密な細胞画像を対象とした細胞トラッキング

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Background

Drug Discovery

Control  Drug A  Drug B

- Drug screening (cell based assay)
  To analyze drug effects for target cells, the cell behavior assay is required.
  ex) anticancer drug

Regenerative Medicine

Subculture

- Cell quality control
  It is considered that cell behaviors are one of the important measures to evaluate cell quality.
  ex) RPE(Retinal Pigment Cell)

To analyze cell behaviors including cell migration, and division, cell tracking is required.
Time-lapse of microscope images
Cell behavior metrics

Time-lapse images are captured by microscope.

Cell tracking system
Difficulties of cell tracking and detection

» Detection
  – Inhomogeneous background and noise.
  – Low contrast.
  – Touching cells.
  – Chips of cells

A chip of the cell
Difficulties of cell tracking and detection

» Similar Appearance

⇒ Appearance-based methods have limitations.
Difficulties of cell tracking and detection

» Change in the number of cells
  – Cell division.
  – Enter or exit.

Cell division

Enter / Exit
Cell tracking methods can be classified into three groups.

**Motion filter approach:**
Ex) Particle filter

**Model-based-contour evolution approach:**
Ex) Level set tracking

**Detection-and-association approach:**
Ex) Multi hypotheses tracking (MTH)
Related works

**Motion filter approach**: [Smal, 2006]

- Particle filter: represents a probability distribution over the state of the object by using a set of weighted samples.

**Disadvantage**
- Tend to confuse closed objects.
Related works

Model-based-contour evolution approach: [Yang, 2005][Li, 2008]
- Level set tracking:

Disadvantage
- A part of the cell contour may be optimized to the boundary of a nucleus or to the other cells.
Related works

**Detection-and-association approach:** [Al-Kofahi, 2006][Padfield, 2011]

- Multi hypotheses tracking (MTH):

**Disadvantage**
- Detection errors directly propagate the association step.
Overview of cell tracking methods

Input: Time-lapse Image sequence

Cell detection

Mitosis detection

Cell division

Detection Results

Frame-by-frame association

Reliable tracklets

Global association

Mitosis Event

Detection Results

Mitosis Event

Output: Cell Trajectory

Biological application
Cell tracking for partially overlapping cells by partial contour matching

Delineating cells requires time sequence.

It is difficult for a human to separate manually by observing only one image!
Outline of Chapter 2

- Identifying touching cells by association.
- Re-segmenting touching cells by contour matching.

Diagram:

Input:
- Time-lapse Images

Preprocessing
- Mitosis detection
- Cell detection

Association
- Frame-by-frame association
- Global association

Re-segmentation

Output:
- Cell Trajectory
Cell detection

- Model Phase-Contrast and DIC microscope imaging
- Reconstruct “ideal” images without artifact
- Non-invasive images can be treated like fluorescent images.


Associate detected regions and identify clusters

Associate detected regions and identify clusters

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>$\rho$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-&gt;none</td>
<td>0.3</td>
</tr>
<tr>
<td>1-&gt;1'</td>
<td>0.7</td>
</tr>
<tr>
<td>none-&gt;1</td>
<td>0.2</td>
</tr>
<tr>
<td>2-&gt;2'</td>
<td>0.1</td>
</tr>
<tr>
<td>2-&gt;3'</td>
<td>0.1</td>
</tr>
<tr>
<td>2-&gt;2',3'</td>
<td>0.7</td>
</tr>
<tr>
<td>3-&gt;4'</td>
<td>0.2</td>
</tr>
<tr>
<td>4-&gt;4'</td>
<td>0.3</td>
</tr>
<tr>
<td>4-&gt;5'</td>
<td>0.5</td>
</tr>
<tr>
<td>3,4-&gt;4'</td>
<td>0.6</td>
</tr>
<tr>
<td>4-&gt;4',5'</td>
<td>0.1</td>
</tr>
<tr>
<td>5-&gt;5'</td>
<td>0.7</td>
</tr>
<tr>
<td>4,5-&gt;5'</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Constraint:
- **Constraint of translation overlap**
- **Ex**: If 「2→2'」 is in the solution, 「2→any」, 「any→2'」 are not in the solution.
Associate detected regions and identify clusters

