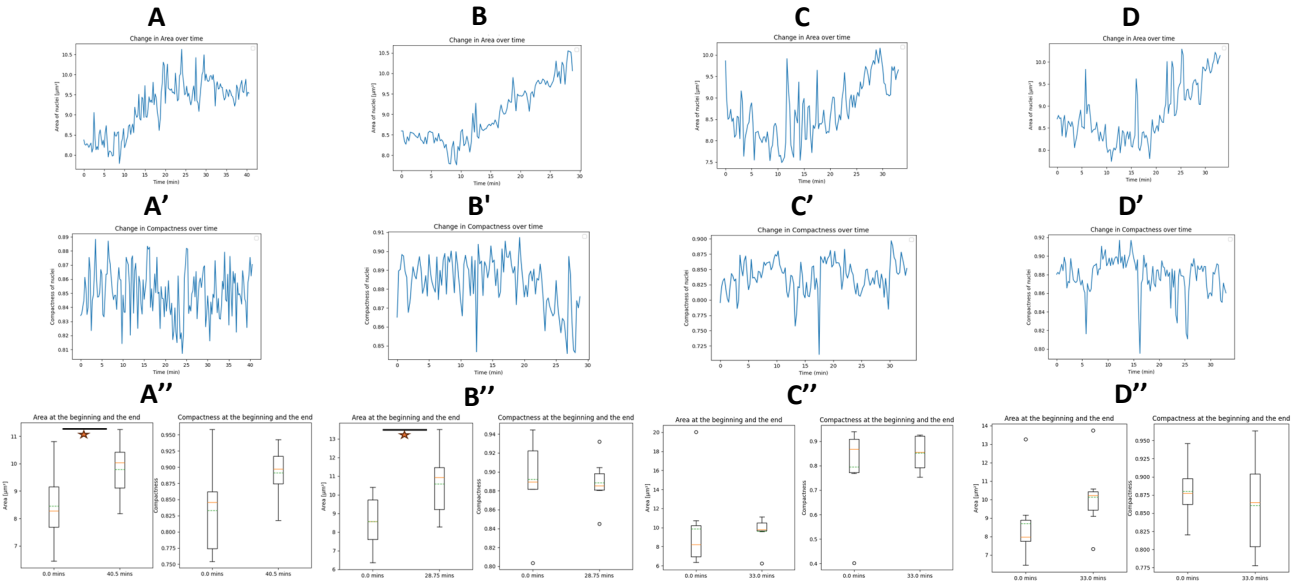
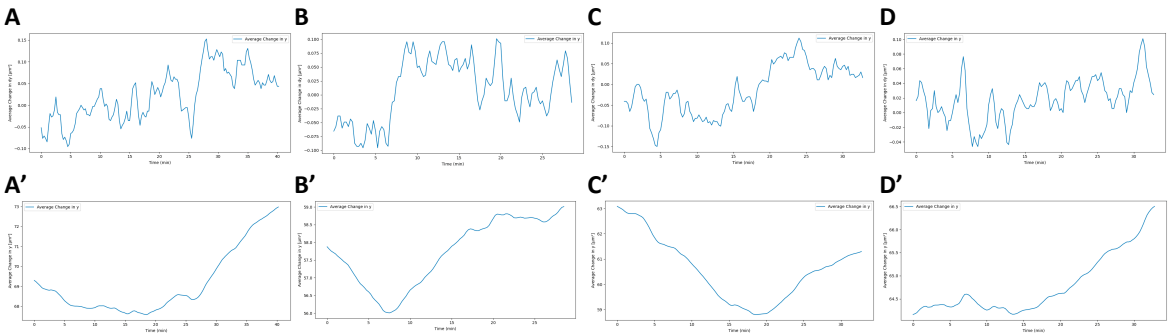


*Figure 4: Overall change in area and compactness (circularity) of cardioblasts in Drosophila embryos over time. Each column represents the correspondng dataset. A, B, C, D) Drosophila nuclei change in area over time. Y-axis: Area of nuclei (µm2). X-axis: Time (min). A', B', C', D') Drosophila nuclei change in compactness over time. Y-axis: Compactness of nuclei. X-axis: Time (min). A'', B'', C'', D'') Drosophila nuclei overall change in area and compactness, comparing the first and last time point. Y-axis:" Area [µm2]" for first boxplot, "Compactness" for second boxplot X-axis: Time (min). Paired t-test analysis of area and compactness for the first/last time point using optical flow tracking algorithm. P-value < 0.05 = statistical significance. * = statistical significance. (Figure A'' * p-value = 0.016, Figure B'' * p-value = 0.027).*

Assessing compactness and area over time in cardiac progenitor nuclei in Drosophila embryo cardioblasts: The first and second dataset showed a statistically significant increase in area when comparing the nuclei in the first and last time point, indicating an overall increase in area of the nuclei (P-value = 0.016 & 0.027 respectively, Figure 4A'', B''). However, there was no statistically significant change in nuclei area in datasets 3 & 4 (Figure 4C'', D''). Despite no statistical significance, the area increases over time across all datasets (Figure 4A, B, C, D). When assessing compactness (a measure for circularity), there was no statistically significant change in circularity across all 4 datasets (Figure 4A'- D', A'' – D'').



