Measuring calcium concentration in contracting smooth muscle

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1. Abstract

The timing of the onset of uterine contractions and their synchronicity are key to successful childbirth. Uterine contractions are initiated by rises in intracellular Ca^{2+} concentration, which are caused and controlled by myometrial action potentials. The precise mechanisms responsible for the initiation and coordination of human uterine contractions remain unclear. Confocal imaging of slices of contracting uterine tissue loaded with Ca^{2+}-sensitive dye allows the visualisation of Ca^{2+} dynamics. Accurate measurements of the calcium signal in a particular part of the muscle as a function of time would enable further investigation of the relationship between Ca^{2+} concentration and muscle contraction. However, this is currently not possible because as the calcium signal increases at a particular point in the muscle, the muscle moves significantly (Figure 1).

2. Aim

To develop a computer program that will account for the transitory deformations in images of thin slices of contracting uterine tissue loaded with Ca^{2+}-sensitive dye, so that the Ca^{2+} intensity of any point in the image may be tracked over the duration of the experiment.

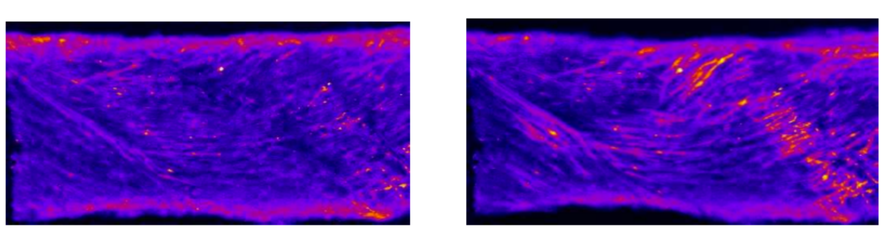


Figure 1: False colour images of a slice of contracting uterine tissue loaded with Ca^{2+}-sensitive dye. *Left*: Pre-contraction. *Right*: During contraction

3. The idea

- Initial examinations of the images revealed that there are many isolated spots of high intensity in each image frame.
- The idea is to first accurately track the movement of any of these landmarks.
- If we can accurately track the movement of enough landmarks, we can then characterise the movement of any point within the image by interpolating between the known trajectories.

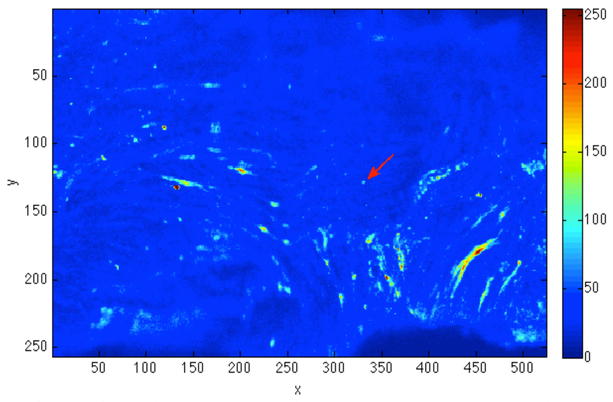


Figure 2: A single frame of an image of a uterine tissue slice, loaded with Ca^{2+}-sensitive dye, imported into MATLAB. An example of a landmark is indicated

4. Description of a landmark

- Examination of a 7×7 pixel region of interest (ROI) about a landmark in a single frame revealed that it was well described by a 2D Gaussian function. We used a nonlinear least-squares algorithm to fit a 2D Gaussian to the ROI:

$$F(x,y) = C + \frac{A}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x-\mu_x)^2}{2\sigma^2}\right) \exp\left(-\frac{(y-\mu_y)^2}{2\sigma^2}\right)$$

