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# COMPARISON OF INTERACTIONS BETWEEN CONTROL AND MUTANT MACROPHAGES

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**Abstract.** This paper presents a preliminary study on macrophages migration in *Drosophila* embryos, comparing two types of cells. The study is carried out by a framework called `macrosight` which analyses the movement and interaction of migrating macrophages. The framework incorporates a segmentation and tracking algorithm into analysing motion characteristics of cells after contact. In this particular study, the interactions between cells is characterised in the case of control embryos and *Shot3 mutants*, where the cells have been altered to suppress a specific protein, looking to understand what drives the movement. Statistical significance between control and mutant cells was found when comparing the direction of motion after contact in specific conditions. Such discoveries provide insights for future developments in combining biological experiments to computational analysis.

**Keywords:** Segmentation, Cell Tracking, Track analysis, Macrophages

## 1 INTRODUCTION

Cell migration is highly involved in development and adult life, in maintaining homeostasis with processes such as wound healing and immune response [1,2]. Moreover, pathological conditions such as cancer or autoimmune disease, are related to dysfunctional cell migration. At present, many aspects of cell migration are known, however no single model is able to integrate all the cues driving motion [3]. Macrophages are highly migratory cells of the immune system that have ranges of functions ranging from tissue repair to immune responses to foreign pathogens [4]. However, excessive migration can be related to autoimmune disease and cancer [5]. An ideal model system to study in vivo cell migration are the embryonic macrophages of *Drosophila megalonaster*, as they are amenable to high spatio-temporal resolution live imaging [6]. In Stramer2010, contact inhibition of locomotion was described in these cells, showing that these cell-cell interactions are needed for functional migration. In the present study, macrophages from control embryos were compared to *Shot3* embryos.

Cell tracking is defined as the linking between objects in a temporal context. In this work, tracking of cells is achieved by segmenting the cells to obtain positions and then linking between the same object in two positions in consecutive time frames. Segmentation and tracking of cells is a widely studied area [7,8]. However, few studies have been made on identifying patterns in the migration or providing a biological context to the tracks obtained. In previous work, the analysis of macrophages' movement has been studied in the context of the cell-shape evolution [9], as well as the comparison of movement patterns of interacting cells from non-interacting [10]. In [11], a framework was presented to analyse the tracks of migrating macrophages, analysing the movement related to the interactions.

In this work, a study on novel data is presented, where time sequences of control and mutant macrophages were acquired and an underlying difference in the motion is searched for. The main contribution in this work consists of the use of a software framework to provide quantitative measurements to provide comparative quantitative measurements of different conditions. Figure 1 represents the differences in movement patterns hypothesised in this work: to distinguish through image analysis cases of (a) control and (b) mutant cells.

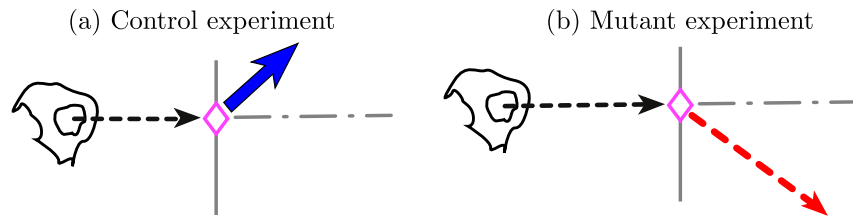


Fig. 1: Illustration of the hypothesis behind this paper. Different movement patterns from control to mutant experiments are represented by the different types of line and colours in the diagram.

## 2 MATERIALS

Fourteen time sequences of macrophages in *Drosophila* embryos were acquired following the protocol described in [6,12], with nuclei labelled in red and microtubules in green. Each image in the time-lapse sequences was obtained every ten seconds at a pixel density of  $0.21\mu m$ . The 14 experiments consist of images of size  $(n_w, n_h, n_d) = (512, 672, 3)$ .

The datasets are classified as control or mutant experiments. Four control and ten Shot3 mutant experiments were analysed, in which mutation affects the cytoskeletal crosslinking. The number of frames within the control experiments range between 137 and 272, while the mutant experiments range between 135 and 422 frames. Figure 2 shows a comparison between four frames of a control experiment against four frames of a mutant experiment.

