The image segmentation and cell tracking in macrophage data

WORKSHOP: DISCRETE DUALITY FINITE VOLUME METHOD AND APPLICATIONS

MARSEILLE, OCT. 17, 2022

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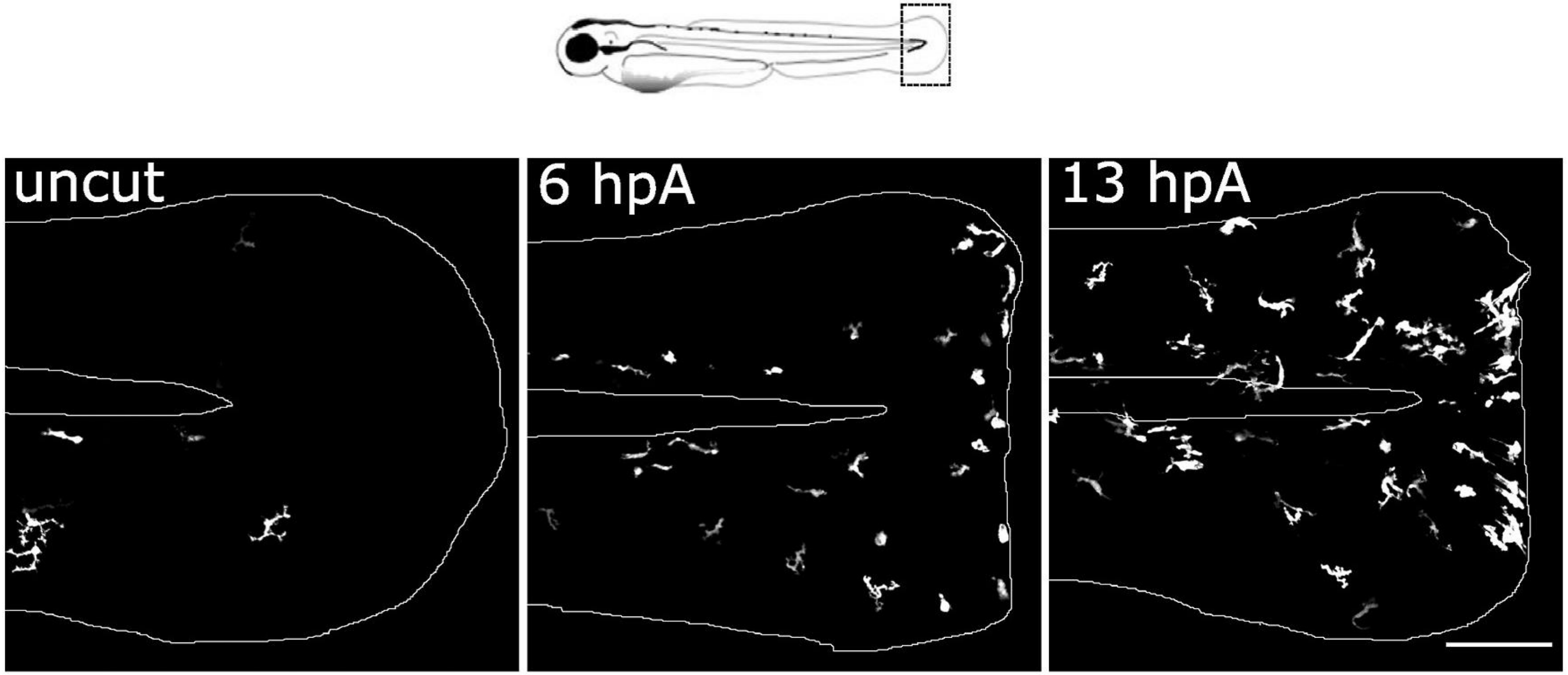
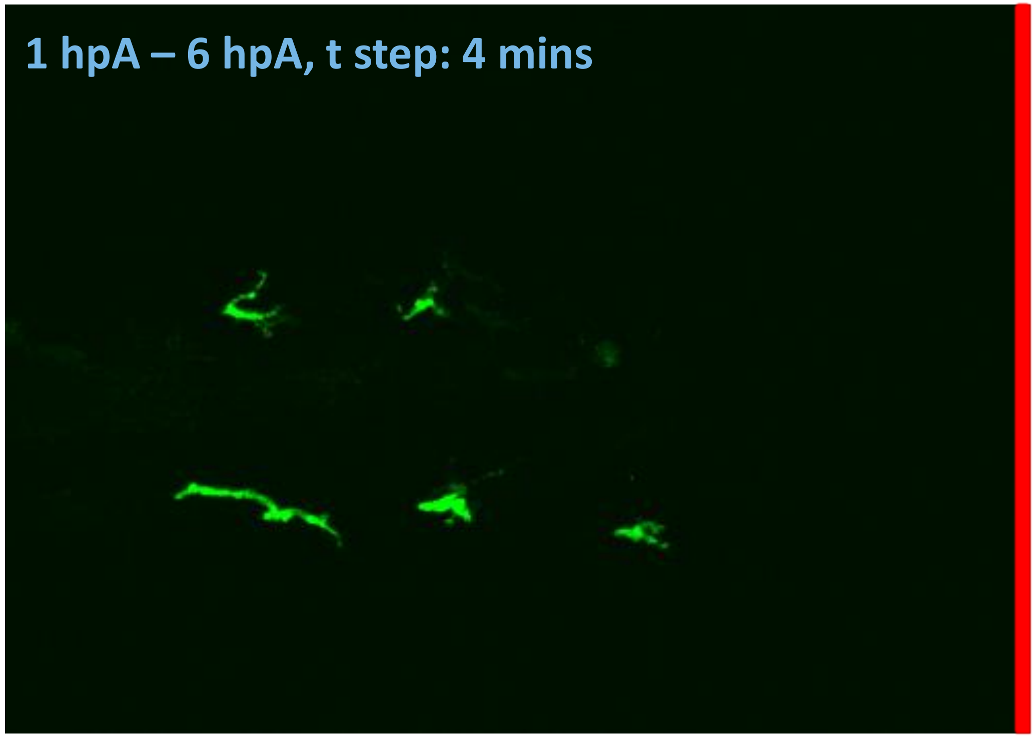
Macrophages?

Sipka, Tamara, et al. "Macrophages undergo a behavioural switch during wound healing in zebrafish." *Free Radical Biology and Medicine* (2022).

