The Time Series Image Analysis of the HeLa Cell Using Viscous Fluid Registration

Soichiro Tokuhisa and Kunihiko Kaneko

Department of Intelligent Systems, Graduate School of Information Science and Electrical Engineering, Kyushu University
744 Motooka, Nishi, Fukuoka, Japan
{tokuhisa,kaneko}@db.is.kyushu-u.ac.jp

Abstract. Optical microscopy image analysis is important in the life science research. To obtain the motion of the cell, we use the viscous fluid registration method based on fluid dynamics. Viscous fluid registration deforms an image at time t to the next image at time t+1. In this algorithm, there is a problem that an object cannot be divided into two. In other words, the divided objects from one are connected by thin line because the velocity field on the connected thin line is zero. To solve this problem, we suggest a new viscous fluid registration algorithm for the object division. This algorithm is only added similarity maximization step to correct the displacement in the near pixels in the original viscous fluid registration. Using this method, one object is divided into two, and divided objects are not connected. We experiment the anaphase detection based on a nucleus identification using laser scanning microscope HeLa cell images. Experimental result shows that 74 in 76 cells are tracking well and 6 cells in the anaphase are detected. In three scenes in the cell division which can not be divided into two using original viscous fluid registration, suggested algorithm can be divided into two cells completely.

Keywords: HeLa cell, cell division, cell tracking, viscous fluid registration.

1 Introduction

Microscopy image analysis is important in the life science research. Cell phase identification and analysis of the cell features are attracted. The abnormal cell detection method helps early detection of a cancer, diabetes, and the gene deficit disease. Therefore early treatment for disease is enabled and helps medical care.

In the field of the cell analysis, various image processing methods are performed in cell segmentation and the phase identification. For example, it is suggested the accurate automated method that cell segmentation using watershed algorithm and the cell phase identification using Markov model by Zhou et al. [1] and Chen et al. [2]. Dzyubachyk et al. suggested the multiple level-set framework that each cell have each level-set function [3].

We think that the cell movement can be expressed by the viscous fluid, so the cell movement can be described as the time series image matching technique. We use the

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image registration method applied in the medical image processing such as the CT image and MRI image. Though this method has a large calculation cost, it is obtained the smooth displacement because it is based on the fluid dynamics. The displacement contains vectors $\boldsymbol{x}_t - \boldsymbol{x}_{t-1}$. It means that a pixel at position $\boldsymbol{x}_t$ at time t is moved from a pixel at position $\boldsymbol{x}_{t-1}$ at time t-1.

There are some kinds in a microscopy images such as an optical microscopy image and confocal laser scanning microscope. The three-dimensional cell image can be constructed using confocal laser scanning microscope. In this experiment, we use these cell images in Fig.1 that are obtained by live cell imaging [4] of the HeLa cell with mCherry-NLS[5] via the confocal laser scanning microscope created by Yuki Tsujimura in RIKEN.

When an object is divided into two objects, viscous fluid registration cannot to be separate correctly. So, we suggest the new algorithm that is added one calculation step in the viscous fluid registration which step is the similarity maximization step to correct the displacement in the near pixels.

When this method is used, we can divide an object to two objects completely. Using viscous fluid registration with similarity maximization step, it will be performed a smooth cell movement analysis. The displacement can be use for the time series image analysis like Fig.2. The displacement is useful for anaphase detection because the corresponding time series cell at time t and cell at time t+1 can be detected. The cell in anaphase is defined the first time of the cells called daughter cell which was divided one cell into two in this article. From the cell identification result, it can be confirmed the movement of the daughter cells after the cell division. Cell identification result shows that 74 in 76 cell in 17 images is success in tracking.

