Chapter 3

Automated Segmentation of the First Mitotic Spindle in Differential Interference Contrast Microcopy Images of *C. elegans* Embryos

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Abstract

Differential interference contrast (DIC) microscopy is a non-fluorescent microscopy technique that is commonly used to visualize the first mitotic spindle in *C. elegans* embryos. DIC movies are easy to acquire and provide data with high spatial and temporal resolution, allowing detailed investigations of the dynamics of the spindle—which elongates, oscillates, and is positioned asymmetrically. Despite the immense amount of information such movies provide, they are normally only used to draw qualitative conclusion based on manual inspection. We have developed an algorithm to automatically segment the mitotic spindle in DIC movies of *C. elegans* embryos, determine the position of centrosomes, quantify the morphology and motions of the spindle, and track these features over time. This method should be widely useful for studying the first mitotic spindle in *C. elegans*.

Key words DIC microscopy, *C. elegans*, Mitotic spindle, Active contour method, Centrosome

1 Introduction

Automated analysis of DIC movies is challenging because of the complex nature of the images. Fluorescence microscopy of spindle-associated proteins, such as gamma-tubulin::GFP [1], provides high-contrast images that are more readily amenable to automated image analysis, but requires genetic manipulation that could produce unintended perturbations, is laborious if large numbers of different genetic backgrounds need to be studied, and requires the use of expensive confocal microscopes to obtain high resolution images. Thus, DIC is still widely used to study the first mitotic cell division in *C. elegans* embryos, and it is highly desirable to extract quantitative data from the obtained movies in a robust fashion.

In this chapter, we introduce an algorithm, based on active contour methods [2], to segment spindles in DIC images of *C. elegans* embryos and determine the position of the centrosomes—the

David J. Sharp (ed.), *Mitosis: Methods and Protocols*, Methods in Molecular Biology, vol. 1136,
DOI 10.1007/978-1-4939-0329-0_3,

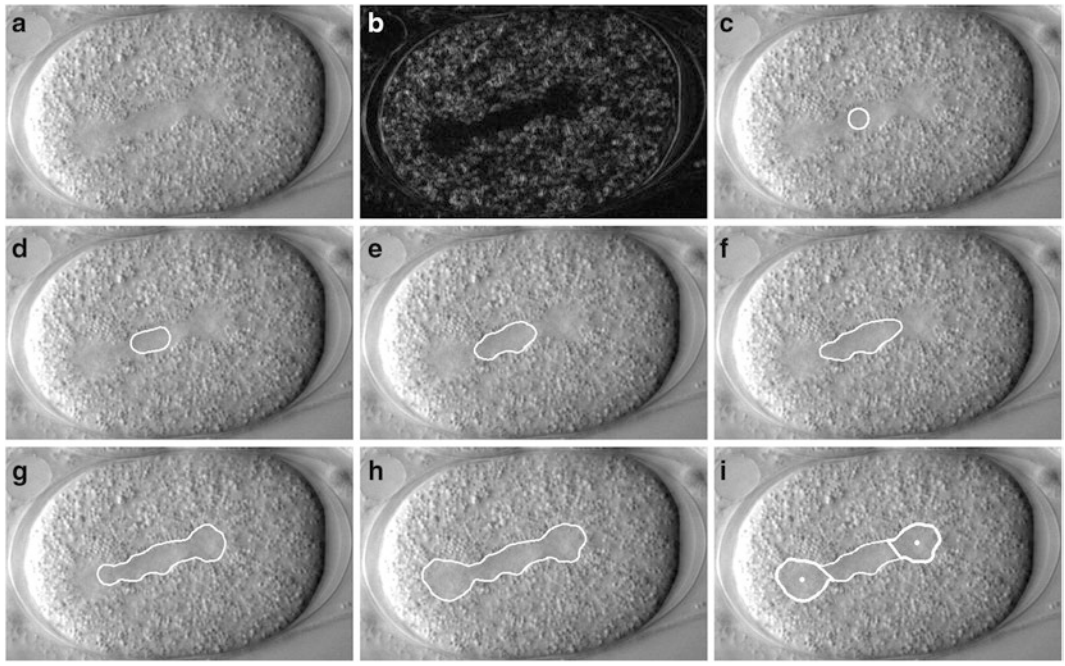

Fig. 1 Segmentation of the first *C. elegans* mitotic spindle in a DIC microscopy image: (**a**) Raw image. (**b**) Smoothed Gaussian derivative of the raw image in (**a**). (**c**–**h**): Time evolution of the active contour starting from an arbitrary shape in (**c**). (**i**): Centrosome positions for segmented spindle

organizing centers that define the bipolar structure of the spindle. In this algorithm, we use a deformable contour and define internal and external forces such that the contour maintains a smooth shape and evolves from an initial configuration to find the shape of the spindle. These forces are derived from a potential function, which has only two free parameters.