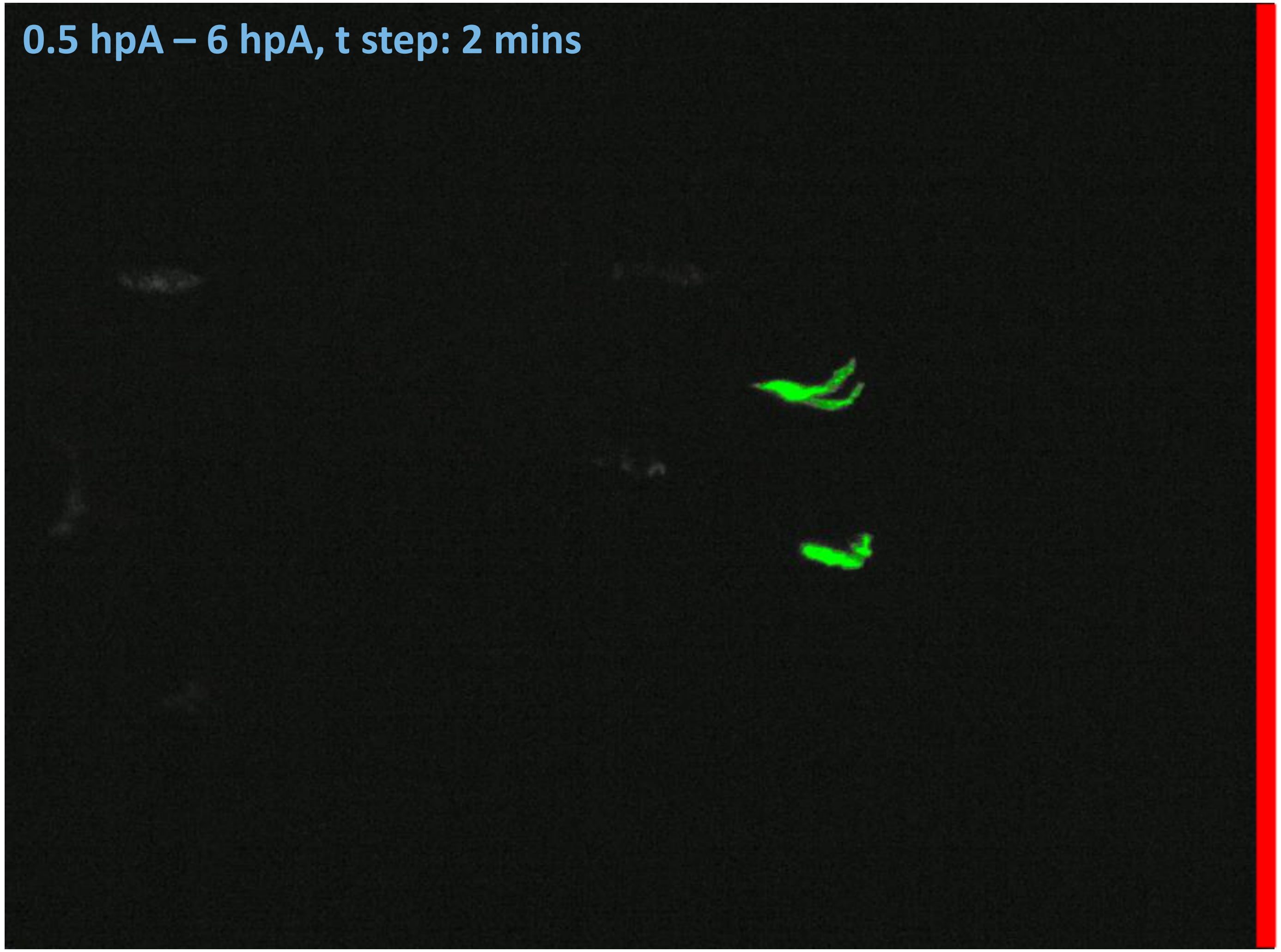


Macrophages in microscopy videos – from Montpellier University

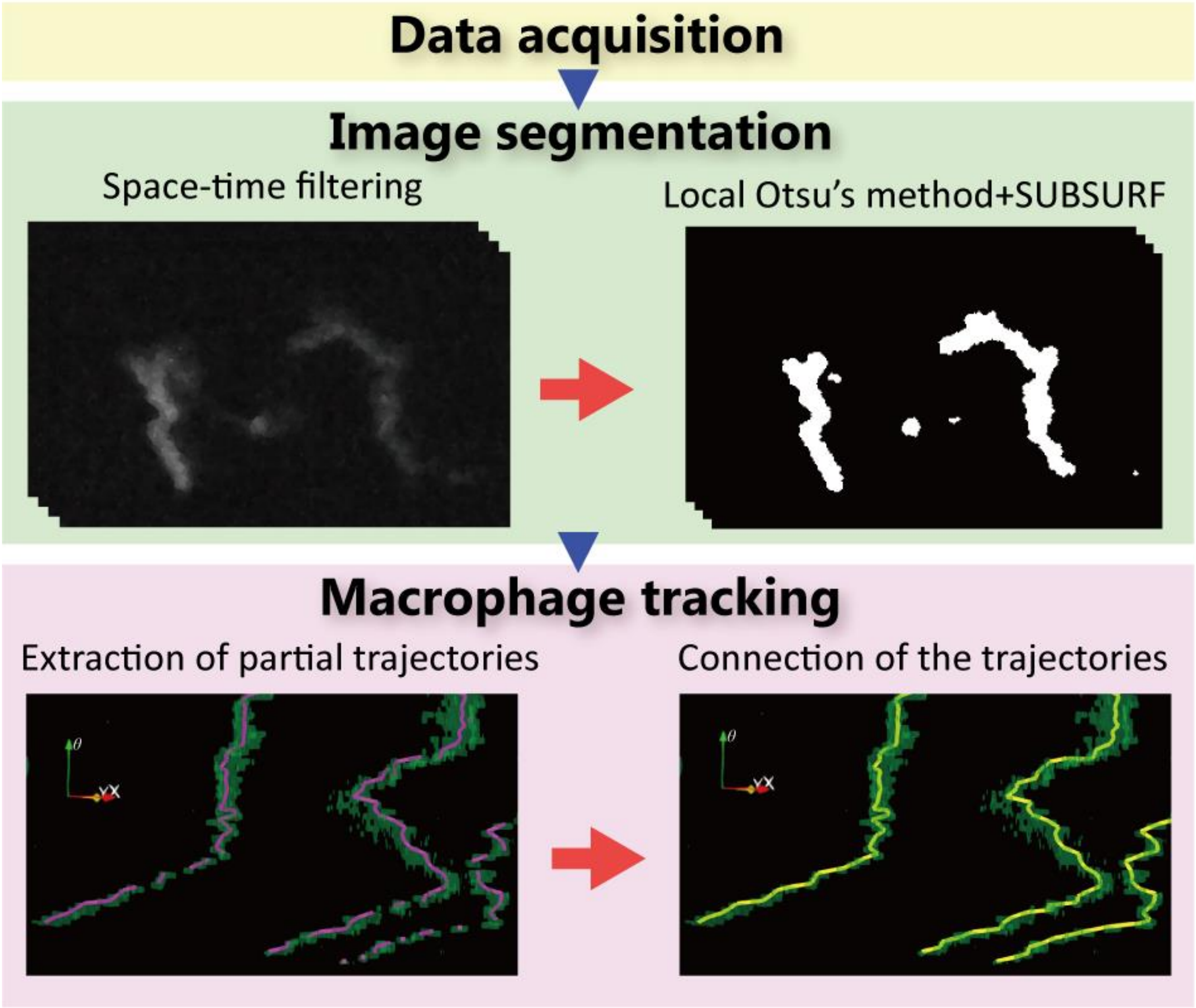
Site of wound



Site of wound



Procedure of macrophage segmentation & tracking



1. *Filtering by considering the temporal coherence*
2. *Segmentation by using local thresholding and removal of the remaining noise*

1. *Extraction of trajectories when segmented cells overlap in time*
2. *Connection of trajectories obtained from #1 by approximating the direction of the cell movement.*

Image segmentation of macrophages
- space-time filtering

Sarti, Alessandro, Karol Mikula, and Fiorella Sgallari. , IEEE Transactions on Medical Imaging (1999), "Nonlinear multiscale analysis of three-dimensional echocardiographic sequences."

$$\frac{\partial u}{\partial t} = \boxed{clt(u)}\boxed{\nabla \cdot \left(g(|\nabla G_\sigma * u|)\nabla u\right)}$$

Perona-Malik (anisotropic diffusion)

$$g(s) = \frac{1}{1 + Ks^2}, \quad K > 0, s = \nabla u$$

To measure the time coherence for moving objects

$$clt(u) = \min_{w_1, w_2} \frac{1}{(\Delta\theta)^2}\left(| < \nabla u, w_1 - w_2 > | + |u(\mathbf{x} - w_1, \theta - \Delta\theta) - u(\mathbf{x}, \theta)| + |u(\mathbf{x} + w_2, \theta + \Delta\theta) - u(\mathbf{x}, \theta)|\right)$$

w_1, w_2: arbitrary vectors in space

θ : time increment between discrete time slices

$clt \rightarrow$ **0 at a pixel** (i, j) **There is motion coherence (objects)**

$clt \rightarrow$ **large at a pixel** (i, j) **There is no motion coherence (noise)**

Image segmentation of macrophages

- space-time filtering

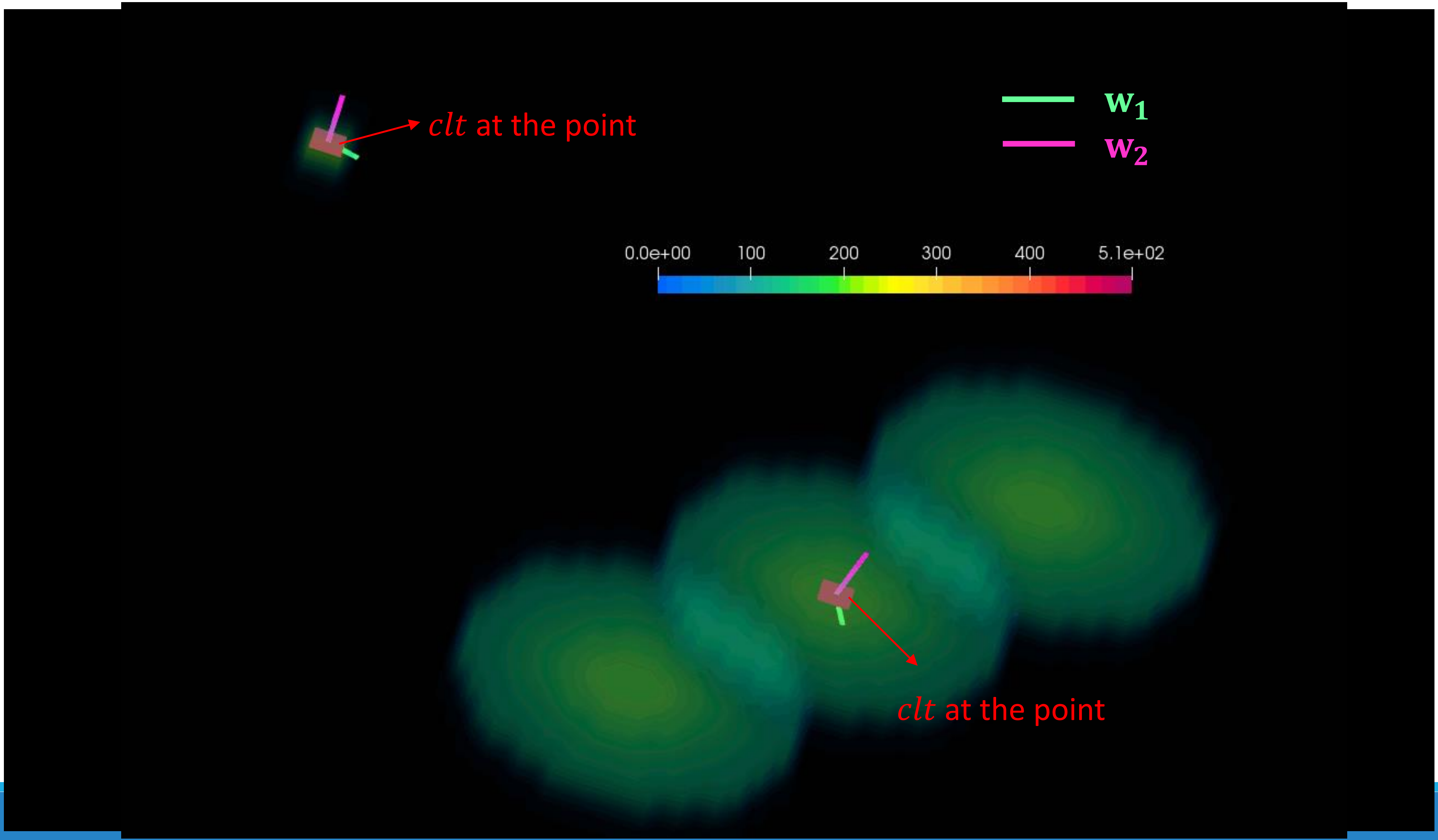


Image segmentation of macrophages

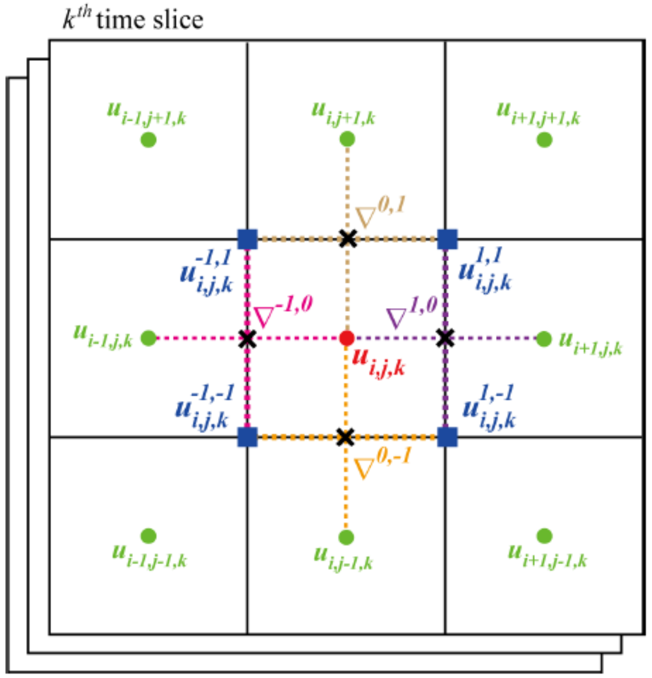
- Discretization of space-time filtering equation

