2D tracking of single micro-organism based on parallel level set method with intensity constraint

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We propose a 2D microorganism tracking system using parallel level set method (LSM) and column parallel vision system (CPV). The proposed new energy function of LSM constrains the amount of light intensity inside detected object contours to control the number of detected objects. This algorithm is implemented in CPV system and computational time for each frame is 2 [ms], approximately. Based on the single target detection and automated stage PD controlling, the single microorganism is tracked to be in the middle of visual field under a microscope. We demonstrated a tracking experiment for about 37 s which shows that a single paramecium can be kept tracking even if other paramecia appear in the visual field and contact with tracked paramecium.

Key Words: level set method, visual servoing, microscope.

1 Introduction

It is difficult for manual microscopes to observe a motile cell, since the motile cell can quickly go out of the visual field of manual microscopes. The authors have developed self-window algorithm and visual servo to realize an automatic microscope which can track the motile cell with the high spatial resolution [1, 2]. However, self window algorithm is not robust for collision problems which is a tracked cell colliding with another cell[3]. Therefore, in order to solve the collision problem, we adopts Level Set Method (LSM) as our image processing framework [4], because the formulation of LSM is expressed as differential expressions but the self window is expressed as boolean expressions. There are wider varieties of operations in differential expressions than in boolean expressions. But most of LSM models are also not robust for the collision problem. That because LSM models extract all boundaries of objects when two or more objects exist in the visual field. So we propose a new energy function of LSM model which constrains an amount of a light intensity within detected object to limit the number of detected object to "1".

To reduce computational time of the proposed LSM model, we use the column parallel vision (CPV) system [5]. CPV system is a combination of a high speed camera and a parallel processor. To verify performance of proposed LSM model, we demonstrate a tracking experiment of the single paramecium by utilizing the proposed LSM method, CPV system, a microscope and a XYZ automated stage. The paramecium is a benchmark of the cell tracking, since the paramecium moves quick relative to size (5 diameter/s) [1].

2 Materials and Method

2.1 General LSM

We suppose a closed boundary curve $C(x, y, t)$ in a two dimensions image (Fig. 1). A typical energy function of

Fig. 1: The properties of level set function ϕ . Ω and $\bar{\Omega}$ represent the inside and outside area of a boundary curve C , respectively. ϕ is positive in Ω , negative in $\bar{\Omega}$ and zero on C .

C is defined by

$$E(C) = E_{in} + E_{out}, \quad (1)$$

where E_{in} and E_{out} denote internal and external energy functions, respectively. This 2D boundary curve C is represented implicitly as a 3D level set function $\phi(x, y, t)$:

$$C(x, y, t) = \{(x, y) | \phi(x, y, t) = 0\}, \quad (2)$$

which means that the boundary curve of objects is given by the zero level set at anytime. ϕ is supposed to have properties as shown in Fig. 1. ϕ evolves from an initial position to the contour of the object by minimizing the energy function of ϕ :

$$\frac{\partial \phi}{\partial t} = -\frac{\partial E(\phi)}{\partial \phi}. \quad (3)$$

2.2 LSM to detect multiple objects

Chan and Vese (CV model) proposed the following energy function of ϕ [6]:

$$\begin{aligned} E_{in} &= \alpha \cdot \text{Length}(C) + \beta \cdot \text{Area}(C) \\ &= \alpha \int |\nabla H(\phi)| dx dy + \beta \int H(\phi) dx dy \end{aligned} \quad (4)$$

$$\begin{aligned}
E_{out} &= \gamma \{ \lambda_1 F_1(\phi) + \lambda_2 F_2(\phi) \} \\
&= \gamma \{ \lambda_1 \int (I - I_{in})^2 H(\phi) dx dy \\
&\quad + \lambda_2 \int (I - I_{out})^2 [1 - H(\phi)] dx dy \}. \quad (5)
\end{aligned}$$

The integral interval of all the integrations in this paper is the whole image. α and β are weight parameters of $Length(C)$ and $Area(C)$, respectively. This model fixes $\beta = 0$. γ is weight parameters of E_{out} . $I(x, y)$ indicates the image intensity at position (x, y) . I_{in} and I_{out} represent average intensities of inside and outside of C , respectively. λ_1 and λ_2 are fitting parameters of inside and outside of C , respectively. $H(\phi)$ is the Heaviside function. E_{in} works as the curvature of ϕ to keep the curve smooth. E_{out} becomes its minimum value $E_{out} = 0$ when $(I - I_{in})^2 = 0$ and $(I - I_{out})^2 = 0$ as C on the boundary of the object. So E_{out} drives the boundary curve C towards the contour of the object.

