

Two-Point Optical Manipulation of Cell Junctions in the Early Epithelium of the *Drosophila* Embryo 2 3

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Abstract 6

Laser manipulation is widely used to study mechanics from the molecular to the tissue scale. We implemented optical tweezers to directly manipulate single cell-cell junctions in a developing tissue. We further extended the approach to two-point laser manipulation to enable extensive remodeling of cell-cell junctions. Here, we describe two-point laser manipulation and its implementation to probe the mechanics of cell junctions in the *Drosophila* embryo. 7 8 9 10 11

Key words Optical tweezers, Tissue mechanics, Force measurements, Epithelia, Morphogenesis 12

1 Introduction 13

Tissue shaping is determined by how forces are applied by and to cells, and how cells respond to these forces. There are several ways to probe cell mechanics in tissues [1, 2], but they are often indirect and inferred from the analysis of the molecular content (e.g., cytoskeletal constituents) or from cell shapes. Such approaches require information on the relationship between molecular content and force application or cell shape and cell mechanical response. A more direct way to probe cell mechanics consists in applying controlled forces and measure the resulting cell and tissue deformation. Optical forces offer the unique possibility to manipulate structures locally, enabling, for example, manipulation of cell junctions [3]. By locally deflecting single-cell junction, single-point manipulation can provide direct measurements of tension and rheological properties [4]. We extended this approach to two-point manipulation, which enables to exert forces in various configurations. Here, we describe the method and its application to the early *Drosophila* embryo. 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

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3.1	Calibration of the Optical Trap Position	61
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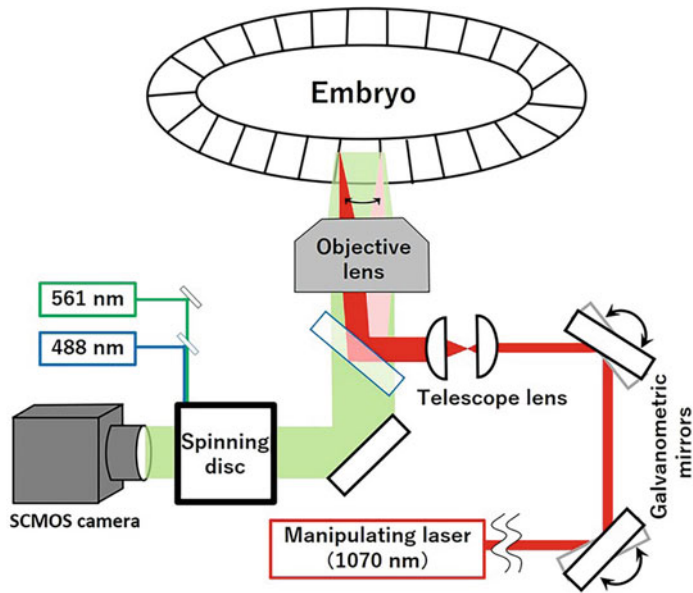


Fig. 1 Setup for two-point optical manipulation. Cell junctions are manipulated by the IR laser (1070 nm). The laser focus position is controlled by two galvanometric mirrors (x-y plane)

3.2 Recording of a Calibration Movie

1. Put a drop of a low-concentration solution of red fluorescent beads ($r = 500 \text{ nm}$) on a coverslip, and place it on the sample holder. Beads in solution are easily trapped by the laser beam. Make sure that the concentration is low enough so that only one bead is trapped at a time.
2. Adjust the z position to find the particles close to the coverslip. This z-position will be within a few μm of the position where the experiments will be carried out in the embryo.
3. Turn on the image acquisition software.
4. Select the parameters for image acquisition: exposure time, gain, time between two images, number of images.
5. Turn on the infrared laser (IR laser, 1070 nm) and set the power to 1 W. The IR laser is used to trap a bead but also here to image it, as it is able to excite the red fluorescence by two-photon excitation.
6. The galvanometers and the shutter are controlled via a national instrument card. The angles of the galvanometer mirrors correspond to voltages. We designed a program in C++ (OTC program) with the Qt library to control the card that sends the voltages to the mirrors and the shutter. The National Instrument card can also record the voltages and the camera trigger, which we will then use to match the mirror positions to the trap position in the image. We can provide the code that generates this program on request. Control the angle of dual-axis