- Here, F is the fitted function in the 2 dimensions, x and y, and there are 5 parameters to fit:

$$A, C, \mu_x, \mu_y, \sigma^2$$

- The function, F, is fitted to the data, D, such that the squared error, r, is minimised:

$$r = \sum_x \sum_y (D(x,y) - F(x,y))^2$$

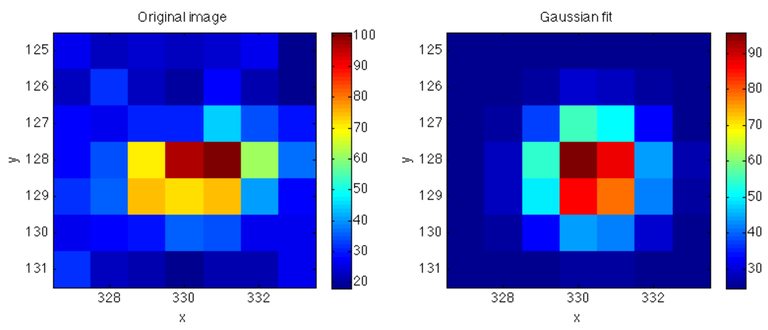


Figure 3: The image of a selected ROI around a landmark *(left)* and the fitted Gaussian function, evaluated at integer values *(right)*

5. Tracking a landmark

- The fitted mean values, (μ_x, μ_y), are used to approximate the coordinates of the centre of the landmark.
- A new 7×7 pixel ROI in the following frame is selected, centred about the coordinates of the fitted mean values, (μ_x, μ_y), from the previous frame.
- The algorithm is used to fit a new Gaussian function to this ROI, to obtain new values, $(\mu_x, \mu_y)'$.
- This process is iterated through a number of frames, to obtain a series of coordinates approximating the centre of the landmark, $(\mu_x, \mu_y)^\tau$, where $\tau = 1, 2, \ldots, T$ indicates the number of the frame in the sequence.
- This sequence of coordinates describes the position and therefore the trajectory of the centre of the landmark throughout a series of frames

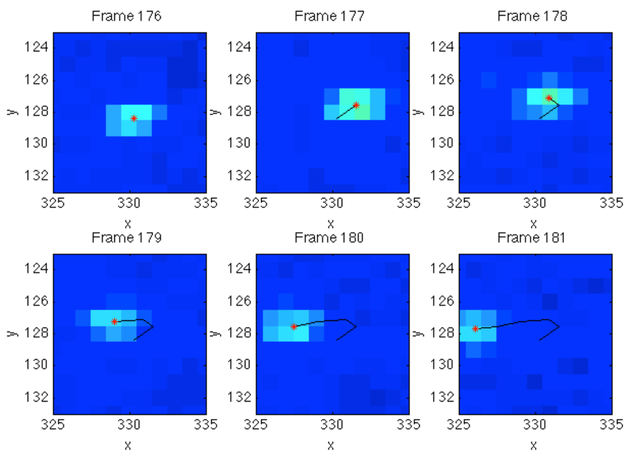


Figure 4: A landmark is tracked through 6 frames. *Red* *: fitted mean, (μ_x, μ_y). *Black line:* trajectory taken by (μ_x, μ_y)

6. Tracking multiple landmarks

- Filtering and thresholding were used to automate the selection of multiple landmarks in the first frame.

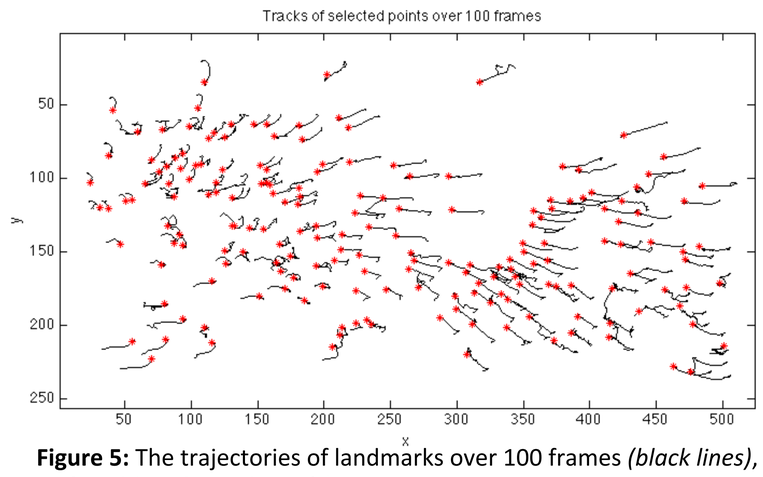


Figure 5: The trajectories of landmarks over 100 frames *(black lines)*, end position shown by red *