By using the semi-implicit scheme,

$$\frac{u_k^{n+1} - u_k^n}{\tau_F} = clt(u_k^n)\nabla \cdot \left(g(|\nabla u_k^{\sigma;n}|)\nabla u_k^{n+1}\right),$$

$u_k^n(\mathbf{x}) = u(n\tau_F, \mathbf{x}, k\Delta\theta)$: Numerical solution in k^{th} time frame in n^{th} filtering step

By using the diamond cell approach,



$$\nabla^{1,0}u^n_{i,j,k} = \frac{1}{h}(u^n_{i+1,j,k} - u^n_{i,j,k}, u^{1,1}_{i,j,k} - u^{1,-1}_{i,j,k}), \qquad \nabla^{-1,0}u^n_{i,j,k} = \frac{1}{h}(u^n_{i-1,j,k} - u^n_{i,j,k}, u^{-1,1}_{i,j,k} - u^{-1,-1}_{i,j,k}),$$

$$\nabla^{0,-1}u^n_{i,j,k} = \frac{1}{h}(u^{1,-1}_{i,j,k} - u^{-1,-1}_{i,j,k}, u^n_{i,j-1,k} - u^n_{i,j,k}), \qquad \nabla^{0,1}u^n_{i,j,k} = \frac{1}{h}(u^{1,1}_{i,j,k} - u^{-1,1}_{i,j,k}, u^n_{i,j+1,k} - u^n_{i,j,k}),$$

$$\boxed{u^{n+1}_{i,j,k} = u^n_{i,j,k} + \frac{\tau_F}{h^2}\, clt(u^n_{i,j,k}) \sum_{|l|+|m|=1} g(|\nabla^{l,m}u^{\sigma;n}_{i,j,k}|)(u^{n+1}_{i+l,j+m,k} - u^{n+1}_{i,j,k})}$$

$$l, m \in \{-1, 0, 1\},\ |l| + |m| = 1$$

Image segmentation of macrophages

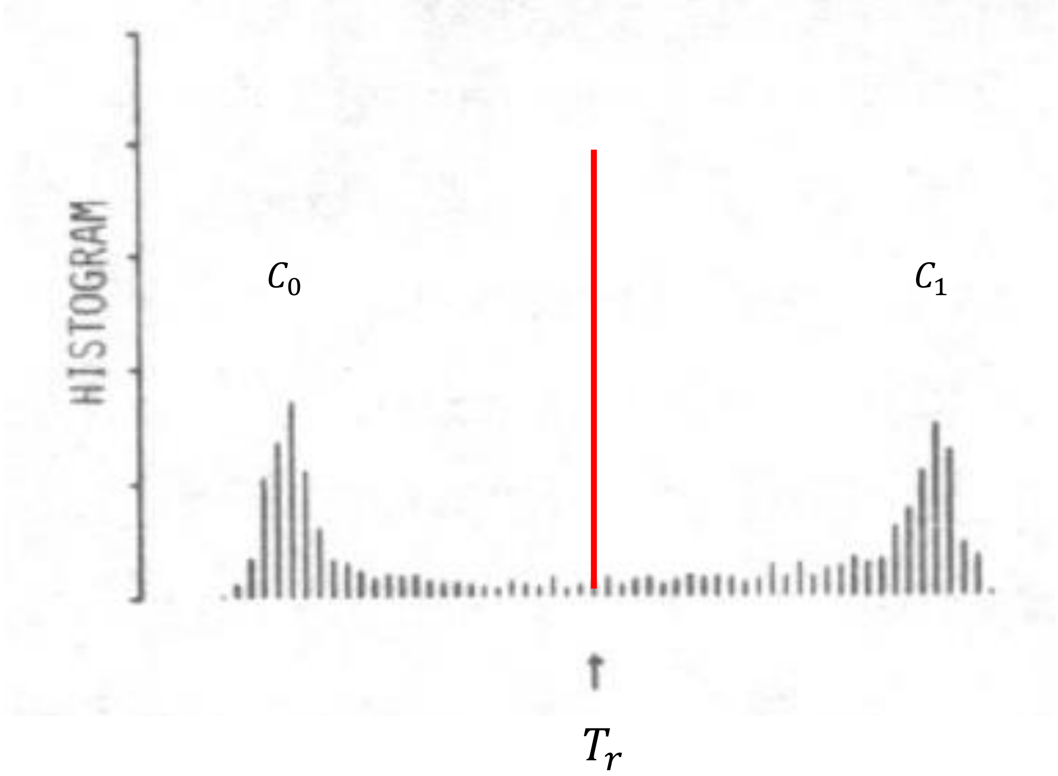
- Local Otsu’s method (thresholding)

Calculation of the optimal threshold using global Otsu’s method in a local window

Global Otsu's method

Let us suppose there are two classes, C_0 and C_1 represented by a threshold intensity k.

OTSU, N.: *A threshold selection method from gray-level histograms*, IEEE transactions on systems, man, and cybernetics 9(1) (1979), 62-66.



Searching for a threshold T_r that maximizes $\sigma_B^2(T_r)$

$$\boxed{\sigma_B^2\,(T_r^*) = \max_{0 \le T_r < L} \sigma_B^2\,(T_r)}$$

Variance between two classes is defined by

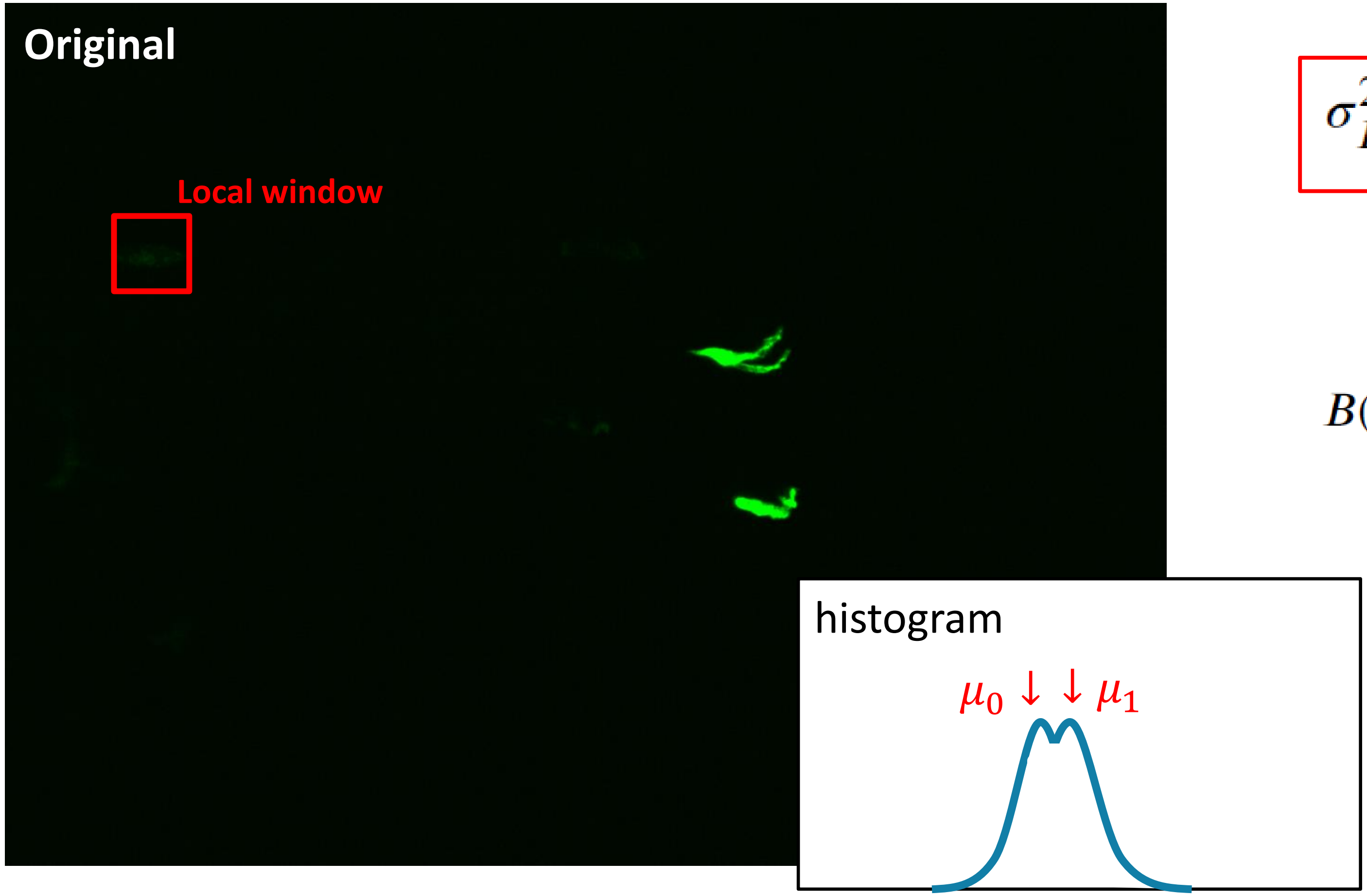
$$\sigma_B^2\,(T_r^*) = \frac{\left(\mu_T \omega(T_r) - \mu(T_r)\right)^2}{\omega(T_r)(1 - \omega(T_r))}$$