2.3 LSM to detect only single object

For tracking only one paramecium and parallel implementation, we propose a novel level set energy function based on CV model as follow:

$$\begin{aligned}
E_{in} &= \alpha \cdot Elasticity(\phi) + \beta \cdot Cell_Intensity(\phi) \\
&= \frac{\alpha}{2} \int |\nabla^2 \phi| dx dy + \beta \int I H(\phi) dx dy - S_0 \quad (6)
\end{aligned}$$

$$\begin{aligned}
E_{out} &= \gamma \{ \lambda_1 \int |I - I_{in}| H(\phi) dx dy \\
&\quad - \lambda_2 \int |I - I_{out}| [1 - H(\phi)] dx dy \}. \quad (7)
\end{aligned}$$

where $Q = |\int I H(\phi) dx dy - S_0|$. Then the evolving form of the level set function ϕ can be derived from minimizing energy with respect to ϕ as follow:

$$\begin{aligned}
\frac{\partial \phi}{\partial t} &= \alpha \nabla^2 \phi - \delta(\phi) [\beta sign(Q) I + \\
&\quad \gamma (\lambda_1 |I - I_{in}| - \lambda_2 |I - I_{out}|)], \quad (8)
\end{aligned}$$

where $\delta(\phi)$ is the one-dimensional Dirac function. Four modifications from CV model are summarized as follows:

1. Due to the architecture of CPV system (see below), the internal energy is constructed as $\nabla^2 \phi$. When the spatial changes of ϕ becomes bigger, $\nabla^2 \phi$ also becomes bigger. Therefore, $\nabla^2 \phi$ has smoothing ability for ϕ .
2. The light intensity emitted by an individual object is always constant regardless of its position and posture [7]. To extract the boundary of only single object among two or more objects, we add the new energy function $Q = |\int I H(\phi) dx dy - S_0|$ in (8). $\int I H(\phi) dx dy$ denotes the light intensity inside object boundary and S_0 denotes an ideal value of the light intensity in a single paramecium. Apparently, when E_{out} obtains minimum, Q approximates 0 which means that actual light intensity inside the object boundary is close to S_0 . By consequence,

Q maintains the light intensity inside the object boundary constant to detect only one object. After some experimental test, we find out that a range of light intensity emitted by a individual paramecium is equal to about 300790 ~ 360210 under our conditions.

Fig. 2: Flow chart of the implementation of the proposed LSM and the visual servoing. $T1$, $T2$ and $T3$ represent the average time for 1st frame, the average time for other frames, and the average time for each iteration, respectively.

3. To cut down the computational cost, $|I - I_{in}|$ and $|I - I_{out}|$ are adopted instead of $(I - I_{in})^2$ and $(I - I_{out})^2$ in (7).
4. To discretize the (8), first, the Dirac function $\delta(\phi)$ is approximated to

$$\delta(\phi) \approx \frac{1}{1 + |\phi|}. \quad (9)$$

Second, we use the finite differences implicit scheme: let h be the discrete space step, Δt be the discrete time step, and $(x_i, y_i) = (ih, jh)$ be the grid points of the image.

Then $\phi_{i,j}^{n+1}$ can be approximately computed by applying above modification:

$$\begin{aligned}
\frac{\phi_{i,j}^{n+1} - \phi_{i,j}^n}{\Delta t} &= \alpha \frac{\phi_{i+1,j}^n + \phi_{i-1,j}^n + \phi_{i,j+1}^n + \phi_{i,j-1}^n - 4\phi_{i,j}^n}{8} \\
&\quad - \frac{1}{1 + |\phi_{i,j}^n|} \{ \beta sign(A) I - \gamma [\lambda_1 |I_{i,j} - I_{in}(\phi^n)| \\
&\quad - \lambda_2 |I_{i,j} - I_{out}(\phi^n)|] \}, \quad (10)
\end{aligned}$$

where n denotes an iteration number.

2.4 Implementation of LSM and visual servoing

The proposed LSM and the visual servoing for each frame is implemented as Fig. 2:

1. Initialize ϕ as $\phi_0(i, j) = 64 - |I(i, j) - 64| + |I(i, j) - 64|$ for the 1st frame, or the ϕ of previous frame for the rest of frames. Set $\alpha = \beta = \gamma = 1$, $\lambda_1 = 1$, $\lambda_2 = 0.7$, $n = 0$, respectively. S_0 is set as 330500, which is an intermediate value of the ideal range.
2. Define a *flag* to represent the sign of *current* ϕ and it is initialized as:

$$flag(i, j) = \begin{cases} 1, & \phi \geq 0 \\ 0, & \phi < 0. \end{cases} \quad (11)$$

Also define a *mask* to represent the sign of *previous* ϕ . Initialize *mask* as $mask(i, j) = 1$ for the 1st frame, or

$$mask(i, j) = \begin{cases} 1, & \phi \geq 0 \\ 0, & \phi < 0, \end{cases} \quad (12)$$

for the rest of frames.