7. Interpolating between trajectories

- The trajectories of landmarks described as

$$\mathrm{X}_c = \{(x_c^\tau, y_c^\tau),\ \tau = 1, \ldots, T,\ c = 1, \ldots, C\}$$

for each landmark, c, at each time frame, τ.

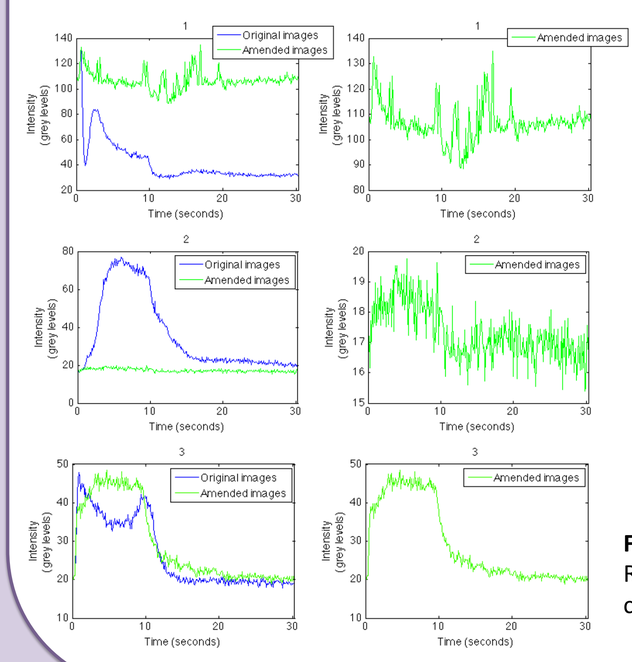
- We infer the trajectories of the remaining pixels, X, as a weighted sum of the known trajectories, according to the distance between the pixel of interest and each trajectory ($d_{\mathrm{X},\mathrm{X}_c}$):

$$\mathrm{X} = \frac{\sum_{c=1}^{C} f(d_{\mathrm{X},\mathrm{X}_c})\mathrm{X}_c}{\sum_{c=1}^{C} f(d_{\mathrm{X},\mathrm{X}_c})}$$

- We choose $f(d_{\mathrm{X},\mathrm{X}_c}) = \exp\left(\frac{d_{\mathrm{X},\mathrm{X}_c}}{\lambda}\right)$, where λ is a spatial scaling constant.

8. Measuring Ca^{2+} signal

- The intensity of any point in the first image takes the value of the pixel described by its trajectory in each subsequent image.



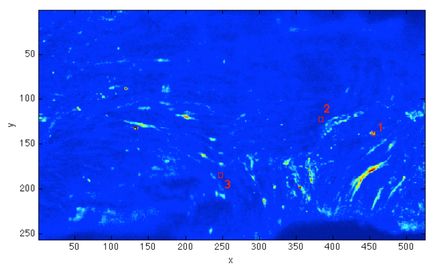


Figure 6: ROIs selected for intensity profiles

Figure 7: The intensity profiles of selected ROIs (see Figure 6) of amended images compared to original images

9. Conclusion

- We have developed a program that can account for the movement in confocal microscopy images of contracting uterine tissue, using the trajectories of selected landmarks.
- This can be used to obtain more accurate measurements of the Ca^{2+} signal in a particular part of the muscle as a function of time.
- The method of motion tracking could have applications in other areas where quantitative measures from images are hard to obtain due to movement within the sample.

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