2 Methods

1. Obtain a time-lapse DIC microscopy movie of the first mitotic division of a *C. elegans* embryo as described in [3] (*see* **Note 1**).
2. Select a frame of the movie to initiate the image-processing steps (*see* Fig. 1a). The pixel intensity at position (i,j) of this image is denoted by $I(i,j)$.
3. Calculate the smoothed gradients of the image along the x and y directions

$$G_x(x,y) = \sum_{i,j} I(i,j) \frac{i-x}{2\pi\sigma^4} \exp\left(-\frac{(x-i)^2+(y-j)^2}{2\sigma^2}\right)$$

$$G_y(x,y) = \sum_{i,j} I(i,j) \frac{j-y}{2\pi\sigma^4} \exp\left(-\frac{(x-i)^2+(y-j)^2}{2\sigma^2}\right)$$

Here, σ is the width of the smoothing Gaussian filter (*see* **Note 2**).

4. Calculate the gradient image $G(x,y) = \sqrt{G_x^2(x,y) + G_y^2(x,y)}$ (*see* Fig. 1b).
5. Calculate the normalized gradient image $N(x,y) = G(x,y)/\max(G)$, where $\max(G)$ is the maximum value of $G(x,y)$.
6. Define an arbitrary closed curve $\Gamma(s) = (x(s), y(s))$ (s is the arc length) inside the spindle region as an initial guess for spindle shape (*see* **Note 3** and Fig. 1c).
7. The final shape of the spindle is determined by a curve $\Gamma_m(s)$ that minimizes the energy functional $E[\Gamma(s)]$

$$E\left[\Gamma(s)\right] = \oint_{\Gamma[s]} \frac{\alpha}{2} |\Gamma_{ss}|^2 \, ds + \iint_R \left(N(x,y) - \beta\right) dx dy,$$

where $\Gamma_{ss} = \partial^2\Gamma/\partial s^2$. The first term is a bending energy that keeps the contour smooth. The second integral is over the area enclosed by the curve $\Gamma(s)$. A value of β is chosen such that the second term makes the curve grow if it is inside the spindle region and shrink if it is outside the spindle region. This energy can be minimized by iteratively updating the contour using a gradient decent method (*see* **Notes 4** and **5** and Fig. 1c–h).

8. To dissect a centrosomal region from the minimal curve $\Gamma_m(s)$, first rotate and center the contour along the x-axis such that the center of the spindle is located at $x = 0$. For the region of the contour that is located in $x > 0$, connect the inflection points or the negative curvature points on the two sides of this region. The centrosome position is chosen to be the geometric center of the dissected area (*see* Fig. 1i). Repeat this procedure for the region in $x < 0$.
9. To segment the spindle for the next frame of the movie, choose $\Gamma_m(s)$ of the current frame as initial curve for the minimization. Then iterate until all images in the movie have been segmented (*see* Fig. 2a–f).