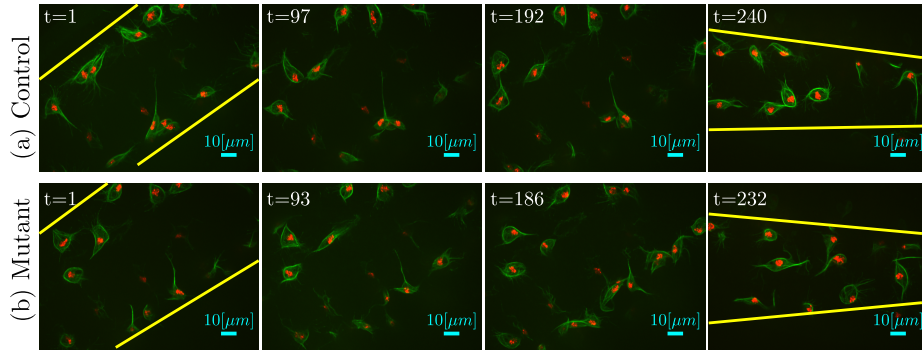


Fig. 2: Comparison between five frames of (a) control against five of (b) mutant experiments. The datasets were chosen because they had a similar number of frames and thus a similar spacing between the frames in both experiments could be shown ( $\approx 95$ ). Yellow lines have been manually added to the first and last frames on both experiments to showcase the apparent change of focus of the microscope as time evolves.

Overlapping events, defined as *clumps* are relevant to the study of interactions caused by cell-cell contact, as presented in figure 3. Given certain circumstances, cells have been shown to align their microtubules and drastically change their direction of movement [12]. The contact observed in certain *clumps* suggest an alteration of the migration patterns of the cells involved. This type of interaction was measured first in [11], where cell-cell contact was shown to be influential in the movement of cells.

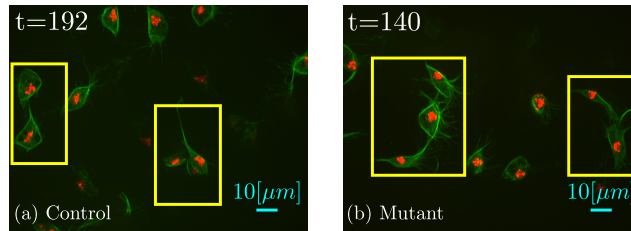


Fig. 3: Representation of *clumps* in control and mutant experiments. Both datasets present overlapping events, where *clumps* are formed. The detail of two frames from figure 2 is shown, highlighting *clumps* in both types of experiment.

### 3 METHODS

Macrosight [11] is a framework for the analysis of moving macrophages capable of segmenting the two layers of fluorescence in the dataset presented previously, and apply the keyhole tracking algorithm inside the PhagoSight framework [13] on the centroids of the segmented nuclei.

Figure 4 shows an illustration of the flow of information in **macrosight**. Each track generated  $\mathcal{T}_r$  contains information on the (i) position  $\mathbf{x}_t$  at a given time frame  $t$ , (ii) track identifier  $r$ , (iii) velocity  $v_t$ , and whether the cell is part of a clump.

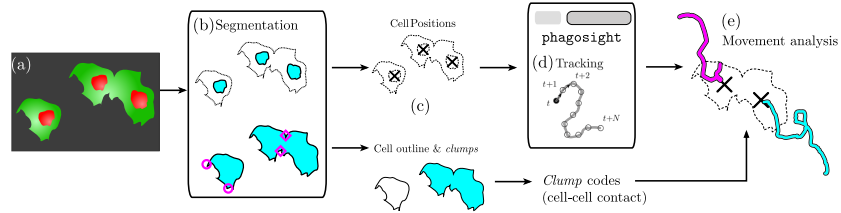


Fig. 4: Illustration of part of the **macrosight** framework. (a) Represents the original image sequences. The two levels of fluorescence are segmented in (b) based on a hysteresis threshold where the levels are selected by the Otsu1979 algorithm. The segmentation of the red channel provides the positions necessary to produce the tracks (d) of the cells using the keyhole tracking algorithm [13]. Finally, the tracks' information is combined with the clump information from the segmented green channel to allow analysis of movement based on contact events (e).

Each *clump* can be uniquely identified through a code  $c(r, q)$ , where  $r > q$  indicate that at a certain time frame  $t$ , tracks  $\mathcal{T}_r$  and  $\mathcal{T}_q$  belong in the same clump. This allows for each interaction to be analysed. Several tracks can come together into a single clump, thus the *clump* codes evolve. Figure 5 represents the evolution of a given track  $\mathcal{T}_2$  and its involvement in two different clumps.