$\omega_{\#}$: probability of class # , #=0,1

$\mu_{\#}$: mean of class # , #=0,1

Image segmentation of macrophages
- Local Otsu's method

The optimal threshold in a certain window of size $s \times s$ for every pixel centered in (i, j) is calculated



$$\boxed{\sigma_B^2(T^*_{i,j}) = \max_{0 \le T_{i,j} < L} \sigma_B^2(T_{i,j})}$$

$$B(i, j) = \begin{cases} 1, I(i, j) > T^*_{i,j} \text{ and } \bigstar \text{ is fulfilled} \\ 0, \text{otherwise} \end{cases}$$

$\bigstar$
$$\boxed{\frac{|\mu_0(T^*_{i,j}) - \mu_1(T^*_{i,j})|}{\mu_0(T^*_{i,j})} > \delta,}$$

Image segmentation of macrophages
- Global Otsu's method

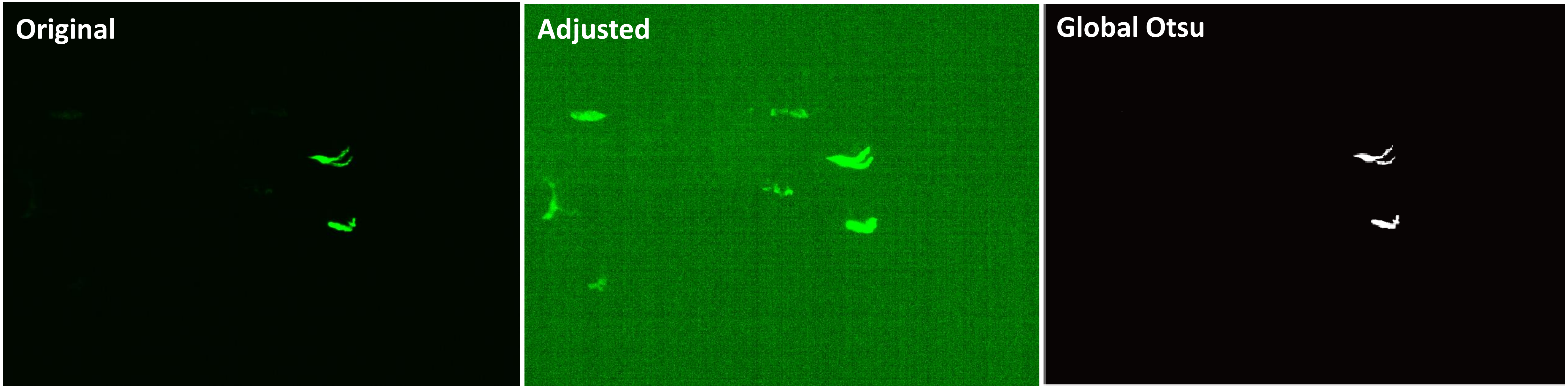
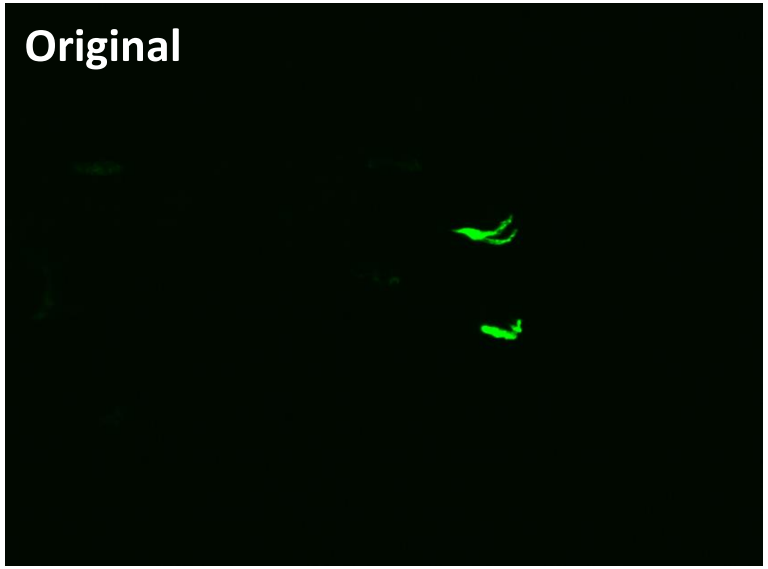
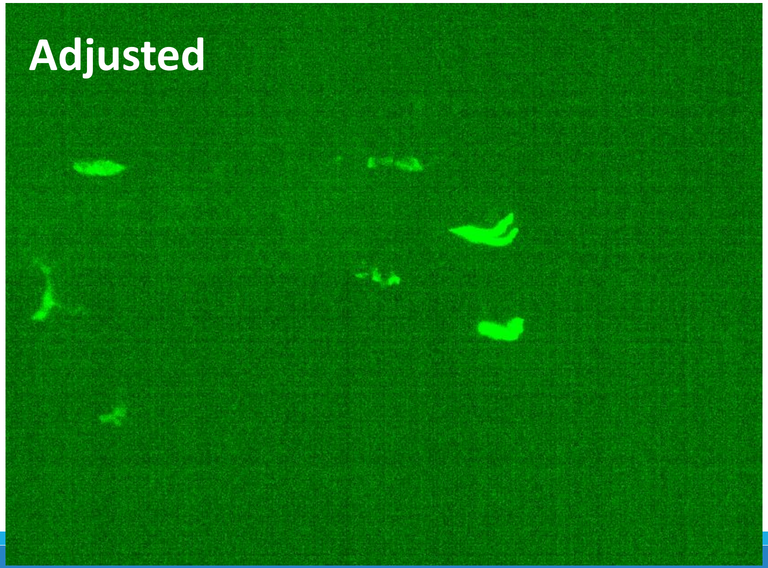
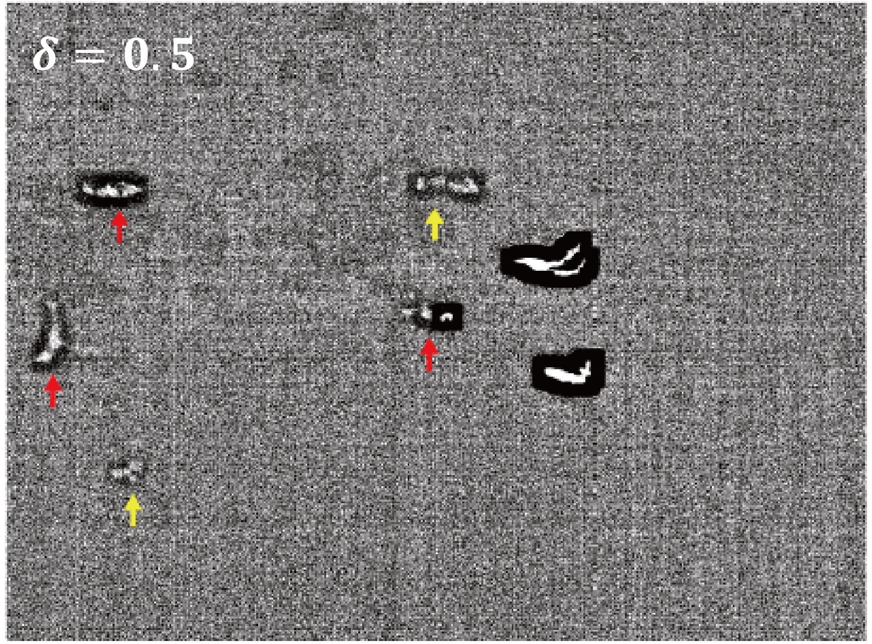