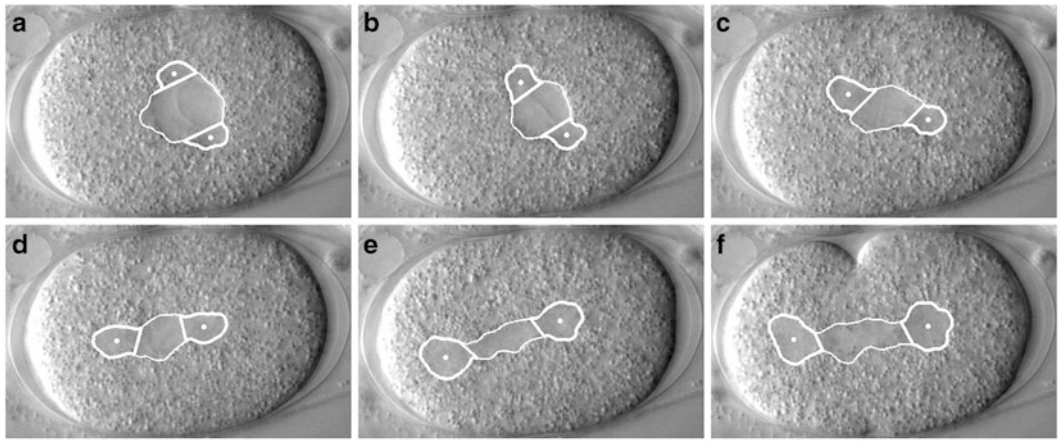

Fig. 2 Tracking spindle for a time-lapse movie: (**a**)–(**f**) segmented spindle and centrosomes for sequential frames of a time-lapse movie

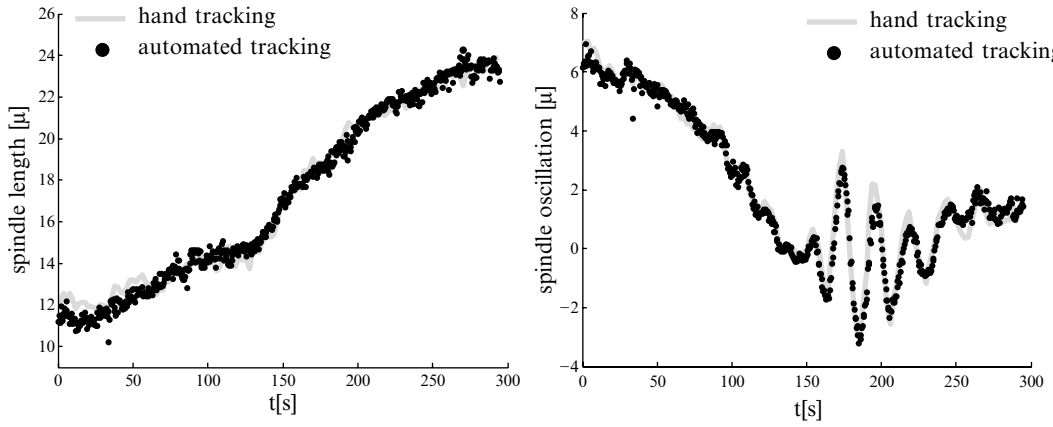

Fig. 3 Spindle length and spindle oscillation as a function of time for embryo shown in Fig. 2. *Gray solid line* is manual tracking

10. Now the positions of the centrosomes have been calculated for each image. The length of the spindle (i.e., the distance between centrosomes) can be plotted as a function of time, allowing spindle elongation to be studied in detail. The oscillations of the spindle can be studied by plotting the Y position of the centrosome as a function of time. We confirmed that the presented automated tracking procedure gives the same results as manually tracking the spindle length and spindle oscillation by hand (Fig. 3a, b).

3 Notes

1. The spindle should appear as a connected smooth region inside the cell surrounded by granule lipid particles.
2. The value of σ should be large enough such that spindle appears as a smooth black region in the derivative image (*see* Fig. 1b).
3. Although this method is robust to variation in shape of the initial curve, to ensure efficient minimization, choose the initial curve not too different from the spindle shape.
4. The first term in $E(\Gamma(s))$ ensures smoothness of the curve by preventing formation of sharp corners with high curvatures. The second term is the sum of pixel intensity of the normalized derivative image inside the region enclosed by the curve $\Gamma(s)$. A balance between the second term and the third term, which is a constant multiplied by the area of this region, determines the final shape of the spindle.
5. One method to minimize the energy functional $E[\Gamma(s)]$ is the steepest descent method. In this method, we assume that the

contour is a function of both arc length and time, i.e., $\Gamma = \Gamma(s,t)$. The steady solution of the equation

$$\Gamma_t(s,t) = -\frac{\delta E[\Gamma]}{\delta \Gamma} = -\alpha \Gamma_{ssss}(s,t) - \left(N\left(\Gamma(s,t)\right) - \beta\right)\vec{n},$$

where $\vec{n} = \left(y_{s,} - x_s\right)$ is the normal vector to the curve $\Gamma(s,t)$, gives us the minimal curve.

Acknowledgment

This work was supported by grant RGP0034/2010 from the Human Frontiers Science Program.

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