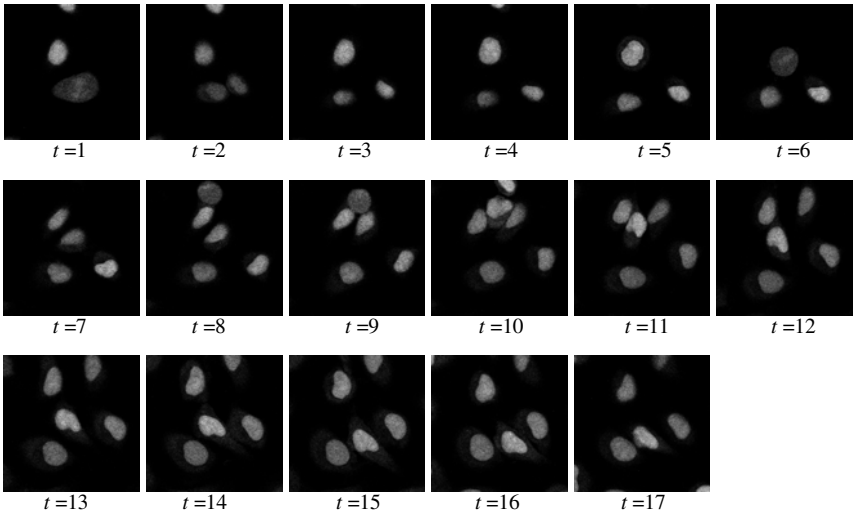

Fig. 1. Time series image data created by Yuki Tsujimura in RIKEN

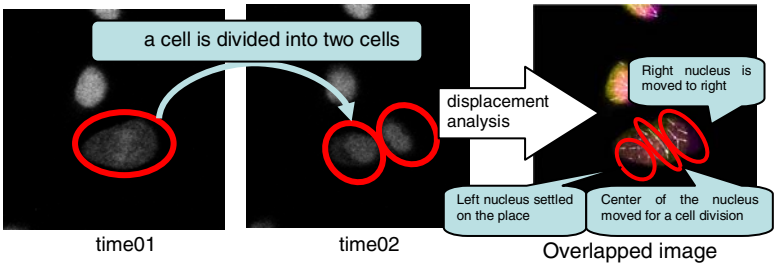

Fig. 2. Example of the time series image analysis. The displacement calculated by viscous fluid registration shows the white arrow in right image named "Overlapped image". These arrows show a pixel in time01 is moved to a pixel in time02.

2 Viscous Fluid Registration

Viscous fluid registration is the non-rigid image registration method [6] to deformed one image to the other image based on viscous fluid dynamics. It is used for adjustment the shape and appearance of two medical images. When this application implemented by C++ language is given a template image, a study image and parameters as input data, it put the output files containing a transformed image and a total displacement file. The deformed image is deformed a template image into a study image. Viscosity fluid registration is calculated three fields the number of repetition times. A force field, a velocity field and a displacement are included in three fields (Fig.3). The number of repetition is defined in an input parameter. In addition, a multi-resolution method is implemented for speedup of calculation and robustness of a registration. Using the example image set that are sample01 (contains in a black square) and sample02 (contains in a white "C"), sample01 is deformed the shape, and the application put the deformed image that is resembles the shape of "C" (Fig.4). The parameters are viscosity=20, rMin=1, rMax=8, calculation number=100. The parameters of the image of the cell are viscosity=60, rMin=1, rMax=8, calculation number=10. There is the explanation of the parameters to 2.1 and 2.2, and we explain the viscous fluid registration algorithm in the section 2.1. In the section 2.2, we explain the two-dimensional viscous image registration workflow using multi-resolution method.

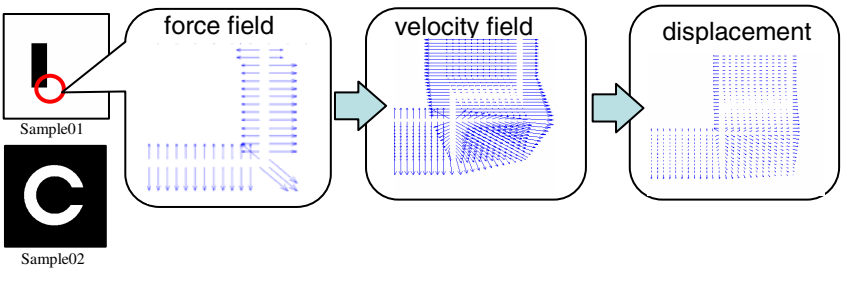

Fig. 3. Example of a force field, a velocity field and a displacement calculated first time