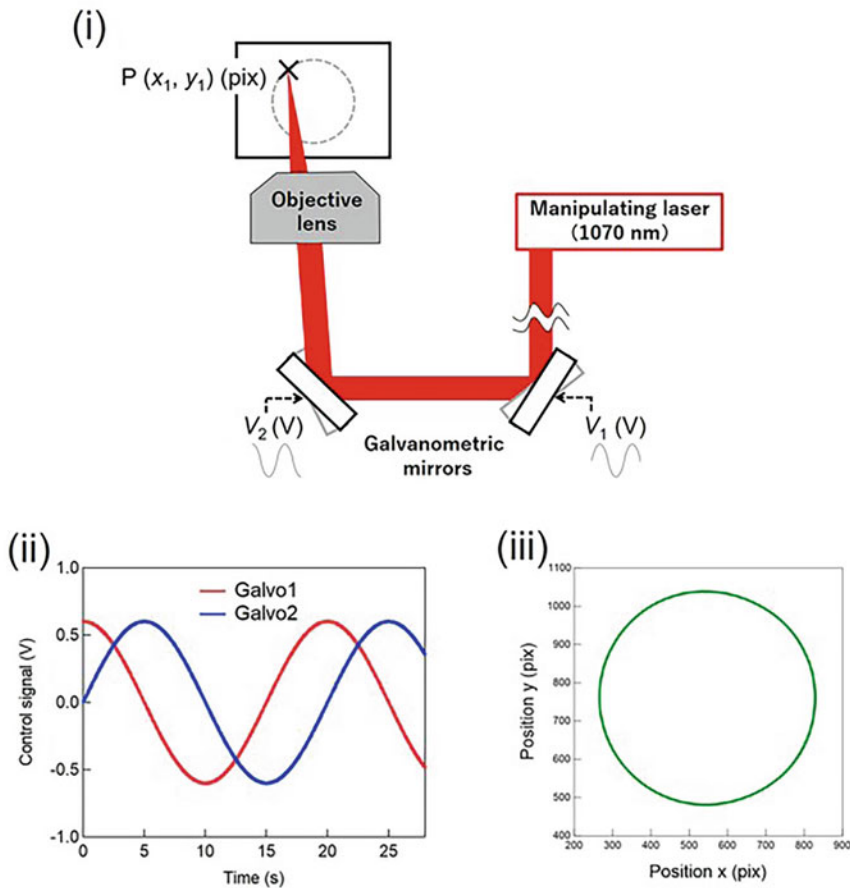


Fig. 2 Calibration of the optical trap position and control voltages of galvanometers

- galvanometric mirrors to make the laser focus trace a circle in x - y plane (Fig. 2). The control signals are two sine waves of amplitude $V1$ and $V2$ (volts) and out of phase with each other by $\pi/2$ (rad) and 10-second period (to draw the circle slowly). Figure 3 shows the interface of the optical tweezers control (OTC) program.
7. Start the image acquisition (1000 images with a high frame rate, e.g., 50 fps).
 8. Run the software that opens the IR laser shutter and controls the dual-axis galvanometric mirrors.
 9. Wait that the trapped bead completes at least two full circles, and stop the optical tweezers.
 10. Stop the image acquisition. Note that a tiff movie and a text file with control voltages of galvanometers are created.

OTC

noFileName SamplingRate 1000 samp/sec bufferSize 1000

C:/TEMP/ Browse...

☒ Waveform 1 parameters

Choose the waveform you want
Sinusoidal

WaveForm's' frequency : 1 Hz
nbPeriod 1

set the amplitude of the waveform in volt
amplitude 0.0000 (Volt)

set the phase lag in rad
phase 0.000 (rad)

set the offset of the waveform in volt
Offset 0.0000 (Volt)

☒ Waveform 2 parameters

Choose the waveform you want
Sinusoidal

WaveForm's' frequency : 1 Hz
nbPeriod 1

set the amplitude of the waveform in volt
amplitude 0.0000 (Volt)

set the phase lag in rad
phase 0.000 (rad)

set the offset of the waveform in volt
Offset 0.0000 (Volt)

☒ AI Parameters

nb AI 4 buffer size 1000 1000 (sa.s-1) n buffer 1

acquisition overall length... ☒ Wait for it...

☒ Double tweezer parameters

V0 V0(x) -0.153 (Volt) V0(y) 0.090 (Volt)

V1 V1(x) -0.333 (Volt) V1(y) 0.198 (Volt)

update ! Continuous Voltage

Generate! Record! reset AOs one Pulse!!

console Zone !!