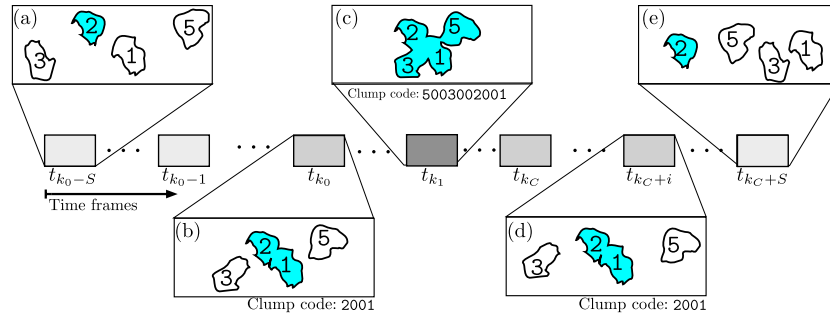


Fig. 5: Illustration of clump codes to the different time frames for a particular track  $\mathcal{T}_2$ . The horizontal axis represents the time, and the detail of five frames is presented to illustrate the evolution of track  $\mathcal{T}_2$  as it interacts with other cells. In (a) and (e), track  $\mathcal{T}_2$  is not in contact with any other cell, thus no clump is present. (b) Represents the moment when  $\mathcal{T}_2$  and  $\mathcal{T}_1$  start interacting in clump 2001. Following in (c), tracks  $\mathcal{T}_3$  and  $\mathcal{T}_5$  become present in the clump, thus the *clump* code changes to 5003002001.

### 3.1 Movement analysis experiments

The events of interest in this paper consist of analysing cell-cell contact events of two cells. The change of direction  $\theta_x \in (-\pi, \pi)$  is calculated by taking the positions of the tracks  $\mathcal{T}_r$  and  $\mathcal{T}_q$  up to  $S$  frames prior the first contact at time frame  $t_{k_0}$ , as well as the positions up to  $S$  frames after the last time frame of contact  $t_{k_c}$ . The time in clump  $TC = t_{k_c} - t_{k_0}$  refers to the number of frames the two tracks interact in a given instance of the clump, and it is not taken into consideration for the calculation of angle  $\theta_x$ . A diagram of the calculation of  $\theta_x$  is provided in figure 6, where the positions on the image  $\mathbf{x} = (x, y)$  get translated and rotated into new frame of reference  $(x', y')$ .

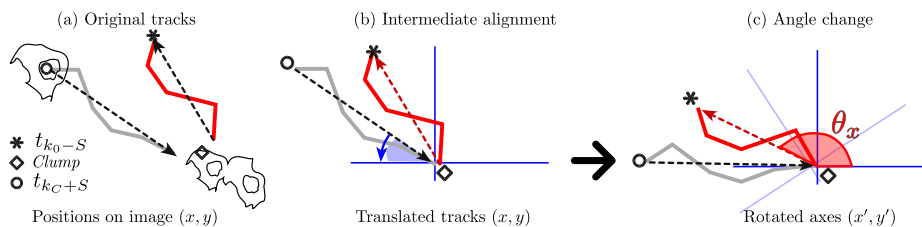


Fig. 6: Illustration of direction change ( $\theta_x$ ) measurement. Three markers represent different positions of a given track. The markers are ( $\circ$ ) represents  $S$  frames before contact; ( $\diamond$ ) represents the starting instant of the clump; and ( $*$ ) represents the position where the experiment is finalised. Notice the translation and rotation into the new frame of reference  $(x', y')$ .

### 3.2 Selection of experiments

All available datasets were segmented and tracked. The tracks' information was searched to find types of *clumps* which fulfil the criteria: (i) **only two cells interacting**; (ii) **full interaction**, where at least one of the cells would enter and exit the *clump*; and (iii) **immediate reaction**, with a value of  $S$  ranging from 3 to 5.

The changing direction angles,  $\theta_x$ , for each case were calculated, recording the time in clump  $TC$  and change of direction. It is worth noting that a single clump could provide more than one experiment in different time spans, as the two interacting tracks could interact with each other back and forth.

## 4 RESULTS

After the processes of segmentation, tracking, and selection of suitable experiments, twenty four control and thirty nine mutant cases were selected for analysis. Table 1 shows the number of cases per dataset selected, it is worth noting the different numbers of experiments fitting the criteria between datasets.

Table 1: Number of suitable experiments per dataset. Notice that not all datasets provided the same number of experiments for the analysis, as one or more of the selecting criteria would not be fulfilled. Also, the mutant datasets 01,02 and 09 did not provide any suitable experiments due to clumps always involving more than two cells.

Dataset ID	$n$ experiments	Dataset ID	$n$ experiments		
CONTROL01	14	MUTANT03	10	MUTANT07	3
CONTROL02	4	MUTANT04	2	MUTANT08	2
CONTROL04	4	MUTANT05	2	MUTANT10	4
CONTROL05	2	MUTANT06	9	MUTANT11	7
TOTAL	24			TOTAL	39

The resulting tracks representing changes of direction are shown in figure 7 for (a) control and (b) mutant. Differences can be observed in the displacement of the cells towards and from the centre, in the horizontal direction  $x'$ .