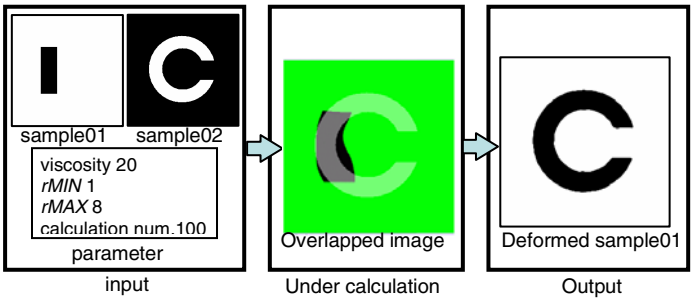

Fig. 4. Example of the viscous fluid registration

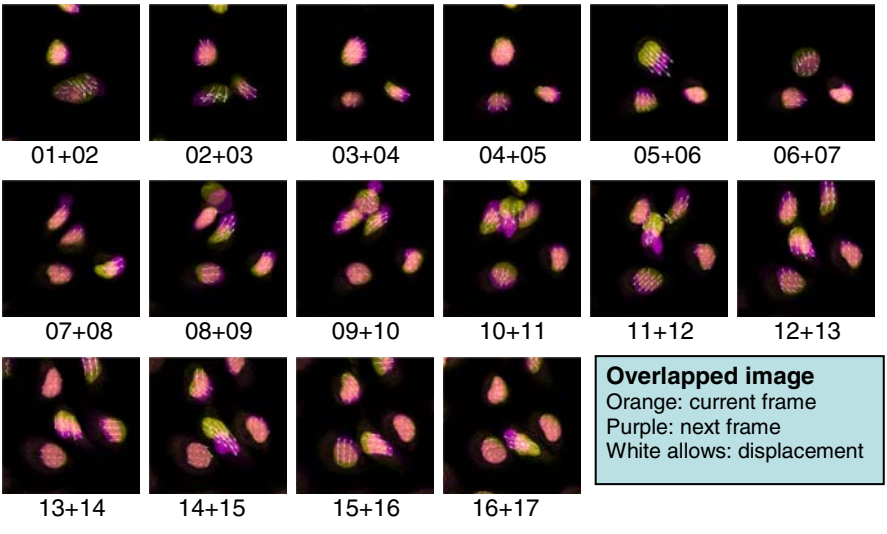

Fig. 5. Overlapped current frame image (orange) on next frame image (purple)

2.1 Viscous Fluid Registration Algorithm

Viscous fluid registration is the image registration method to align two images by deforming one image to the other [7] [8]. A viscous fluid registration is based on the theory of a viscous fluid dynamics. This algorithm contains the calculation of three vector fields: a force field, a velocity field, and a displacement. These vector fields are a set of two-dimensional vectors that are x-axis and y-axis values because we perform two-dimensional viscous fluid registration for all images. We use sum of squared difference as an image similarity measure. Sum of squared difference is used to calculate the force field. The force field is equal to the derivative of image similarity measure. The force field equation can be written as follow:

$$\boldsymbol{f}(\boldsymbol{x}, \boldsymbol{u}(\boldsymbol{x}, t)) = -[T(\boldsymbol{x} - \boldsymbol{u}(\boldsymbol{x}, t)) - S(\boldsymbol{x})]\nabla T(\boldsymbol{x} - \boldsymbol{u}(\boldsymbol{x}, t)) \quad (1)$$

where $\boldsymbol{f}(\boldsymbol{x}, \boldsymbol{u}(\boldsymbol{x}, t))$ is the force field at point $\boldsymbol{x}$ and at time t that depends on the displacement $\boldsymbol{u}(\boldsymbol{x}, t)$,. $T(\boldsymbol{x})$ is the pixel value at point $\boldsymbol{x}$ on template image T, and $S(\boldsymbol{x})$ is the pixel value at point $\boldsymbol{x}$ on study image S. ∇T is calculated by sobel filter. The velocity field is equal to the convolution of the force field with the Gaussian kernel. The velocity field $\boldsymbol{v}(\boldsymbol{x},\boldsymbol{u}(\boldsymbol{x},t))$ can be written as follow:

$$\boldsymbol{v}(\boldsymbol{x}, \boldsymbol{u}(\boldsymbol{x}, t)) = G_\sigma (\mathrm{f}(\boldsymbol{x}, \boldsymbol{u}(\boldsymbol{x}, t)) \tag{2}$$