Maximizing sum of scores

$\mathbf{x}^* = \arg\max_{\mathbf{x}} \mathbf{\rho}^T \mathbf{x}, \quad \text{s.t.} \quad \mathbf{C}^T \mathbf{x} \leq 1$

Object ID: 1 2 3 4 5 1' 2' 3' 4' 5'

Constraints:

- $1 \rightarrow \text{none}$: $\rho = 0.3$
- $1 \rightarrow 1'$: $\rho = 0.7$
- none $\rightarrow 2'$: $\rho = 0.2$
- $2 \rightarrow 2'$: $\rho = 0.1$
- $2 \rightarrow 3'$: $\rho = 0.1$
- $2 \rightarrow 2', 3'$: $\rho = 0.7$
- $3 \rightarrow 4'$: $\rho = 0.2$
- $4 \rightarrow 4'$: $\rho = 0.3$
- $4 \rightarrow 5'$: $\rho = 0.5$
- $3,4 \rightarrow 4'$: $\rho = 0.6$
- $4 \rightarrow 4', 5'$: $\rho = 0.1$
- $5 \rightarrow 5'$: $\rho = 0.7$
- $4,5 \rightarrow 5'$: $\rho = 0.1$

Cell ID at t-1

Blob ID at t

Constraint: $\mathbf{C}^T \mathbf{x} \leq 1$
Associate detected regions and identify clusters

Maximizing sum of scores

\[ x^* = \arg \max_x \rho^T x, \quad \text{s.t.} \quad C^T x \leq 1 \]

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>( \rho )</th>
<th>( C )</th>
<th>( x )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1→none</td>
<td>0.3</td>
<td>1 0 0 0 0 0 0 0 0</td>
<td>1 or 0</td>
</tr>
<tr>
<td>1→1'</td>
<td>0.7</td>
<td>1 0 0 0 0 1 0 0 0</td>
<td>1 or 0</td>
</tr>
<tr>
<td>none→</td>
<td>0.2</td>
<td>0 0 0 0 0 0 1 0 0</td>
<td>1 or 0</td>
</tr>
<tr>
<td>2→2'</td>
<td>0.1</td>
<td>0 1 0 0 0 0 1 0 0</td>
<td>1 or 0</td>
</tr>
<tr>
<td>2→3'</td>
<td>0.1</td>
<td>0 1 0 0 0 0 0 1 0</td>
<td>1 or 0</td>
</tr>
<tr>
<td>2→2',3'</td>
<td>0.7</td>
<td>0 1 0 0 0 0 1 1 0</td>
<td>1 or 0</td>
</tr>
<tr>
<td>3→4'</td>
<td>0.2</td>
<td>0 0 1 0 0 0 0 0 1</td>
<td>1 or 0</td>
</tr>
<tr>
<td>4→4'</td>
<td>0.3</td>
<td>0 0 0 1 0 0 0 0 1</td>
<td>1 or 0</td>
</tr>
<tr>
<td>4→5'</td>
<td>0.5</td>
<td>0 0 0 1 0 0 0 0 1</td>
<td>1 or 0</td>
</tr>
<tr>
<td>3,4→4'</td>
<td>0.6</td>
<td>0 0 1 1 0 0 0 0 1</td>
<td>1 or 0</td>
</tr>
<tr>
<td>4→4',5'</td>
<td>0.1</td>
<td>0 0 1 1 0 0 0 0 1</td>
<td>1 or 0</td>
</tr>
<tr>
<td>5→5'</td>
<td>0.7</td>
<td>0 0 0 1 0 0 0 0 1</td>
<td>1 or 0</td>
</tr>
<tr>
<td>4,5→5'</td>
<td>0.1</td>
<td>0 0 0 1 1 0 0 0 1</td>
<td>1 or 0</td>
</tr>
</tbody>
</table>

Object ID: \[ \begin{array}{ccccccc} 1 & 2 & 3 & 4 & 5 & 1' & 2' & 3' & 4' & 5' \\ \end{array} \]

cell ID       blob ID
Partial contour matching by DP

1. Feature (convex) point detection and matching.
2. Divide each contour into segments using feature points.
3. Partial contour segment matching using dynamic programming.
Tracking results

Input Image

Result of Proposed method

Result of levelset + motion filter [Li, 2008]
Target effectiveness

Image sequence 1
Frames: 1000
Interval: 5 min
Over 3 days

<table>
<thead>
<tr>
<th>Frame</th>
<th>600</th>
<th>800</th>
<th>900</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>0.976</td>
<td>0.943</td>
<td>0.922</td>
<td>0.903</td>
</tr>
</tbody>
</table>
Chapter 3: Cell tracking by spatio–temporal global data association

Previous work  
(frame–by–frame association)

False Positives (FP) cause identity switching errors.