Image segmentation of macrophages

- Local Otsu's method **without** space-time filtering



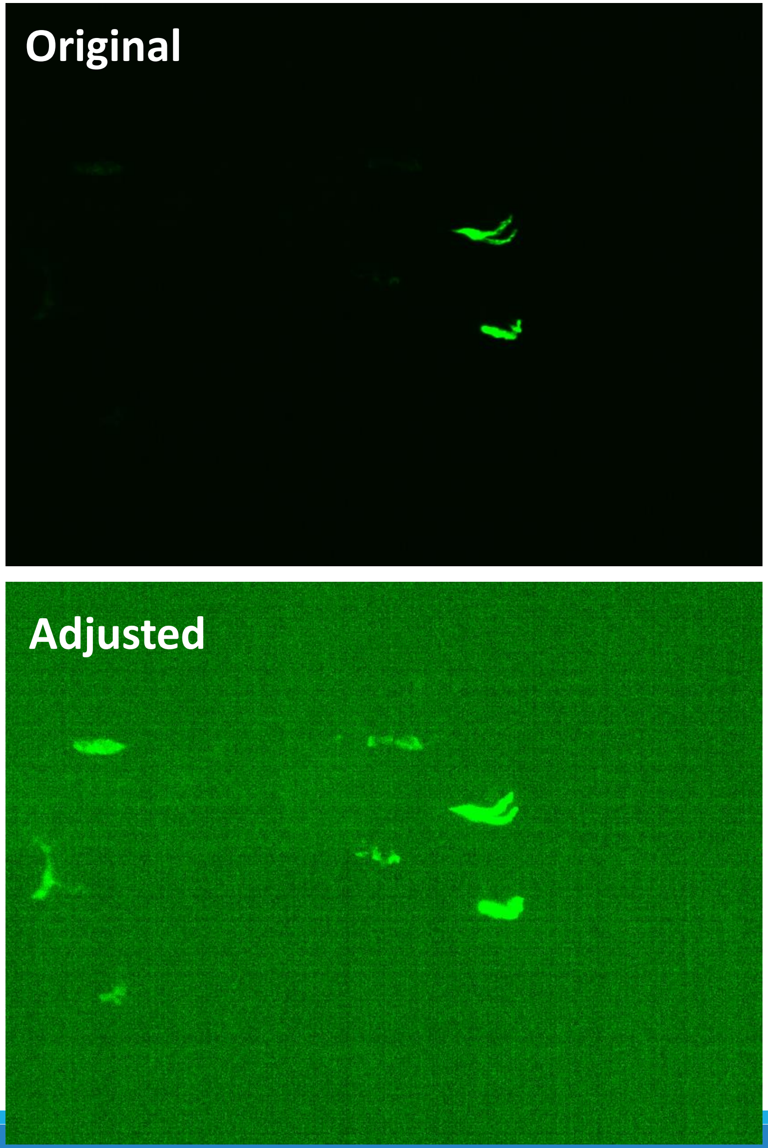


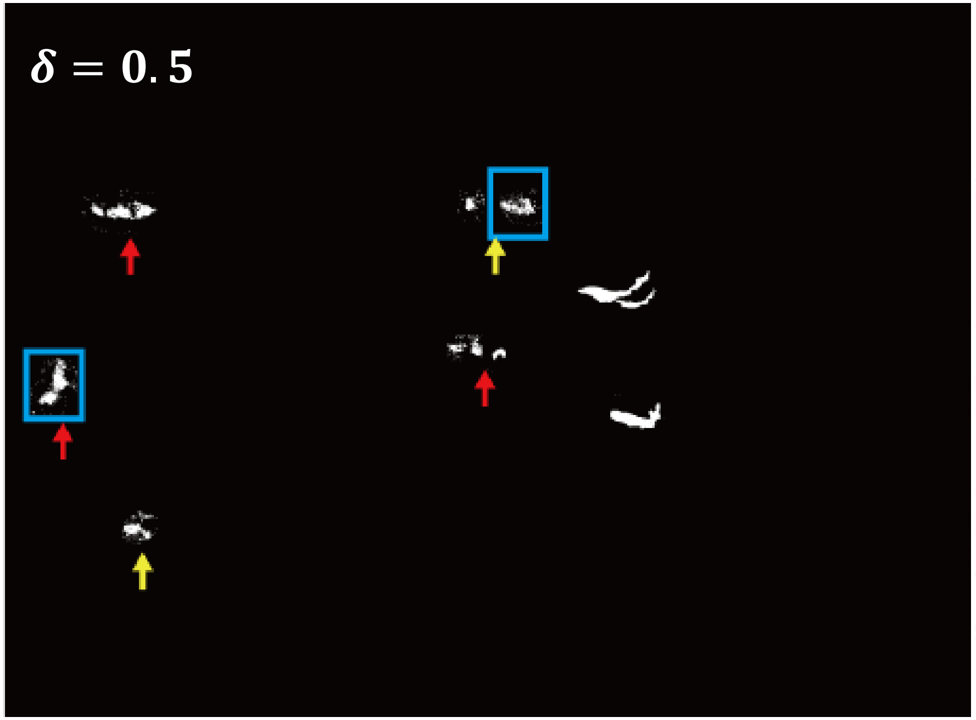


$$\frac{|\mu_0(T^*_{i,j}) - \mu_1(T^*_{i,j})|}{\mu_0(T^*_{i,j})} > \delta, \quad \sigma_B^2(T^*_{i,j}) = \max_{0 \le T_{i,j} < L} \sigma_B^2(T_{i,j})$$

Image segmentation of macrophages

- Local Otsu's method **with** space-time filtering





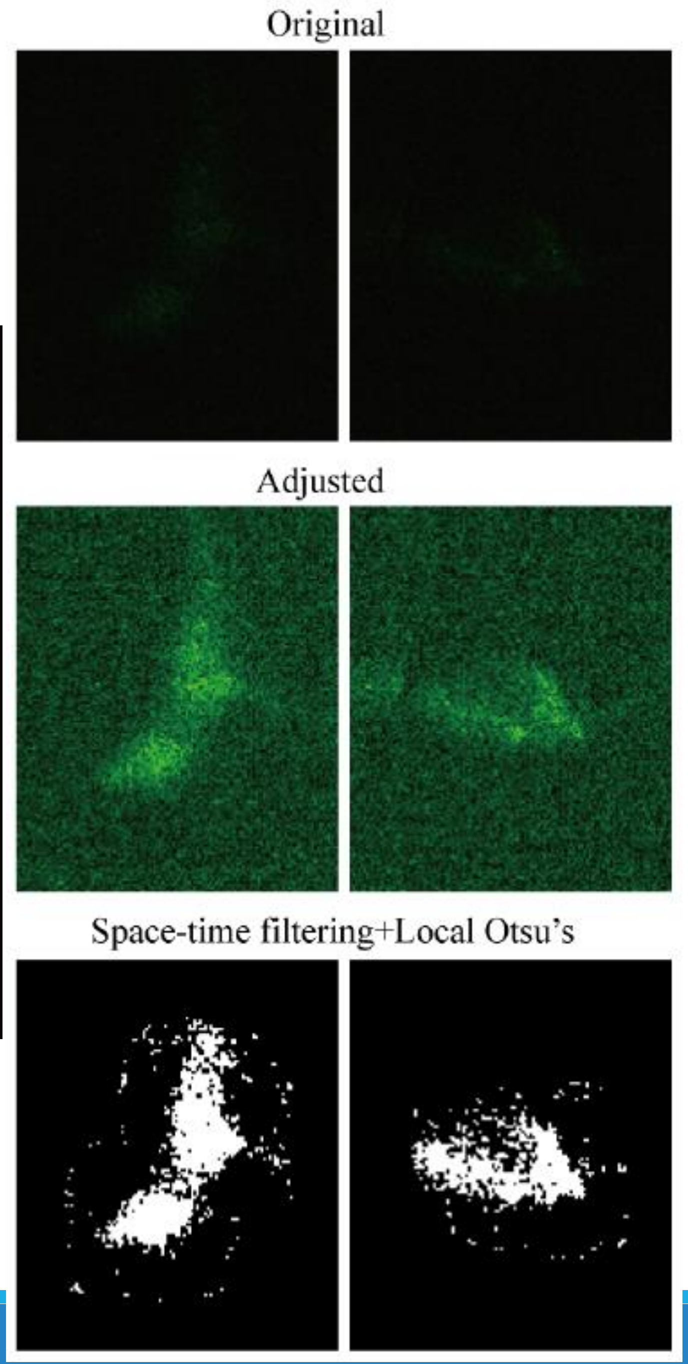


Image segmentation of macrophages

- The Subjective surface segmentation (SUBSURF) method

*The SUBSURF method **remove the artifact** from the local Otsu's method and **smooth the inside of macrophages***

Original image

$$u_t = |\nabla u| \nabla \cdot \left(g \frac{\nabla u}{|\nabla u|} \right) \qquad g(s) = \frac{1}{1 + Ks^2}, \; K > 0, \qquad s = |\nabla G_\sigma * I^0|$$

The final discretization form is given by

$$u_{ij}^{n+1} - u_{ij}^{n} = \frac{\tau}{h^2} \bar{Q}_{ij}^{lm;n} \sum_{|l|+|m|=1} g_{ij}^{lm,\sigma} \frac{u_{i+l,j+m}^{n+1} - u_{ij}^{n+1}}{Q_{ij}^{lm;n}}$$

$$Q_{ij}^{lm;n} = \sqrt{\epsilon^2 + |\nabla^{lm} u_{ij}^{n}|^2} \qquad \bar{Q}_{ij}^{lm;n} = \sqrt{\epsilon^2 + \frac{1}{4} \sum_{|l|+|m|=1} |\nabla^{lm} u_{ij}^{n}|^2}$$

Image segmentation of macrophages

- The Subjective surface segmentation (SUBSURF) method

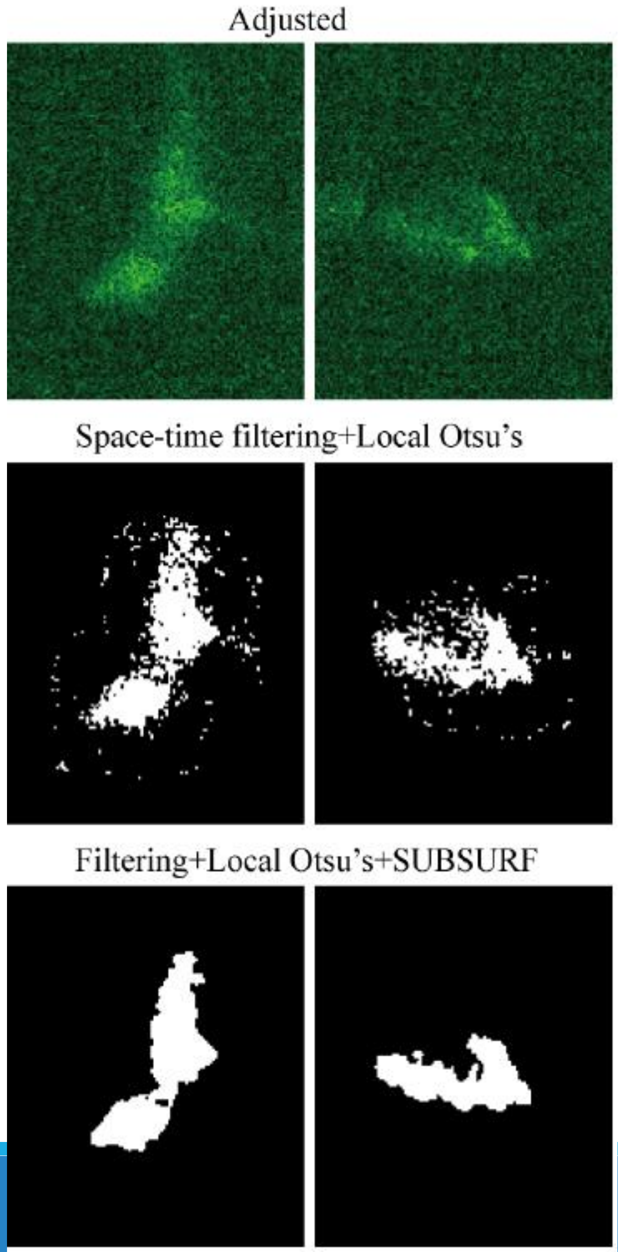
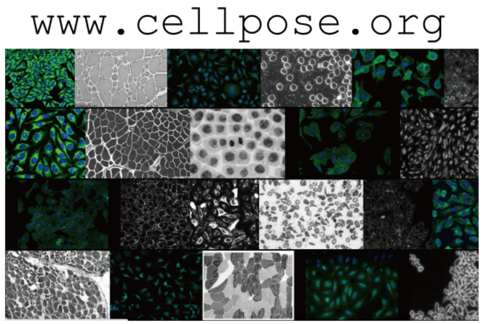
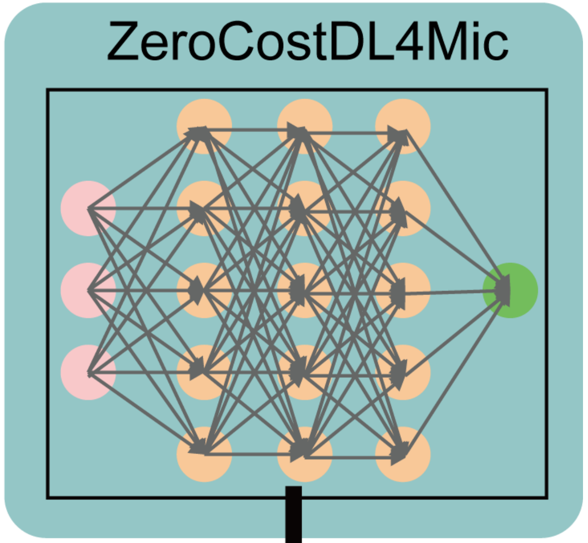