Fig. 3 Interface of the optical tweezers control (OTC) program

3.3 Position Interpolation with Image Analysis

1. Open Matlab and launch the “position2tension.m” script that computes the table converting the control voltages of galvanometers (V_1 , V_2) into the optical trap positions (x_1 , y_1) (Matlab script provided on request).
2. Select a calibration movie (several movies can be used, but generally one is enough), and provide the first and last image numbers in the dialog box corresponding to a sequence with a clear trajectory and a good signal-to-noise ratio.
3. Select the corresponding text file containing the voltages of the galvanometer mirrors during the acquisition. The script detects the bead for each frame with a subpixel resolution by fitting a 2D Gaussian to the bead. It displays a graph showing the bead trajectory.
4. Select the circular path by mouse clicks around it. If necessary, eliminate by clicking any poorly detected points (outliers) to only keep the points that define the circle.
5. Check the quality of the interpolated map for the laser position: If many positions of the bead describing the circle are missing, repeat the operation with another calibration movie with a better signal-to-noise ratio or/and with a slower speed of the galvanometers. These images and the interpolation values are automatically saved in the same folder as the image, with the name convertFct.mat.

3.4 Two-Point Optical Manipulation

Two-point manipulation is obtained by very fast displacement of the laser focus between two positions. The signal to adjust the mirror angles switches between the two values every 4×10^{-3} s (Fig. 4), which is much shorter than the viscous relaxation time of a cell junction ~ 1 s (Ref. 4). Equal time share between two points P_1 (x_1 , y_1) and P_2 (x_2 , y_2) leads to a power split of 50% [5]. If the distance between the two points is within 20 μm , the position of the two focal points is very stable and controlled. This is a long enough distance to manipulate two different junctions belonging to each of two neighboring cells.

3.5 Selecting the Optical Trap Position on the Screen

In single-point manipulation, the motorized stage is moved to superimpose the target junction’s midpoint with the laser focus [4]. For two-point manipulation, the two points must be defined by the user according to the configuration of junctions. We use a file that records the positions of the two traps to communicate between the acquisition software (micromanager in our case) and OT program that controls the tweezers.

1. Select in the image the two positions you want to manipulate. In Micro-Manager, we select the two positions with the tool multipoint selection, we visualize the positions with Analyze>Measure (After selecting only Centroid in Analyze>Set Measurements...)

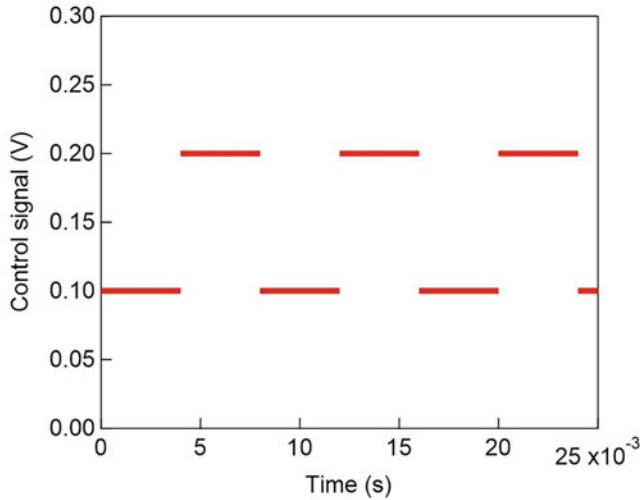


Fig. 4 Control signal sent to the galvanometers for two-point manipulation. In this example for galvanometer 1, the angle alternates between two positions every 4 msec

2. Save the selected positions $P_1(x, y)$ (pix) as a text file. 148
3. Run OTC program. The values of the control voltage are 149
selected from the calibration file (obtained in 2.6) to minimize 150
 $|P_1 - C|$ where $C(x, y)$ is the position in the calibration file. This 151
is performed for the two positions. 152
4. Select the amplitude to be imposed on the laser movement. 153
The two laser foci are moved at constant speed from the initial 154
positions. Select parallel or antiparallel movement. 155
5. Start the image acquisition (with a fast frame rate, such as 156
10 fps). 157
6. Open the shutter of the optical tweezers, and run OTC pro- 158
gram which will start sending voltages to the galvanometers 159
and record the galvanometer voltages, the status of the shutter 160
(5 is open, 0 is closed), and trigger of the camera in a text file. 161
7. After the desired time, close the shutter of the IR laser in OTC 162
program. 163
8. Stop the image acquisition. Check the acquired movie and the 164
corresponding text file giving the galvanometer voltages. 165