Propose a spatio–temporal global data association method.
Outline

- Global spatio-temporal association.
  - Associating cells by binary linear programming.

Input:
- Time-lapse Images

Output:
- Cell Trajectory

Steps:
1. Original Image Sequence
2. Cell detection
3. Mitosis detection
4. Time-lapse Images
5. Frame-by-frame association
6. Global association
7. Re-segmentation
8. Overall trajectories
Cell tracking

- Input image
- Cell Detection
- Mitosis Detection
- Make tracklets
- Hypotheses
- Global Association
- Tracking Results

Find optimal tree structure paths on the all considerable hypotheses
Global data association problem

Input image

Cell Detection
Mitosis Detection

Make tracklets

Hypotheses

Global Association

Tracking Results

An optimal set of trees in any hypotheses are selected.

Detected mitosis event

Detection

Mitosis Detection

Make tracklets

Hypotheses

Global Association

Tracking Results

An optimal set of trees in any hypotheses are selected.

Detected mitosis event

Global data association problem

Input image

Cell Detection
Mitosis Detection

Make tracklets

Hypotheses

Global Association

Tracking Results

An optimal set of trees in any hypotheses are selected.

Detected mitosis event
Global association for tree structure

\[ \tau^* = \arg \max_\tau \prod_{T_i \in \tau} P_{FP}(T_i) \prod_{\tau_k \in \tau} P_{\text{tree}}(\tau_k) \]

\[ P_{\text{tree}}(\tau_k) = P_{\text{ini}}(E_k_0) \prod_{E_k \in \tau_k} P_{\text{edge}}(E_k_i) \prod_{(E_p, E_{c_1}, E_{c_2}) \in B} P_{\text{div}}(E_{c_1}, E_{c_2} | E_{p_i}) \prod_{E_{k0} \in L} P_{\text{term}}(E_{k_i}) \]
Global association for tree structure

- Input image
  - Cell Detection
  - Mitosis Detection
  - Make tracklets
  - Hypotheses
  - Global Association
  - Tracking Results
Tracking results
Tracklets

A tree in which tracklets are associated by global data association.
Results

Tracklets

Trees in which tracklets are associated by global data association.
Quantitative evaluation

Table 1. For a fully annotated sequence

<table>
<thead>
<tr>
<th></th>
<th>Track Purity</th>
<th>Target Effectiveness</th>
<th>Mitosis Branching Correctness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. [2]</td>
<td>0.62</td>
<td>0.70</td>
<td>0.46</td>
</tr>
<tr>
<td>Ours</td>
<td>0.81</td>
<td>0.87</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table 2. For the other four sequences (partially annotated)

<table>
<thead>
<tr>
<th></th>
<th>Target Effectiveness</th>
<th>Mitosis Branching Correctness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ours</td>
<td>Li et al.</td>
</tr>
<tr>
<td>exp1</td>
<td>0.96</td>
<td>0.75</td>
</tr>
<tr>
<td>exp2</td>
<td>0.87</td>
<td>0.7</td>
</tr>
<tr>
<td>exp3</td>
<td>0.87</td>
<td>0.68</td>
</tr>
<tr>
<td>exp4</td>
<td>0.78</td>
<td>0.6</td>
</tr>
<tr>
<td>average</td>
<td>0.87</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Comparison with human annotation

Lineage Tree: Target Effectiveness = 95.63%
Cell tracking methods for addressing difficulties under high-density conditions

Propose tracking and detection methods which solve both detection and association.
Assessment of cell quality for regenerated medicine:

- Cells are cultured until they densely fill the dish. 
  ex.) RPE cells are cultured for 30 days.
- Cell behavior metrics are key element.

⇒ It is important to track cells under high dense conditions.