Image segmentation of macrophages

- Comparison with deep learning-based segmentation



Training networks

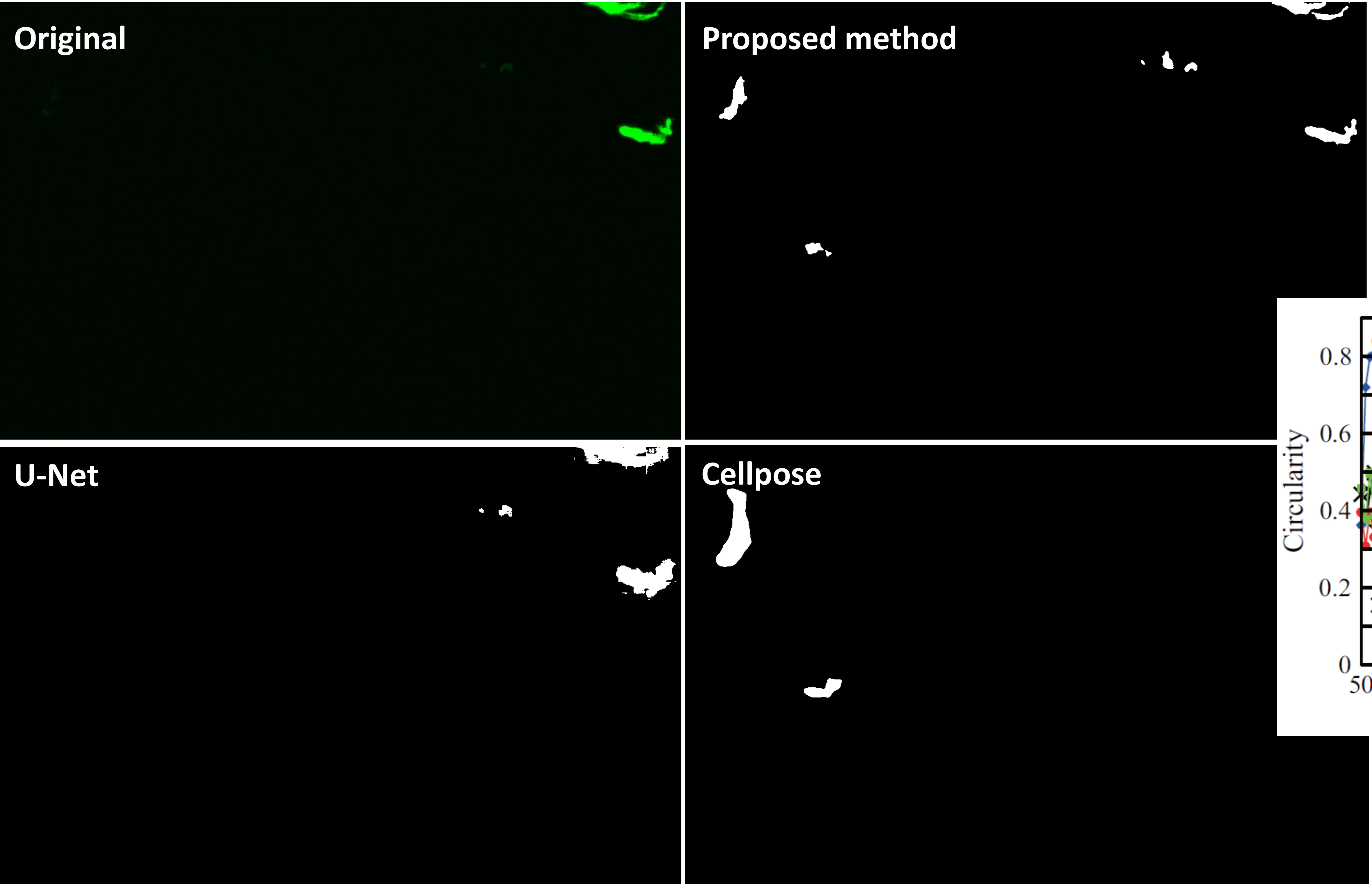


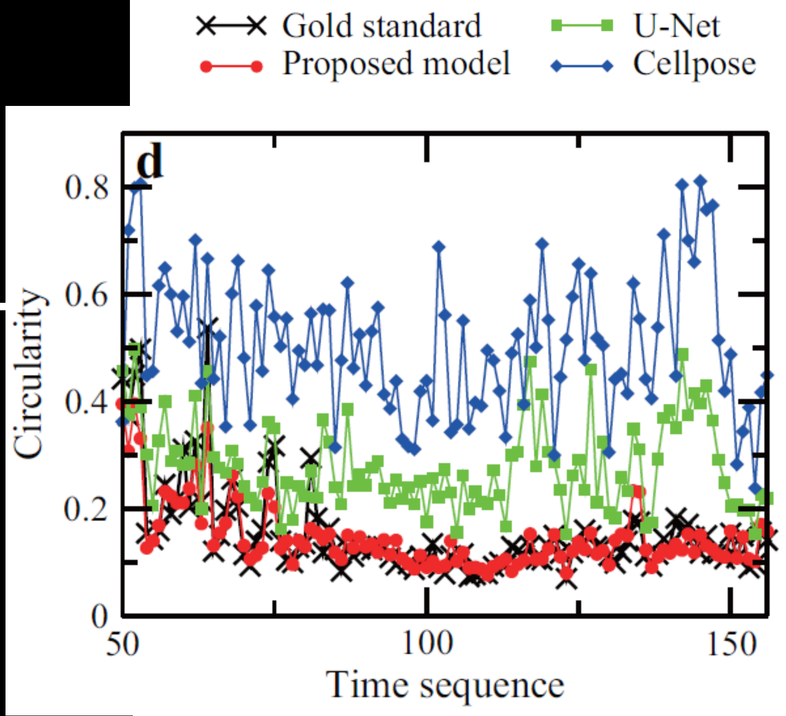
Testing to macrophage data

Stringer, Carsen, et al. "Cellpose: a generalist algorithm for cellular segmentation." *Nature methods* 18.1 (2021)

von Chamier, Lucas, et al. "Democratising deep learning for microscopy with ZeroCostDL4Mic." *Nature communications* 12.1 (2021)