3.6 Sample Preparation

We use the early epithelium of the *Drosophila* embryo as a model 166
system. Experiments were carried out after cellularization and 167
before germ-band extension. Tissue movement is not too fast at 168
this stage. At later stages when tissue movements are fast, such as 169
during germ-band extension, the feedback tracking system of 170
motorized stage is required (details are in 2.11). Experiments 171
were carried out at the adherens cell junctions close to the apical 172

side, where the adhesion molecule E-cadherin is highly enriched 173
and can be imaged using fluorescently labeled lines. 174

1. Put the agarose plate with a paste of yeast in a fly cage of *Drosophila* incubated at 25 °C, and collect *Drosophila* embryos after 1 h. *Drosophila* lay eggs on the paste of yeast. Keep the plate 2–3 h after egg laying to wait until they are growing just before cellularization. 175–179
2. Collect embryos from the yeast on the plate. Dissolve the yeast by adding water and pass the liquid through a sieve (pore size should be slightly smaller than the embryo ~100 μm). 180–182
3. Remove the chorion of the embryos [6]. Soak the embryos in 50% bleach (2.6% sodium hypochlorite) for 50 s, and wash the embryos with water. 183–185
4. Put the washed embryos on an agar pad with a brush. If they are still covered by chorion, remove it by a needle. 186–187
5. Select embryos which are just after cellularization, and align them with a needle or a moist brush. Put the embryos as the target part becomes top. 188–190
6. Add glue on one side of the cover glass, and let it dry for 20 s. Attach the side of the cover glass to the embryos on the agar pad. Here, the target part becomes the bottom. 191–193
7. Cover the embryos with 200 dpi oil to avoid drying. 194
8. Put the cover slide on the stage of the microscope. 195

3.7 Optical Manipulation of Junctions in the Epithelium of the *Drosophila* Embryo

To manipulate the junction stably, the focus of the laser is moved so that it pushes outward from slightly inside the cell boundary rather than over the top of the cell boundary. 196–198

1. Turn on IR laser (1070 nm) and illumination lasers (488 nm laser and 561 nm) ~ 30 min before experiments for their power stabilizing. 199–201
2. Select the junction to target in the tissue (Fig. 5i). 202
3. Move the sample so that the midpoint of the target junction is close to the center of the image before selecting the positions to manipulate on the image, as the laser focus is precisely controlled within a limited range (Fig. 5ii). 203–206
4. Select the positions you want to manipulate in the image. Save these positions as a text file (Fig. 5iii). 207–208
5. Start the movie acquisition (with a fast frame rate ~ 5 fps) (Fig. 5iv). 209–210
6. Select the amplitude **A** to be imposed on the laser movement. 211
7. When you set the amplitude **A** to control the galvanometers, laser focus is moved at a constant speed from the initial location $\mathbf{P}_1(x_1, y_1) + \mathbf{A} \cdot t/T$, where **A** is the amplitude of the displacements of laser foci, *T* is the time period for the laser focus to 212–215

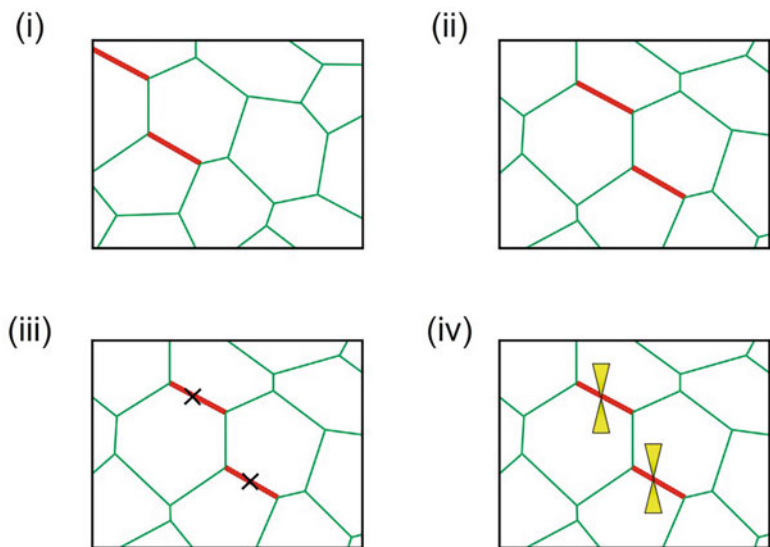


Fig. 5 Steps for two-point optical manipulation in an embryo. (i) Find the junctions to target. (ii) Move the stage to locate the junction close to the center of the image. (iii) Select the positions for manipulation. (iv) The two laser traps are moved to manipulate the two (red) junction