---

day1

---

day21
Cell detection errors under high-density

There are many detection errors under high-density. These errors affect the tracking accuracy adversely.

Cell detection from redundant candidate regions under non-overlapping constraints

Difficulties of cell detection

- Inhomogeneous background and noise.
- Low contrast.
- Touching cells.
- Chips of cells

A chip of the cell
Overview of proposed method

1. **Detect candidate regions**
2. **Generate tree structure**
3. **Compute scores of regions**
4. **Select set of optimal cell regions**

**Detailed Steps:**
- **Detect candidate regions:**
  - Original Image
- **Generate tree structure:**
  - A1, A2, A3 are selected as an optimal region set where selected objects do not overlap each other.
  - Score: A1 = 0.4, A2 = 0.9, A3 = 0.7
- **Compute scores of regions:**
- **Select set of optimal cell regions:**
1. Preconditioning (Yin et. al. 2010)
   A brighter region surrounded by darker regions is detected as a single cell region.

2. Multithreshold
   By using many threshold, candidate regions may include very few false negatives.

\[ g \approx Hf + C \]

\[ (Hf)_j = \sum_{u=1}^{2M+1} \sum_{v=1}^{2M+1} \text{PSF}(u, v)f(x_j^u + u - M, x_j^v + v - M) \]

Positive value: foreground
A small value: background
Computing score of each candidate

(a)

(b)
\[ x^* = \text{arg max}_{x} \rho^T x, \quad \text{s.t.} \quad C^T x \leq 1, \]

Constraint matrix \( C \)

<table>
<thead>
<tr>
<th>ID</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
<th>A8</th>
<th>A9</th>
<th>A10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A6</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>1</td>
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<td>A7</td>
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<td>A8</td>
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<tr>
<td>A9</td>
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<td>0</td>
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<td>1</td>
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<tr>
<td>A10</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Constraint: 
- **Constraint of region overlap**

Ex: If A3 is in the solution, 
\{A1, A5, A6, A8, A9, A10\} are not in the solution since these regions overlap with region A7.
Cell detection results in 2D

- Data set A: Stained zebrafish cells in fluorescent microscopy images.
- Data set B: Stained BAEC in fluorescent microscopy images.
- Data set C: Center nervous system (CNS) stem cell in DIC microscopy images.

### Average performance for all data set

<table>
<thead>
<tr>
<th>Method</th>
<th>Recall</th>
<th>Precision</th>
<th>F-measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otsu thresholding [Otsu79]</td>
<td>0.672</td>
<td>0.938</td>
<td>0.775</td>
</tr>
<tr>
<td>Thresholding with classification [Yin12]</td>
<td>0.609</td>
<td>0.983</td>
<td>0.745</td>
</tr>
<tr>
<td>CellProfiler [Carpenter06]</td>
<td>0.781</td>
<td>0.944</td>
<td>0.851</td>
</tr>
<tr>
<td>Level set [Li10]</td>
<td>0.570</td>
<td>0.909</td>
<td>0.657</td>
</tr>
<tr>
<td>FIJI [Schindelin12]</td>
<td>0.8139</td>
<td>0.8410</td>
<td>0.8233</td>
</tr>
<tr>
<td>Proposed</td>
<td><strong>0.9320</strong></td>
<td><strong>0.9216</strong></td>
<td><strong>0.9262</strong></td>
</tr>
</tbody>
</table>
Cell detection results in 3D

- Data set D
  - Stained Cells in embryo of zebrafish were captured using DSLM.

<table>
<thead>
<tr>
<th>Method</th>
<th>Recall</th>
<th>Precision</th>
<th>F-measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otsu thresholding [Otsu79]</td>
<td>0.447</td>
<td>0.993</td>
<td>0.616</td>
</tr>
<tr>
<td>Proposed</td>
<td>0.906</td>
<td>0.966</td>
<td>0.935</td>
</tr>
</tbody>
</table>
Cell tracking by jointly solving tracklet selection and global association


Main point of the proposed methods

- Jointly solving cell detection and association.

**Existing Methods:**

- Tracking Result at $t-1$

**Proposed Method:**

- Detection Result at $t$

Correct association may **not be** in a set of association hypotheses.