The Gaussian kernel G_σ has two parameters: window size w and standard deviation σ. We define this standard deviation σ as the viscosity. The value of the viscosity determines the degree of freedom of the deformation to limit the movement of the pixel at point $\boldsymbol{x}$. The window size w is defined as follow:

$$w = \frac{6\sigma}{r} \tag{3}$$

where r is the resolution level that allows for reducing the image size. If the multi-resolution method is not used, the resolution level is set to 1. Using this window size w, the velocity at $\boldsymbol{x}$ is convolved in 99.74% force field affected to the viscosity at $\boldsymbol{x}$ according to Gaussian distribution. Displacement $\boldsymbol{u}(\boldsymbol{x}, t)$ is calculated as follow:

$$\mathbf{u}(\mathbf{x}, t_{i+1}) = \mathbf{u}(\mathbf{x}, t_i) - (t_{i+1} - t_i)\ (\mathbf{I} - \nabla \mathbf{u}(\mathbf{x}, t_i))\mathbf{v}(\mathbf{x}, t_i) \tag{4}$$

where the velocity field $\mathbf{v}(\boldsymbol{x},\boldsymbol{u}(\boldsymbol{x},t))$ is equal to the Lagrange derivative of displacement $\mathbf{u}(\mathbf{x}, t)$. Matrix $\boldsymbol{I}$ is the identity matrix. The time $(t_{i+1}-t_i)$ is calculated by the following equation:

$$MAX(|\boldsymbol{v}(\boldsymbol{x}, t_i)|)(t_{i+1} - t_i) = du_{\max} \cdot r \tag{5}$$

where $du_{\max}$ is the maximal movement allowed in one iteration. We set du_{max} as a fixed value of 0.7 (pixel). If $|\boldsymbol{I} - \nabla \boldsymbol{u}(\boldsymbol{x}, t)|$ is less than 0.5, the displacement $\mathbf{u}(\mathbf{x}, t)$ is applied to the image, and the displacement $\boldsymbol{u}(\boldsymbol{x}, t)$ and time t are initially set to 0, because the displacement is singular for large curved deformation. The total displacement is calculated as follow:

$$\boldsymbol{u}_{\text{total}}(\boldsymbol{x}, t_i) = \boldsymbol{u}(\boldsymbol{x}, t_i) + \boldsymbol{u}_{\text{total}}(\boldsymbol{x} - \boldsymbol{u}(\boldsymbol{x}, t_i), t_{i-1}) \tag{6}$$

2.2 Two-Dimensional Viscous Fluid Registration

Viscous fluid registration algorithm requires four input parameters: the maximum resolution level *rMAX*, the minimum resolution level *rMin*, the calculation number k, and the viscosity σ. We use a multi-resolution method that enables fast and robust image registration. In the multi-resolution method, image I_r in the current resolution level r is used. Image I_r is reduced in size by resolution level r. If resolution level r is 2, the image size is reduced by half. Image I_r is defined as follows:

$$I_r(x_r, y_r) = I_1(r \cdot x_r, r \cdot y_r) \tag{7}$$

$$1 \le x_r \le \frac{width}{r} \quad 1 \le y_r \le \frac{height}{r} \tag{8}$$

where x_r and y_r are array numbers and they are constrained by (9). The current resolution level r is taken as 1, 2, 4, 8,…,2^n. The values of the *width* and *height* are obtained by the image width and image height at resolution level 1.

The viscous fluid registration method is calculated three vector field. In the first step, it calculates the force field value using data from two images. In the second step, it calculates the velocity field using the force field. In the third step, it calculates the displacement using the velocity field. When these three steps are finished, it increments the iteration number and repeats the three steps. If the iteration number is equal to the calculation number, the resolution level is divided by 2, and the iteration number is initialized to 0. After initialization, the three calculation steps are repeated. The resolution level indicates the scale of the image. For example, if the resolution level is 1, it indicates the original image, and if the resolution parameter is 4, it indicates that an image size is quarter. At the beginning of a calculation, the resolution level is set to the maximum resolution level. If the resolution level is equal to the minimum resolution level, it generates output data, including the total displacement and the image deformed using displacement.