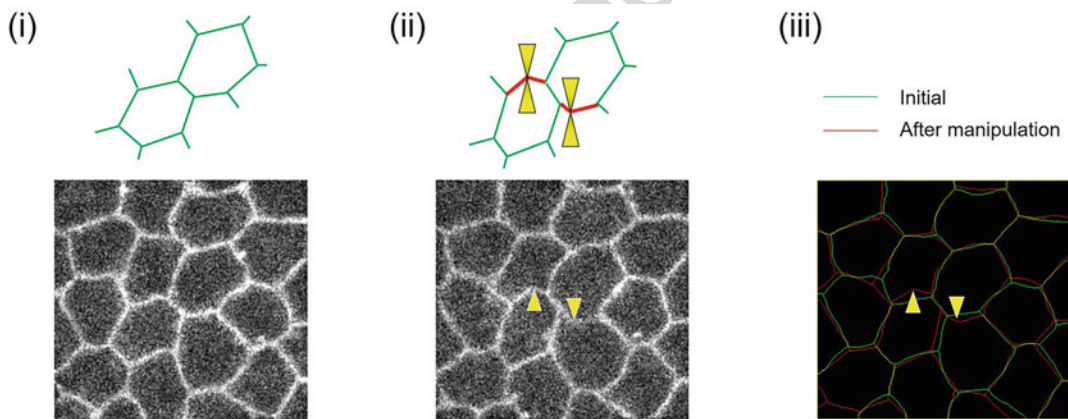


Fig. 6 Applying optical forces to two different cell junctions in an embryo
Images of cells (i) before and (ii) after manipulation. (iii) Both of the two cell junctions were observed to bend after optical force application. The yellow arrows point to junction points that are manipulated by the laser

move, and t is the elapsed time since the manipulation started. 216
The details about how to manage the positions of the two laser 217
foci can be found in (2.10). 218

8. Start the image acquisition (with a fast frame rate, such as 219
10 fps). 220
9. Open the shutter of the optical tweezers. When the optical 221
forces are successfully trapping cell junctions, one observes 222
that cell junctions bend (Fig. 6). 223

10. After the desired time, switch off the trap. 224
11. Stop the image acquisition. Check the acquired movie and the 225
corresponding text file giving the galvanometer voltages. 226
12. For efficient manipulation of the junctions, it is important to 227
pay attention to certain points (*see Notes*). 228

3.8 Extending/ Shrinking the Junction

1. To extend a junction, the two laser foci are moved in opposite 229
directions and away from each other. Acquisition must start as 230
soon as possible after selecting the points to be manipulated in 231
order to avoid drift (mainly due to tissue movement). To 232
achieve this, it is important that the laser focus be controlled 233
quickly and automatically as intended without requiring time- 234
consuming manual settings. After the initial laser foci $P_1(x_1, y_1)$ 235
and $P_2(x_2, y_2)$ are selected, they are automatically moved to the 236
direction away from each other. For example, for horizontal 237
movement, laser foci $P_1(x_1, y_1)$ and $P_2(x_2, y_2)$ are moved to P_1 238
($x_1 + A, y_1$) and $P_2(x_2 - A, y_2)$ if $x_1 > x_2$, or $P_1(x_1 - A, y_1)$ and P_2 239
($x_2 + A, y_2$) if $x_1 < x_2$ (Fig. 7i). The directions in which the laser 240
foci move are automatically determined according to the initial 241
positioning of the two points. 242
2. For junction shrinkage, laser foci are moved toward each other 243
(Fig. 7ii). 244
3. If you would like to approach the two laser foci to the same 245
position gradually, both laser foci are moved at a constant speed 246
to the middle point between two points $P_m((x_1 + x_2)/2,$ 247
($y_1 + y_2)/2$) after the initial laser foci $P_1(x_1, y_1)$ and $P_2(x_2,$ 248
 $y_2)$ are selected (Fig. 7iii). 249