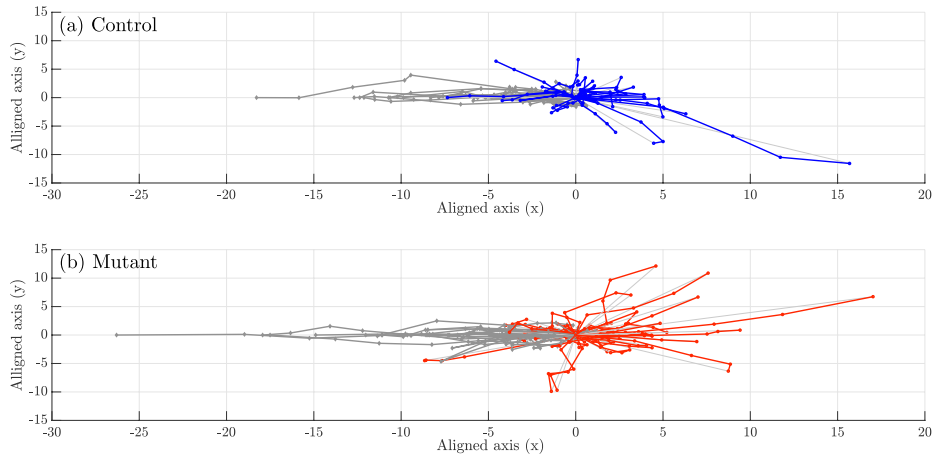


Fig. 7: Comparison of aligned tracks for (a) Control and (b) Mutants experiments. Refer to figure 6 on how to read this figure.

Boxplots showing the change of direction angle  $\theta_x$ , time in clump  $TC$  and distances from the centre in the  $x'$  are presented in figure 8. Notice that in figure 8(a), the angle  $\theta_x$  for mutant experiments appear to be distributed with more cases towards the lower angles, or a smaller change of direction after the contact. However, on its own, this measurement did not provide a statistically significant difference. The data points where  $\theta_x < 90$  were chosen, as seen in figure 9. A t-test was calculated between the remaining angles showing statistical significance ( $p = 0.03 < 0.05$ ).

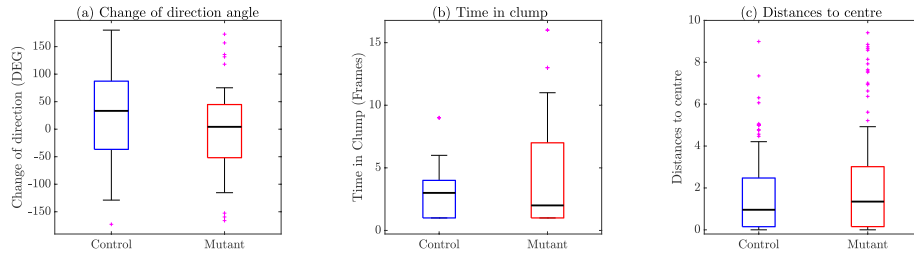


Fig. 8: Comparison of relevant variables between Control (blue) and Mutant (red) experiments. (a) Change of direction angle,  $\theta_x$ , coming from figure 7. (b) Time in clump  $TC$  in frames. Finally, (c) shows the distances to the centre.

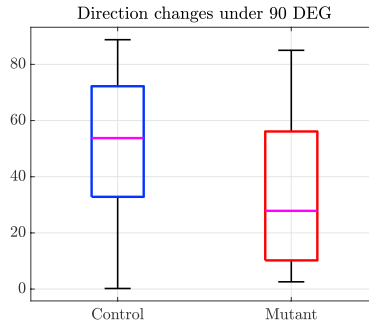


Fig. 9: Change of direction differences between Control (blue) and Mutant (red) experiments for angles under  $90^\circ$ . After observation of figure 7(a), the smaller angles show a significant difference between the control and mutant experiments.

## 5 DISCUSSION

The previous work presented in [11] presented a novel framework for the analysis of macrophages migration in a controlled environment. In this work, the framework was extensively used in different datasets comparing control and mutant cells. While some of the calculations still did not provide a statistically significant difference between control and mutant cells, some insights were found. Apart from the qualitative differences between the measurements presented it can be noted, as seen in figure 9, there is a difference between control and mutant experiments in cells that do not change direction drastically. Future work will improve on the number of variables collected from the tracking.

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