- Comparison with deep learning-based segmentation

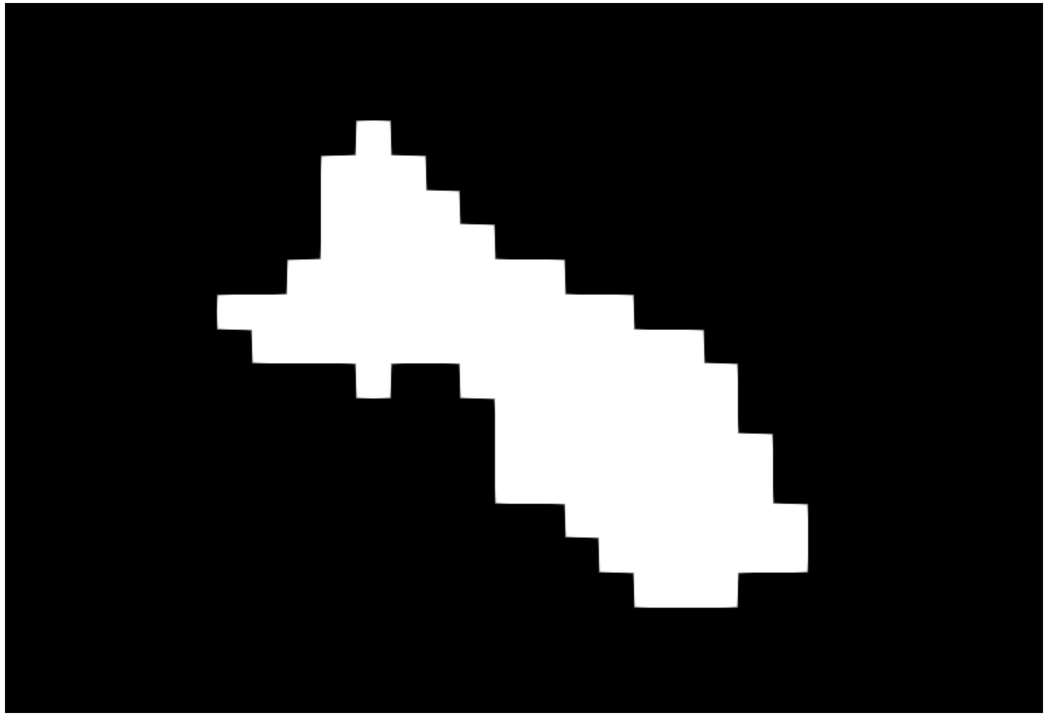


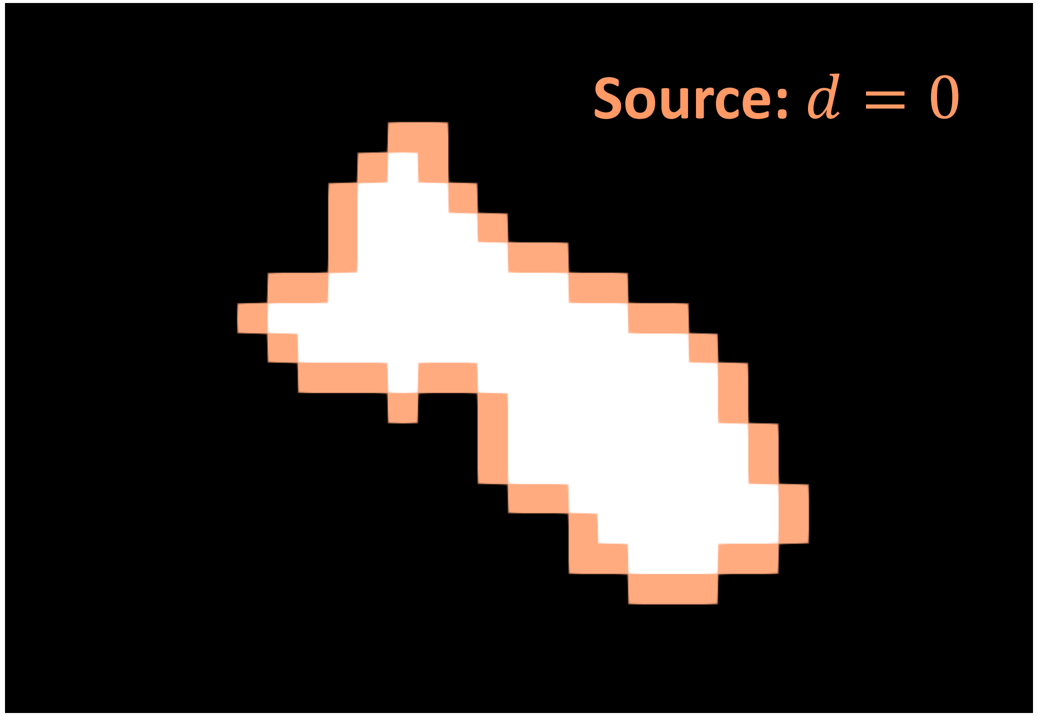


Cell tracking

- The approximate center of the cell

Time-relaxed eikonal equation: $d_t + |\nabla d| = 1$





Cell tracking

- The approximate center of the cell

We solve the unknown function $d(\mathbf{x}, t, \theta)$, $(\mathbf{x}, t) \in \Omega \times [0, T_E]$

By using Rouy-Tourin scheme, (in 2D+time)

$$d_{ij}^{n+1}(\theta) = d_{ij}^{n}(\theta) + \tau_D - \frac{\tau_D}{h}\sqrt{M_{ij}^{10}(\theta) + M_{ij}^{01}(\theta)}$$

$$M_{ij}^{10}(\theta) = \max\Big(D_{ij}^{-1,0}(\theta), D_{ij}^{1,0}(\theta)\Big)$$

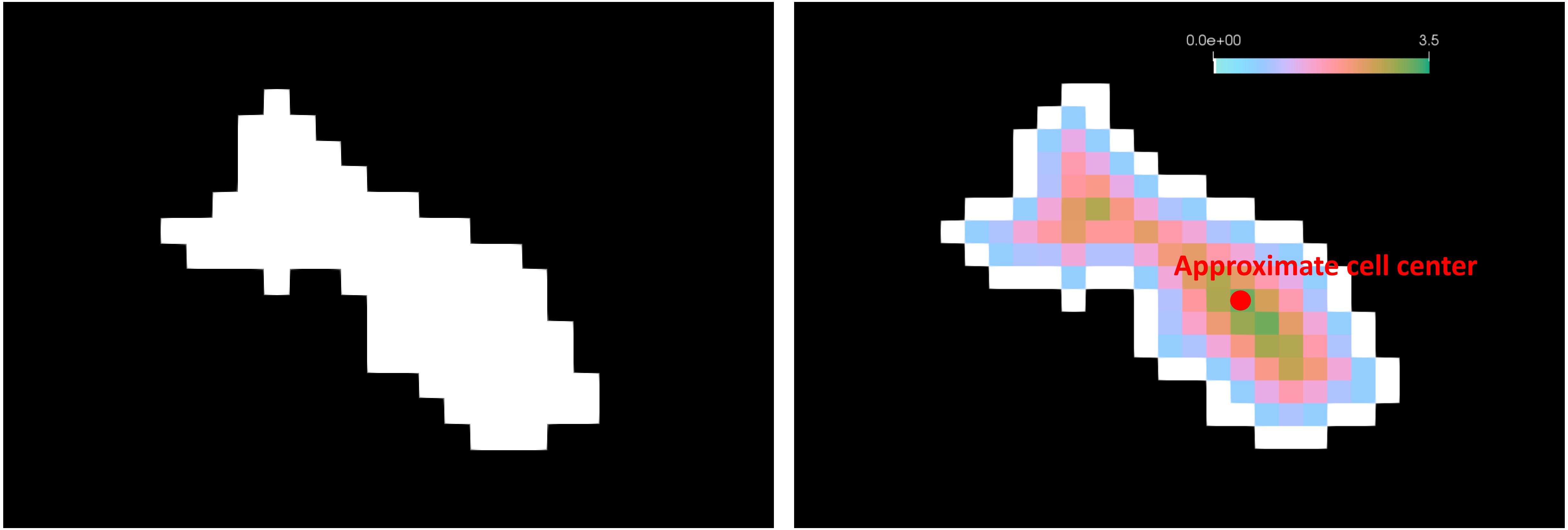
$$M_{ij}^{01}(\theta) = \max\Big(D_{ij}^{0,-1}(\theta), D_{ij}^{0,1}(\theta)\Big)$$

$$D_{ij}^{lm}(\theta) = \Big(\min\Big(d_{i+l,j+m}^{n}(\theta) - d_{ij}^{n}(\theta), 0\Big)\Big)^2$$

$$l, m \in \{-1, 0, 1\}, \ |l| + |m| = 1$$

Cell tracking

- The approximate center of the cell



Cell tracking

- Extraction of trajectories

Partial trajectories

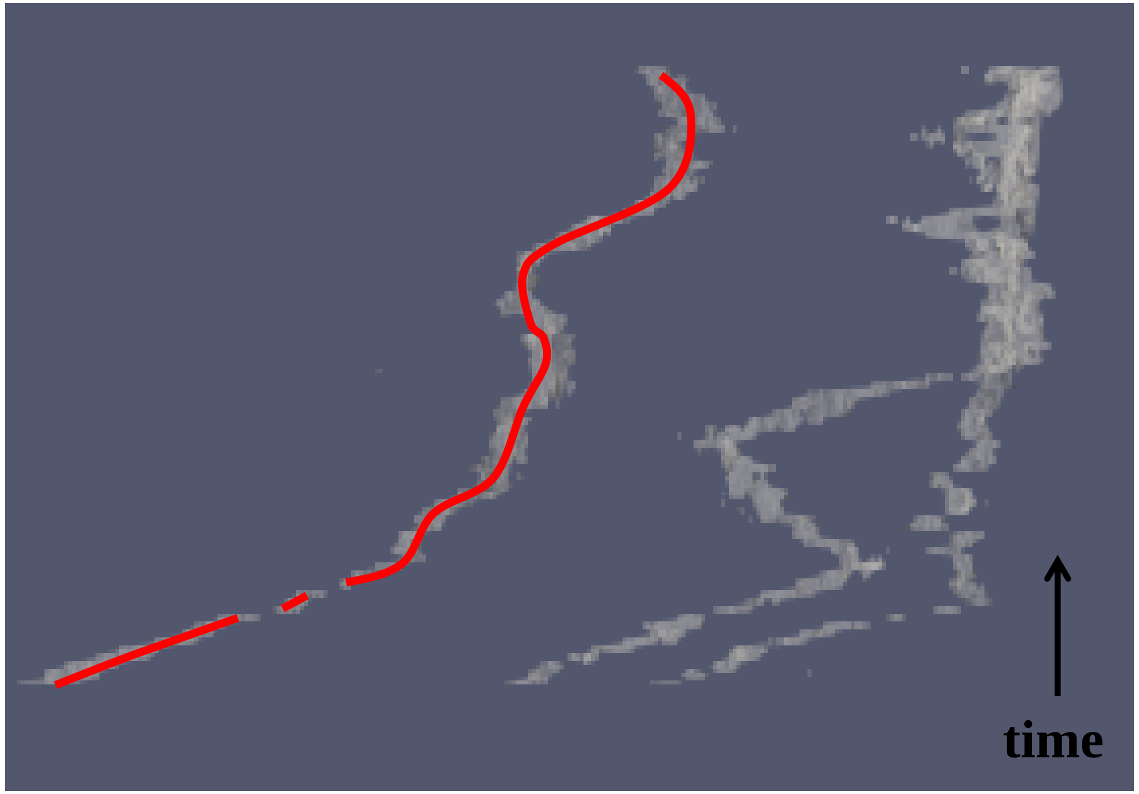
Case 1: when the cells are ***mostly*** overlapped in temporal direction