2.3 Similarity Maximization Step

To divide one object into two, we suggest adding the similarity maximization step in viscous fluid registration. To confirm this technique, we used two image set in Fig.6, left image set is shown that one circle is divided into two circles, and right image set is shown that one square is divided into two squares. Using original viscous fluid registration, two objects are connected by the thin line (see 40calculation in Fig.7). The force field over and under the line have in the opposite direction (see Fig.8). Because the velocity is calculated by the convolution of the force field, the velocity field on the line is calculated to zero. And the displacement is in proportion to the velocity, displacement on the connected line is zero. After all calculation, there are gaps between the displacement on the connected line and over and under the line. Therefore I introduce a similarity maximization step to approximate the displacement at the point $\boldsymbol{x}$ by near the point $\boldsymbol{x}$. The similarity maximization step is described as follow:

$$\mathbf{u}_{\text{total}}(\mathbf{x}, t_i) = MIN\{\left(T(\mathbf{x} - \mathbf{u}(\mathbf{x}, t)) - S(\mathbf{x})\right)^2 \mid \mathbf{x}-1 \le \mathbf{x} \le \mathbf{x}+1\} \tag{9}$$

This equation maximizes an image similarity measure by minimizing the square error of the difference. This step is added after the calculation of the total displacement $\boldsymbol{u}_{\text{total}}$ in (6). Using this step, deformed result is shown in Fig.9, and the deformed grid image is shown in Fig.10. Fig.10 shows that image edge is enhanced in circle grid image using the new step. This means that fluency of the motion vector is decreased, and the issue of connected line was solved.

Fig. 6. two image set. Left is circle division example. Right is square division example

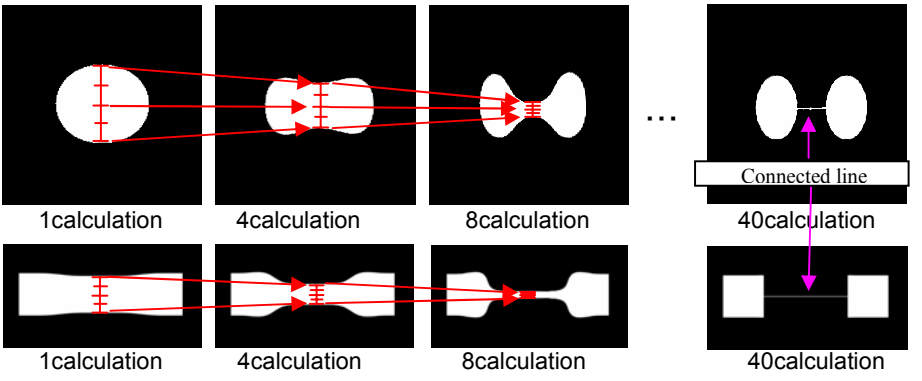

Fig. 7. Cconnected line is not go disappear using original viscous fluid registration. Top images are circle image result. Bottom images are a square image result.

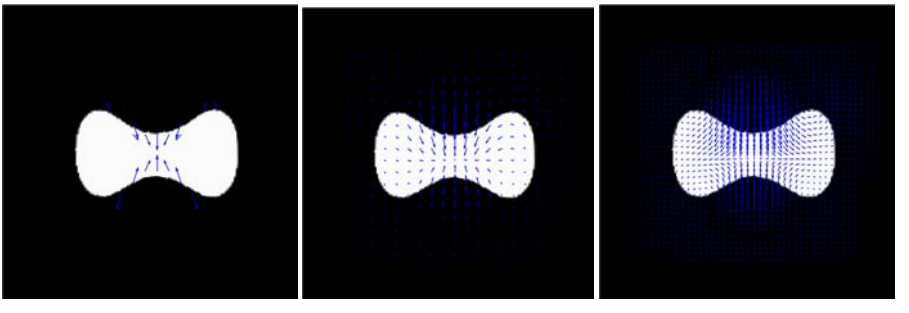

Fig. 8. Force field, velocity field, and displacement at 7calculation in circle image