3. Compute $\nabla^2 \phi$ in E_{in} by using neighbor connections of CPV system (see a next subsection).
4. Update ϕ and *flag* according to (10);
5. Check if the change of I_{in} between two continuous steps is less than a specified threshold ϵ . If yes, go to Step 6. If no, $n = n + 1$ and go back to Step 2.
6. To decrease the affection of inaccurate contour detection, perform an AND operation between a dilated version of *mask* and *flag*. The dilated mask is:

$$mask_{i,j} = mask_{i,j} \mid mask_{i-1,j} \mid mask_{i+1,j} \mid mask_{i,j-1} \mid mask_{i,j+1}. \quad (13)$$

"|" is logical OR operation.

7. Calculate the centroid of tracked object based on *flag*.
8. Move automated XYZ stage to keep the detected object in the center of the view field. PD control is adapted.
9. Check if there is another frame. If yes, go back to the Step 1; if no, stop computing.

2.5 CPV system

The proposed LSM is implemented in CPV system (Hamamatsu Photonics K.K.). CPV system is comprised of camera, processor and controller parts [5]. The camera (12bit monochrome, frame rate is 1000[fps]) includes 512×512 photo-detectors (PDs). There are 128×128 programmable processing elements (PEs) and one summation circuit in the processor part. Each PD, PE and summation circuit can work parallelly. One PE is connected with its 4 neighbor PEs to calculate the local image features, such as ∇^2 . All PEs are connected with summation circuit which can extract global image features, such as I_{in} and I_{out} . Controller is responsible for management and data transmission with PC.

Fig. 3: Photograph of experimental set-up. Arrows indicate light and information flows.

2.6 System setup

As shown in Fig. 3, The camera is mounted on the top of an inverted microscope (BX51, OLYMPUS). This camera captures a bright-field image which is an image with a bright background. Paramecia swimming in a water pool on a slide glass can be observed through a 20-times objective lens (N.A 0.75, UAPO, OLYMPUS, visual field: X: 384 [μ m], Y: 384 [μ m]). The body length of the paramecium ranges from 100 to 200 μ m and the speed of the paramecium is about 1 [mm/s]. The water pool is enclosed with a glass slide, a cover glasses and silicon rubber sheets. The size of the water pool is X: 15 [mm], Y: 15 [mm], Z: 0.05 [mm]. The slide glass is fixed on a XYZ automated stage (HEPHAIST, Stroke: XY axis 25[mm], Z axis 10[mm], Resolution: ± 1 [μ m], Maximum speed: 30[mm/s]). This stage is controlled by the visual feedback to track the single paramecium.

3 Experimental Results

We conduct an experiment of tracking a single paramecium to examine the performance of our model. First of all, when only one paramecium (called as "target") enters the visual field, we start the program by hand. Then the target is kept tracking and result is recorded in movies (see the attached movie). In Fig. 4, consecutive image sequences are chosen from 2809 ms to 4249 ms out of the movie of 37.9 s. White curves represent the computed contours of target. At 2809 ms, there is only target in the visual field. At 2969 ms, another paramecium appears in the visual field, the target is still kept in the middle of visual field. At 3289 ms and 3609 ms, another paramecium contacts with the target. However, tracking still proceeds successfully. It turns out our model is robust for the collision problem. To show how our model works in real time, we investigate average computational time for each frame from 2809 ms to 4249 ms. The average time for 1st frame T1 is 2056.6 [μ s] and the average time for other frames T2 is 1101.4 [μ s] (Fig. 2B). The average time for each iteration T3 is 897.2 [μ s]. This difference between T1 and T2 is probably because ϕ is initialized as ϕ of the last frame and the position change of paramecium between consecutive frames is very small. These results represent that the implementation of the proposed LSM on CPV system works in real time of about 500 [Hz].

The trajectory of the target paramecium during the

Fig. 4: A continuous image sequence and zero level sets.

attached movie is shown in Fig. 5 which shows the range of target swimming is X: (-3000 [μm] $\sim$ 1000 [μm]), Y: (-2500 [μm] $\sim$ 1000 [μm]). This range is far beyond the visual field of CPV system. It turns out that our method can track a single moving paramecium to keep it in the middle of visual field by using light intensity constraint.

4 Discussion

In this paper, we proposed a parallel LSM model for two dimensional tracking of the single paramecium by using CPV system. To solve the collision problem and detect only one paramecium, we constructed a new energy function to constrain the amount of the light intensity of the detected object. After parallelization, simplifications and discretizations, the proposed LSM model was implemented in CPV system. Based on the single con-

Fig. 5: Trajectory of the target paramecium.

tour of the target paramecium and PD control of the automated XYZ stage, we successfully tracked the single paramecium for more than 37s (Fig. 4 and movie). Even if the collision problem happened, the contour of target paramecium was detected roughly. Inaccurate boundary detection of the target paramecium causes inaccurate calculation of the centroid of the target paramecium. But the error of centroid is not big enough to make the paramecium tracking failure. Therefore, our model is robust for dealing with the problem of collision.

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