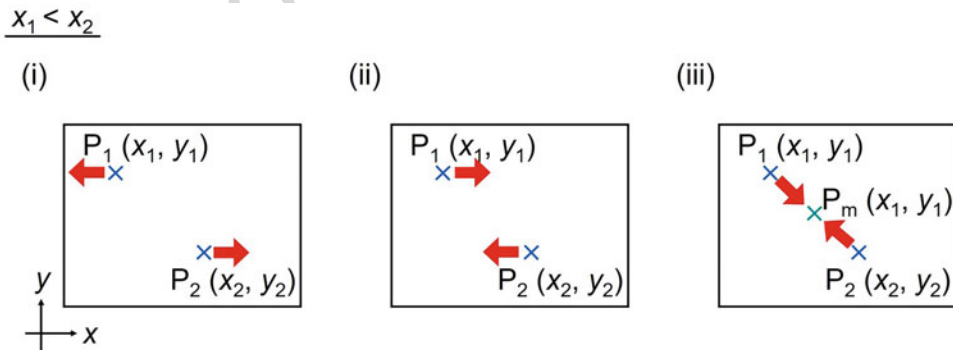


Fig. 7 Controlling the two laser focus positions. The two laser foci are moved along parallel directions away or toward each other

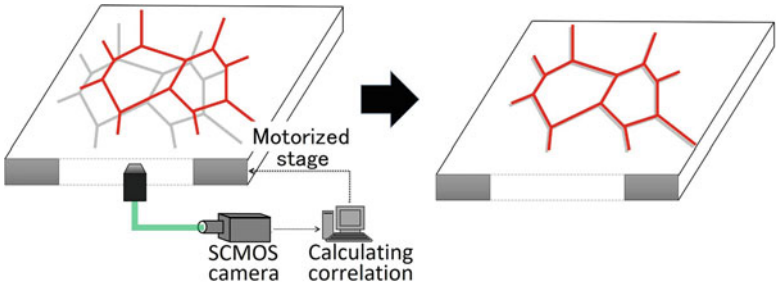


Fig. 8 Tracking tissue movement by image correlation. Tissue movement (translation here) is analyzed by calculating correlation between two images and corrected by moving the motorized stage

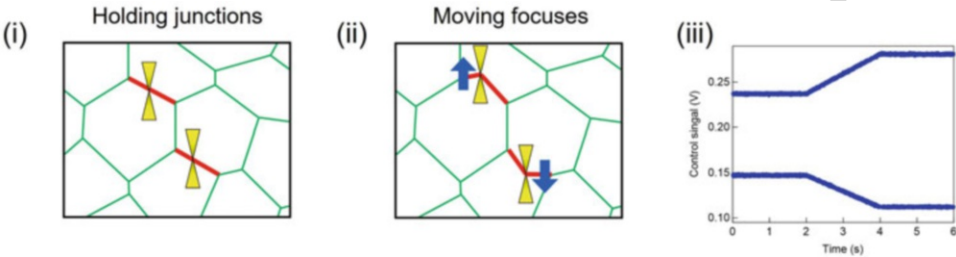


Fig. 9 Efficient manipulation of junctions. For efficient manipulation of junctions, laser foci hold the junctions first (for 2 s) and then are moved

3.9 Feedback Control for Motorized Stage

In a developing tissue, junction positions are not static: they fluctuate, move, and rearrange. Moreover, the cells can respond to the applied forces and change their shape. Therefore, we introduce a feedback control to maintain the target junction at the same position so that the manipulation is well controlled. We calculate correlation between two images to correct potential drifts during the acquisition (Fig. 8).

1. Run the macro in Micro-Manager that calculate the displacement of the tissue by correlation.
2. The motorized stage is then moved to correct the calculated drift. The target area is moved to the center of the imaging window.

4 Notes

1. Before moving laser foci, it is important to hold the junction well by laser manipulation. A few seconds after (2 s) the laser is turned on and junctions are trapped, laser foci are moved. Laser foci are kept at $P_1(x_1, y_1)$ and $P_2(x_2, y_2)$ in the first t_m seconds. After t_m seconds, laser foci are moved in constant speed from the initial place $P_1(x_1 +, y_1) + A^*(t - t_m)/T$ and $P_2(x_2 +, y_2) - A^*(t - t_m)/T$ (Fig. 9).

2. In the experiments, cells are selected close to the coverslip, in the flat part of the embryo to avoid curvature effects. 270
271
3. Laser foci should be moved close to the center of the image. If not, calibration becomes less precise due to large angular movements of the galvanometers. 272
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4. Be aware that bleaching of the fluorescence can be fast under IR illumination. Thus, it is easier to work with a stable fluorescence marker. 275
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