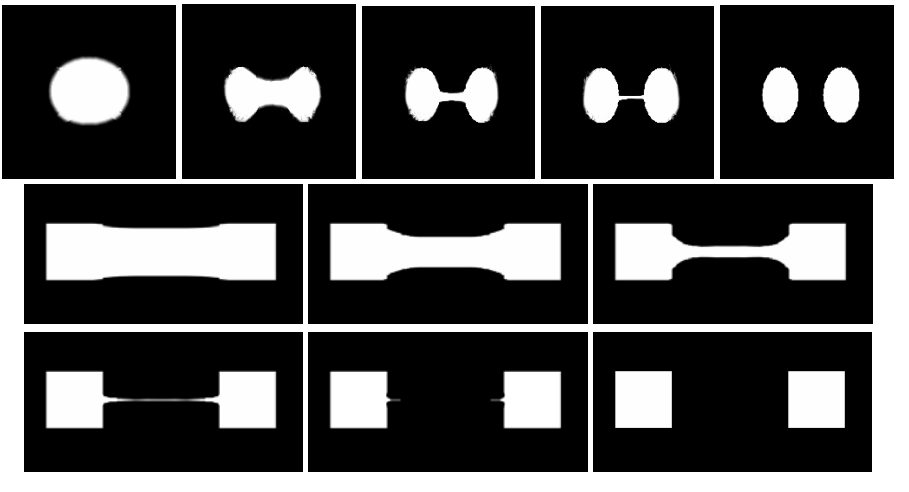

Fig. 9. Disappear connected line adding Similarity maximization step in registration. Top line images are circle image result. center and bottom line images are sqare image result.

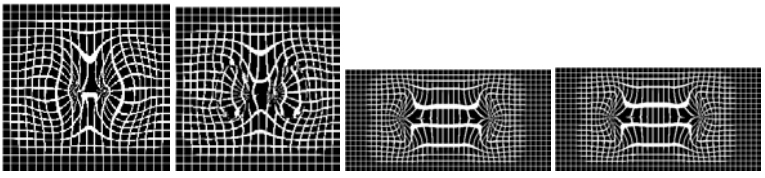

Fig. 10. Deformed grid image. Left two images are deformed circle grid result. Right two images are deformed square grid result. In these set, left is not using step result, right is using step result.

3 Experiment

We experiment the detection of the anaphase in the time series HeLa cell images. Anaphase detection consists of the three steps: image processing, viscous fluid registration and cell identification. We use 17 bitmap images in Fig.1. These images are 350*350 size and 8bit grayscale data created by Yuki Tsujimura in RIKEN. These images contain 76 cells. The resolution of the image is 0.286um in x and y direction and time span between images is 1 hour.

At first we generate a label image by image processing. This step is described in 3.1. In the next step, we perform viscous fluid registration time series images. This second step is described in 3.2. Finally, we perform time to time cell identification by using of the displacement calculated by viscous fluid registration. If corresponding cell is not found after the registration, these cells make pair of a nearest cell in the previous time image. After three steps, we make cell identification in all time, so we can detect a cell in anaphase because these cells are divided into two. This last step and anaphase detection are described in 3.3.

3.1 Image Processing

For the cell identification, we generate a label image from grayscale image using hybrid image processing. This image processing is implemented by the octave language. This method contains five processes: thresholding, median filter, erosion, labeling and dilation(Fig.11). Thresholding process make binary image from grayscale image. Median filter process removes white noise in the cell from binary image. Erosion process makes the cell area shrink. Labeling process add label data on the cell. Dilation process makes the cell area inflated. Dilation and erosion is used for division cell because some cells are touching together. After labeling, label image is displayed by a color using color table. So, label image have pixel values such as 0 on the background and 1, 2, 3… on each independent cell. In this process, 17 label images are created. Regulating parameters at each time are described in Table 1.