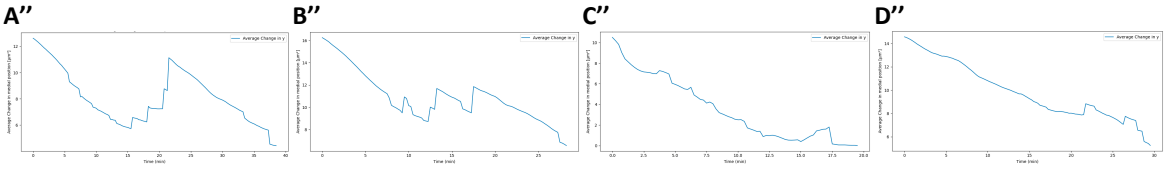


Figure 5: Migration of Cardioblasts in Drosophila embryos over time. Each column represents one dataset (four datasets). A, B, C, D) Nuclei instantaneous anterior (lower y values) and posterior (higher y values) movements tracked over time. Y-axis: Average change in dy (μm²). X-axis: Time (min) A', B', C', D') Nuclei tracking the y-coordinate positions over time in the anterior (lower y values) and posterior (higher y values). Y-axis: Average change in y (μm²). X-axis: Time (min). A'', B'', C'', D'') Nuclei tracking the medial movement over time. Y-axis: Average change in medial position (μm²). X-axis: Time (min).

Oscillatory and directional trends in cardiac progenitor nuclei anterior and posterior movements: Analysis of the anterior and posterior (AP) instantaneous migration of cardiac progenitor nuclei over time revealed an oscillatory migration of AP movement, with a directional instantaneous shift in the posterior direction (Figure 5A, B, C, D). These results were observed across four datasets, with a preference in the posterior direction. Despite the AP movements over approximately 30 minutes showing periodic, oscillatory shifts towards both anterior and posterior, the nuclei tend to move relatively in the posterior direction. (Figure 5A, B, C, D). Analysis of spatial coordinates for cardiac progenitor nuclei revealed a general posterior migratory movement. Datasets 1, 2, and 4 revealed a movement in the posterior direction, indicating a directed migration (Figure 5A', B', D'). Dataset 3 revealed a general movement in the anterior direction, followed by a small shift towards the posterior direction, indicating a potential outlier (Figure 5C'). Despite repeated samples for each dataset, a different trend was shown for the third dataset, and therefore while there seems to be a general posterior migration, the results are partly unclear. Analysis of the medial migration of cardiac progenitor nuclei over time revealed a medial migration over 30 minutes across all four datasets (Figure 5A'', B'', C'', D'').

Table 1: Pearson's correlation analysis between elongation and migration direction of Drosophila embryo cardioblasts. Correlation coefficient and p-value obtained using the optical flow tracking algorithm. P-value < 0.05 = statistical significance.

Migration Direction	Correlation Coefficient	P-Value
Anterior	0.009	0.562
Posterior	-0.012	0.369
Medial	0.021	0.060
Lateral	0.040	0.244

Migration movements in the cardiac progenitor nuclei elongation process: Pearson correlational analysis of elongation of cardiac progenitor nuclei over time revealed anterior, posterior, lateral, and medial movement do not have a significant contribution to the elongation process (Table 1). However, using optical flow for tracking nuclei shows a weak positive medial correlation with a p-value of 0.060 (Table 1). While not statistically significant, the p-value is close to the threshold of 0.05, which could imply that there might be a potential effect worth exploring further using the optical flow tracking algorithm. This pattern aligns with the observed medial movement of the nuclei as they migrate towards each other (Figure 5A'', B'', C'', D''). This consistency between the quantitative data and the visual observations.

*Figure 6: **Validation testing using gold standard PJAMAS segmentation algorithm** [insert citations]. Outputs are shown for dataset 2 only. A) Seeding and watershed segmentation using PJAMAS. B) Average medial-lateral (ML) positional change of nuclei over time. Lower y-value = medial movement, higher y-value = lateral movement. C) Average anterior-posterior (AP) positional change over time. Lower y-value = anterior movement, higher y-value = posterior movement.*

Validation testing of image segmentation and tracking pipeline: The published algorithm PYJAMAS was used as a gold standard for validating this study's algorithm [8]. Through this gold standard validation, our study's findings were supported: average medial positional changes exhibited a declining trend, suggesting nuclei moving towards each other

(medial migration) (Figure 5C'', Figure 6B). However, small differences arose in the validation algorithm's output, initially shifting anteriorly before transitioning posteriorly over time (Figure 6C). Our study mirrored this pattern (anterior to posterior migration), however, with a lower initial y-value compared to the validation algorithm (Figure 5C').