Correct association could **well be** in a set of association hypotheses.
Candidate Cell Region Generation

1. Preconditioning (Yin et. al. 2010)
   A brighter region surrounded by darker regions is detected as a single cell region.

2. Multithreshold
   By using many threshold, candidate regions may include very few false negatives.

\[ g \approx Hf + C' \]

\[ (Hf)_j = \sum_{u=1}^{2M+1} \sum_{v=1}^{2M+1} \text{PSF}(u, v)f(x_j^u + u - M, x_j^v + v - M) \]

Input Image  \rightarrow  Preconditioning Image  \rightarrow  Candidate Cell Regions

Positive value: foreground
A small value: background
Step1-1: candidate detection

- Candidate cell regions are generated by multi-thresholding.
Optimization

Slice : $z-1$ Tracking results

Slice : $t$ Candidate Regions

Hypothesis : $T_{l}^{z-1} \rightarrow A^z_m$

Likelihoods:
- region : circularity
- translation : relative overlap

\[
\rho(h) = P_{con}(A^z_m | T_{l}^{z-1}) P_{TP}(A^z_m)
\]

translation cell

\[
P_{con}(A^z_m | T_{l}^{z-1}) = \frac{T_{l}^{z-1} \cap A^z_m}{T_{l}^{z-1} \cup A^z_m}
\]

\[
P_{TP}(A^z_m) = \frac{4\pi S(A^z_m)}{B(A^z_m)}
\]
Slice : z-1 Tracking results

Slice : t Candidate Regions

Constraint :
○ **Constraint of translation overlap**
  Ex: If 「①→2」 is in the solution, 「①→any」, 「any→2」 are not in the solution.

○ **Constraint of region overlap**
  Ex: If 「①→2」 is in the solution, {1,3} are not in the solution since these regions overlap with region 2.
Optimization

Slice: \( z-1 \) Tracking results

Constraint:

- **Constraint of translation overlap**
  
  Ex: If \( ① \rightarrow 2 \) is in the solution, 
  \( ① \rightarrow \text{any} \), \( \text{any} \rightarrow 2 \) are not in the solution.

- **Constraint of region overlap**
  
  Ex: If \( ① \rightarrow 2 \) is in the solution, 
  \{1,3\} are not in the solution since these regions overlap with region 2.
Tracking results

day1

day21
Tracking results

Original Image

Tracking Result

Frame 1

Frame 10

Frame 20

Frame 30

Frame 40

Frame 50

Frame 60
### Comparison

<table>
<thead>
<tr>
<th>Method</th>
<th>Association accuracy</th>
<th>Target Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frame-by-frame</td>
<td>0.757</td>
<td>0.427</td>
</tr>
<tr>
<td>Frame-by-frame with improved detection</td>
<td>0.881</td>
<td>0.601</td>
</tr>
<tr>
<td>Global association</td>
<td>0.799</td>
<td>0.593</td>
</tr>
<tr>
<td>Global association with improved detection</td>
<td>0.863</td>
<td>0.587</td>
</tr>
<tr>
<td>SelectCandi+association</td>
<td>0.910</td>
<td>0.650</td>
</tr>
<tr>
<td>Proposed</td>
<td>0.911</td>
<td>0.636</td>
</tr>
</tbody>
</table>
Application: drug discovery
wound healing assay

Understanding mechanism of wound healing

Culture cells → scratch for "wound" → Cell growth: healing

Control

Add 10 nM

Add Latrunculin 100 nM
Automated tracking
How cells migrate depending on distances from the wound
How cells migrate depending distances from the wound
Density Slope and Migration Direction

Migration direction

Density slope

$\Delta d_r$
Take cell culture images. Check the tracking results during culturing cells.
To optimize the subculture timing, predicting cell population.

**Microscope**

Time-lapse images are taken.

**Computer Vision**

- detecting cell areas.
- compute cell confluence level curve.
- predicting cell confluence.

**Notification for cell culture**

- A human operator was notified by e-mail 4 hours prior to exceeding a pre-determined cell confluence level.
Automated Cell Culture System

Computer Vision Processing

Cell Lineage Measurement

Time

Stemness Metric (symmetry)

Time-Lapse Microscopy

Sample Cultures

Feedback for Adaptive Sub-Culturing Timing

Time

Laser Tweezers

$L_t$