Table 1. Parameters list in Image processing at each time

time	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
threshold	30	30	30	30	30	30	30	50	70	70	70	30	30	30	30	60	60
erosion and diration repeat numver	0	0	0	0	0	0	0	5	6	5	5	0	0	0	0	0	0

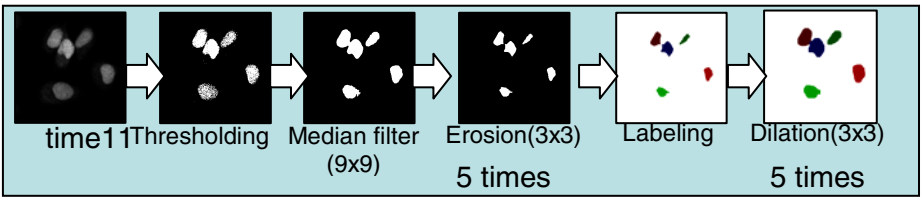

Fig. 11. Image processing flow example at time 10

3.2 Viscous Fluid Registration

In this step, we input two time series images into viscous fluid registration to get the displacement. The label images are not used in this step. The result of the original viscous fluid registration is shown in Fig.12, and the result of the suggested viscous fluid registration added the similarity maximization step is shown in Fig.13. We can find that a cell is divided obviously in suggested technique. As for this technique, limitation of the transformation becomes lax, so the deformed cell shape almost same as input study image.

By the next step, a label image in Fig.11 is deformed using the displacement, and determined the correspondence of the pair of the cells in time *t* and time *t*+1 image. If the cells in time *t* and time *t*+1 image exist in the same position, these cells are treated as the correspondence of the pair. And when cell division occurs, cell at time t is divided into two cells at time t+1, so we can distinguish the cells in Anaphase by checking label numbers are same value or not.

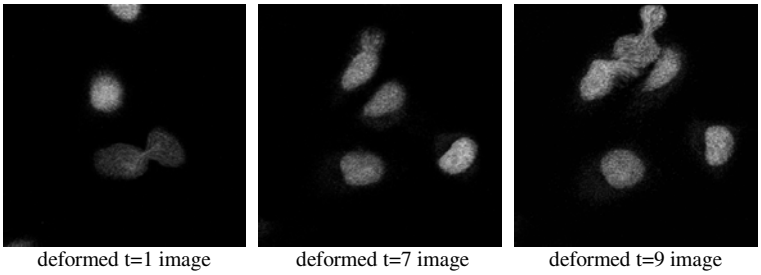

Fig. 12. Original viscous fluid registration result when cell division is occurred

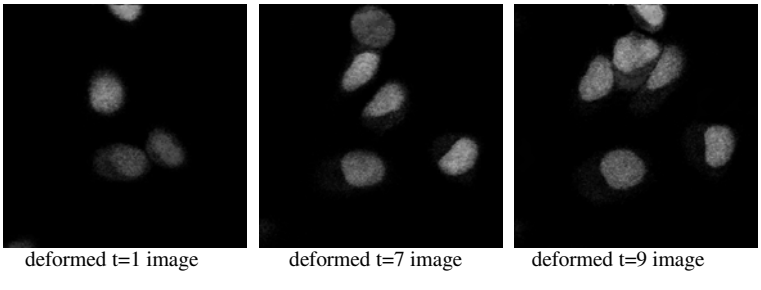

Fig. 13. Suggested viscous fluid registration result when cell division is occurred

3.3 Cell Identification and Anaphase Detection

In this step, cell identification and anaphase detection is performed. The label images and the displacement is used for cell identification. Current time label of cell is taken the correspondence of the next time label. The correspondence of the label is found from the relations of a cell position between deformed current time label and next time label. We calculate histogram of deformed current time label image and next time label image. And next time label value is changed the value of the most overlapped label from deformed current time label image (Fig.14 left). Most overlapped label is found from the histogram. If there are same labels in the next time label image, it found that anaphase is detected. Once anaphase is detected in one label value,