DISCUSSION

The work aimed to understand the development of the heart in Drosophila embryos through a segmentation pipeline and an optical flow tracking algorithm. The primary focus was to determine the association of the lengthening of the heart tube with anterior-posterior movements of cardiac progenitor nuclei. Additionally, the work explored the association of the heart tube formation with changes in cell area and circularity. The study rejected the primary hypothesis as anterior and posterior movements did not play a statistically significant role in the lengthening of the heart tube in Drosophila embryos (Table 1). However, there is a directional trend of cardiac progenitor nuclei in the posterior direction over time (Figure 5 A, B, C, D & Figure 5 A', B', D'). The alternative hypothesis was partially supported, with a statistically significant overall increase in area in the cardiac progenitor nuclei over time in Drosophila embryos heart development for datasets 1 and 2, however there was no significant change in circularity of the nuclei (Figure 4). Validation using the gold standard PYJAMAS mostly confirmed our findings, with slight variation in the AP positional change (Figure 6).

The outliers shown in the results (ex. Figure 5C') could be due to errors with the segmentation algorithm. Addressing the segmentation algorithm, cardiac progenitor nuclei that were segmented tend to appear and disappear over time. To address this issue, the tracking algorithm only takes into consideration the nuclei detected across all frames. The nuclei disappearing and appearing over time could be due to changes in illumination levels which can result in inconsistent nuclei detection. Further optimization of the segmentation algorithm can help, however, a better approach would be to use machine learning to train the algorithm to detect and track the nuclei based on a diverse set of features, such as intensity and shape [9]. Overall, this work developed a segmentation pipeline and tracking algorithm for measuring nuclei area, circulatory, elongation, and the AP movements of Drosophila embryos cardiac progenitor nuclei over time. This study suggests nuclei area and posterior migration are important in the formation of heart tubes in Drosophila embryos.

REFERENCES

[1] Y. Tao and R. A. Schulz, "Heart development in Drosophila," *Model Syst. Study Cardiovasc. Dev. Dis.*, vol. 18, no. 1, pp. 3–15, Feb. 2007, doi: 10.1016/j.semcdb.2006.12.001.
[2] E. J. Ward and J. B. Skeath, "Characterization of a novel subset of cardiac cells and their progenitors in the Drosophila embryo," *Development*, vol. 127, no. 22, pp. 4959–4969, Nov. 2000, doi: 10.1242/dev.127.22.4959.
[3] N. Balaghi, G. Erdemci-Tandogan, C. McFaul, and R. Fernandez-Gonzalez, "Myosin waves and a mechanical asymmetry guide the oscillatory migration of Drosophila cardiac progenitors," *Dev. Cell*, vol. 58, no. 14, pp. 1299-1313.e5, Jul. 2023, doi: 10.1016/j.devcel.2023.05.005.
[4] I. Esteban *et al.*, "Pseudodynamic analysis of heart tube formation in the mouse reveals strong regional variability and early left–right asymmetry," *Nat. Cardiovasc. Res.*, vol. 1, no. 5, pp. 504–517, May 2022, doi: 10.1038/s44161-022-00065-1.
[5] P. Virtanen *et al.*, "SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python," *Nat. Methods*, vol. 17, pp. 261–272, 2020, doi: 10.1038/s41592-019-0686-2.
[6] G. Le Besnerais and F. Champagnat, "Dense optical flow by iterative local window registration," in *IEEE International Conference on Image Processing 2005*, 2005, p. I–137. doi: 10.1109/ICIP.2005.1529706.
[7] S. van der Walt *et al.*, "scikit-image: image processing in Python," *PeerJ*, vol. 2, p. e453, Jun. 2014, doi: 10.7717/peerj.453.
[8] R. Fernandez-Gonzalez *et al.*, "PyJAMAS: open-source, multimodal segmentation and analysis of microscopy images," *Bioinformatics*, vol. 38, no. 2, pp. 594–596, Jan. 2022, doi: 10.1093/bioinformatics/btab589.
[9] M. K. Goshisht, "Machine Learning and Deep Learning in Synthetic Biology: Key Architectures, Applications, and Challenges," *ACS Omega*, vol. 9, no. 9, pp. 9921–9945, Mar. 2024, doi: 10.1021/acsomega.3c05913.