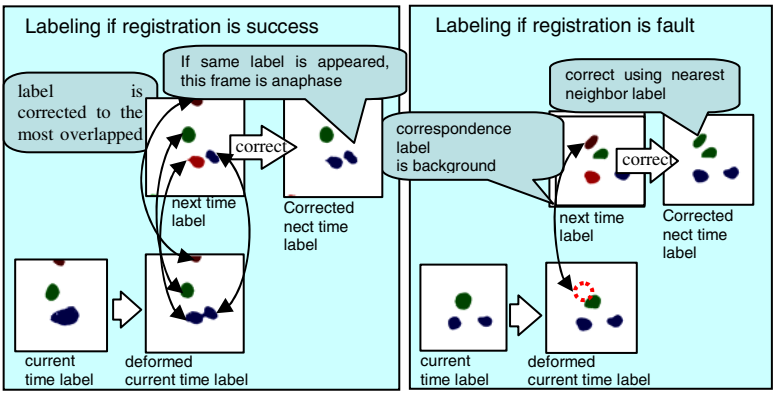

Fig. 14. Procedure of a correcting label

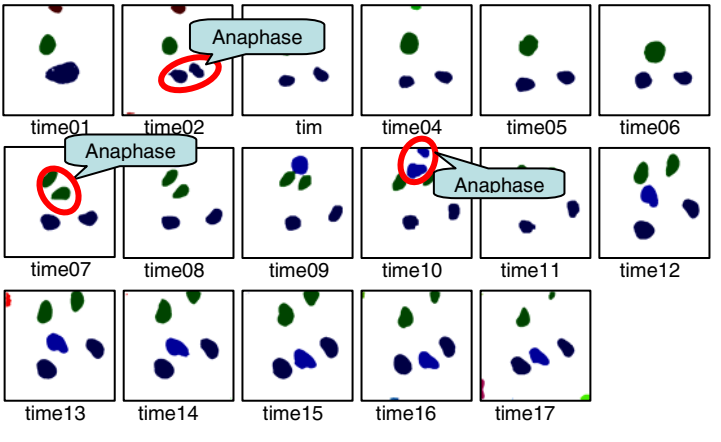

Fig. 15. Result of a detection of anaphase. Dividing cell in anaphase is shown in the red circle

create flag on this label value. This mean anaphase is already found on this label. So, if flag is created, anaphase is not detected on this label. There is the case that registration is fault because the next time cell and deformed cell is not overlapped well. In other words, the corrected label value candidate has the background value (Fig.14 right). When a label candidate became the background, nearest neighbor label is given. In 17 images, two cells are detected by nearest neighbors. This nearest neighbor process is adapted only center of the image. If the center of gravity of a label exists on the area of 40 pixels from boundary of the image, this cell is not regard as in anaphase because this cell moves image boundary to inside and outside. Fig.15 shows the result of corrected all label images.

4 Time Series Image Analysis

To observe the movement of the cell division, all label images are overlapped adapted each image in transparent percentage of 1/17, except for the cell which appeared first is transparency 50% and the cell boundary displays in white and which appeared last time is transparency 0% and the cell boundary displays in black. From this image, it can be observed that three cells are divided into two, and daughter cells are moved to different direction.

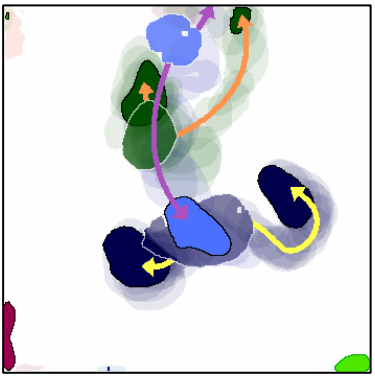

Fig. 16. Overlapped time series label images. This image shows the dividing nucleus movement. The boundary line of nucleus that appears the first is white and that appear the last time is black. A cell color from the second and before last nucleus is semitransparent. Dark blue nucleus is divided and daughter nuclei are moved to left and right (yellow allow), green daughter ones are moved to top and right-top (red allow), and blue ones are moved to top and bottom (purple allow).

5 Conclusion

We can find that divided objects are connected by a thin line using original viscous fluid registration. So we suggest a new viscous fluid registration algorithm for an object division. This algorithm is to add new step in original viscous fluid registration.

We confirmed that connected line which appeared in original viscous fluid registration result is disappeared by adding new step in two sample image set and time series HeLa cell images. We examine the anaphase detection to confirm that new algorithm is worked well. By creating label image and deforming label image using displacement, the cell identification is success 74 in 76 cells. 2 of the remainder cell can be tracked by nearest neighbor processing. A cell in anaphase is detected be cell identification, and daughter cell movement is observed by the overlapped time series label image. Displacement calculated by viscous fluid registration is useful in various ways. Using displacement, we can observe cell movement like Fig.2.

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