Case 2: when the cells are ***barely*** overlapped in temporal direction

Case 3: when the cells are ***not*** overlapped in temporal direction

Cell tracking

- Extraction of partial trajectories



Approximate centers are connected when the cells are overlapped in time

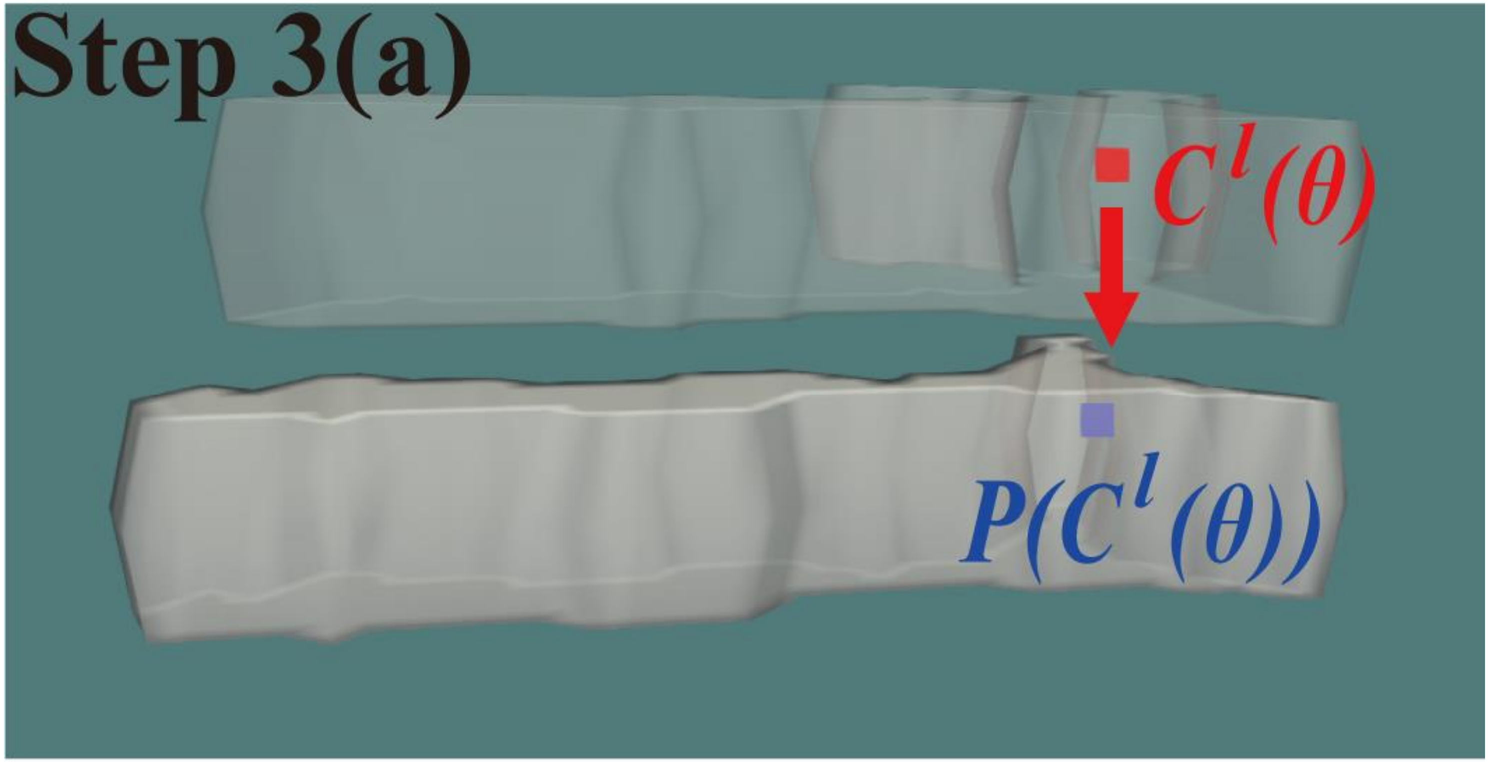
Cell tracking

Steps for extracting partial trajectories

Cell tracking

- Extraction of partial trajectories

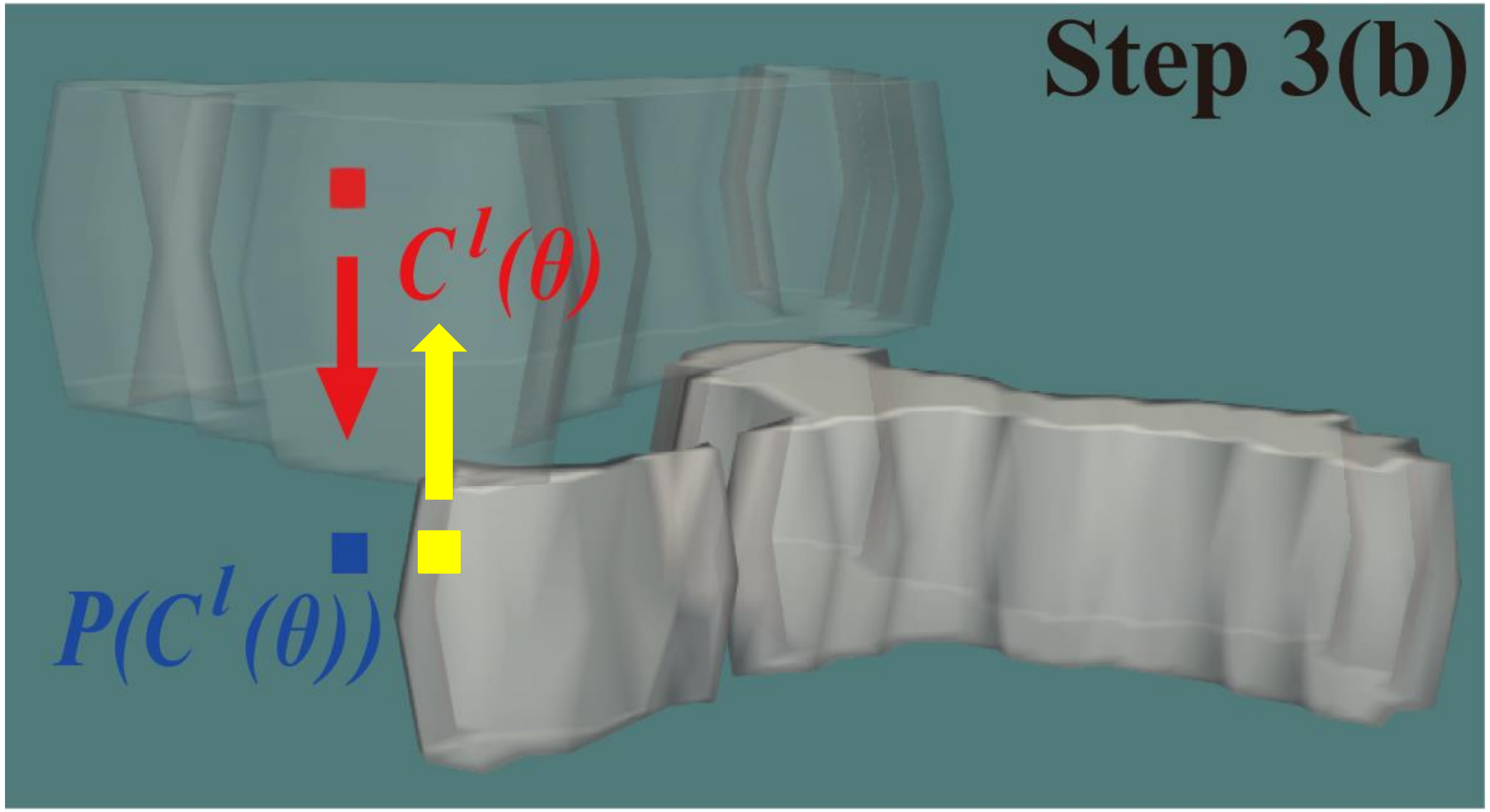
Case 1: when the cells are ***mostly*** overlapped in temporal direction



Cell tracking

- Extraction of partial trajectories

Case 2: when the cells are ***barely*** overlapped in temporal direction



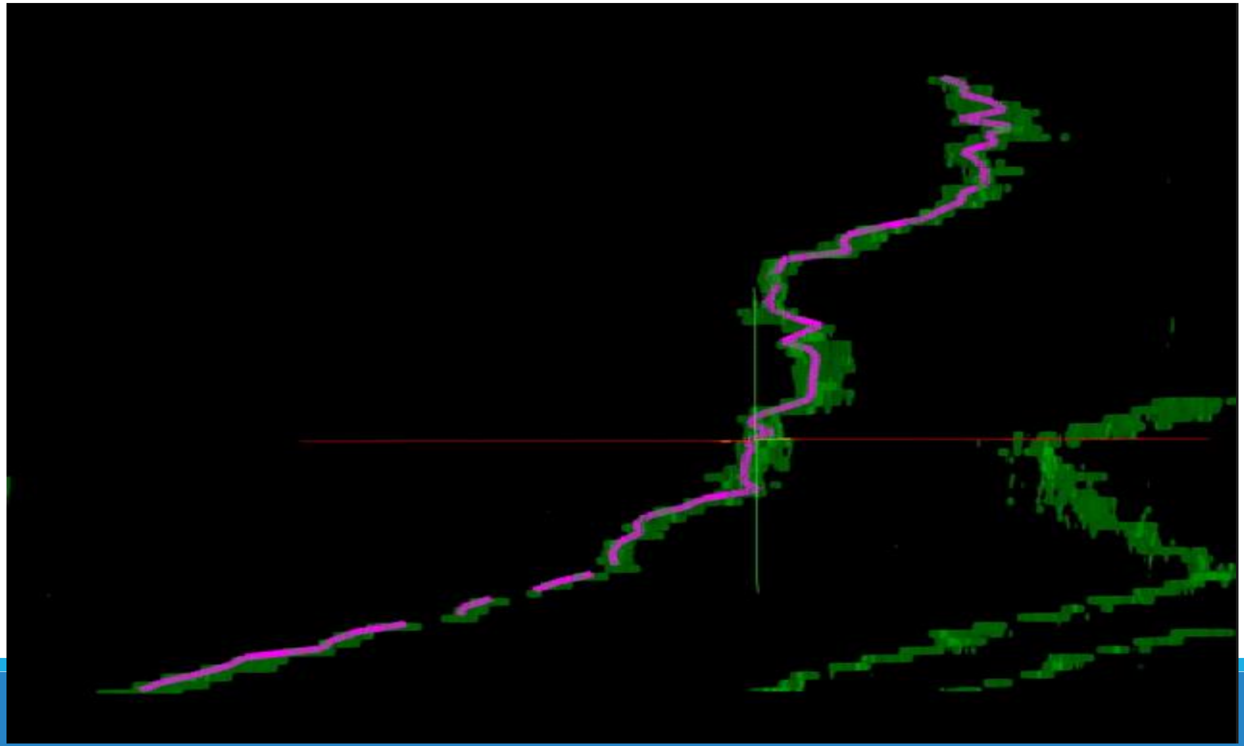
Cell tracking

- Extraction of partial trajectories

The steps are repeated until every cell is inspected for forming partial trajectories

Cell tracking

- Extraction of partial trajectories



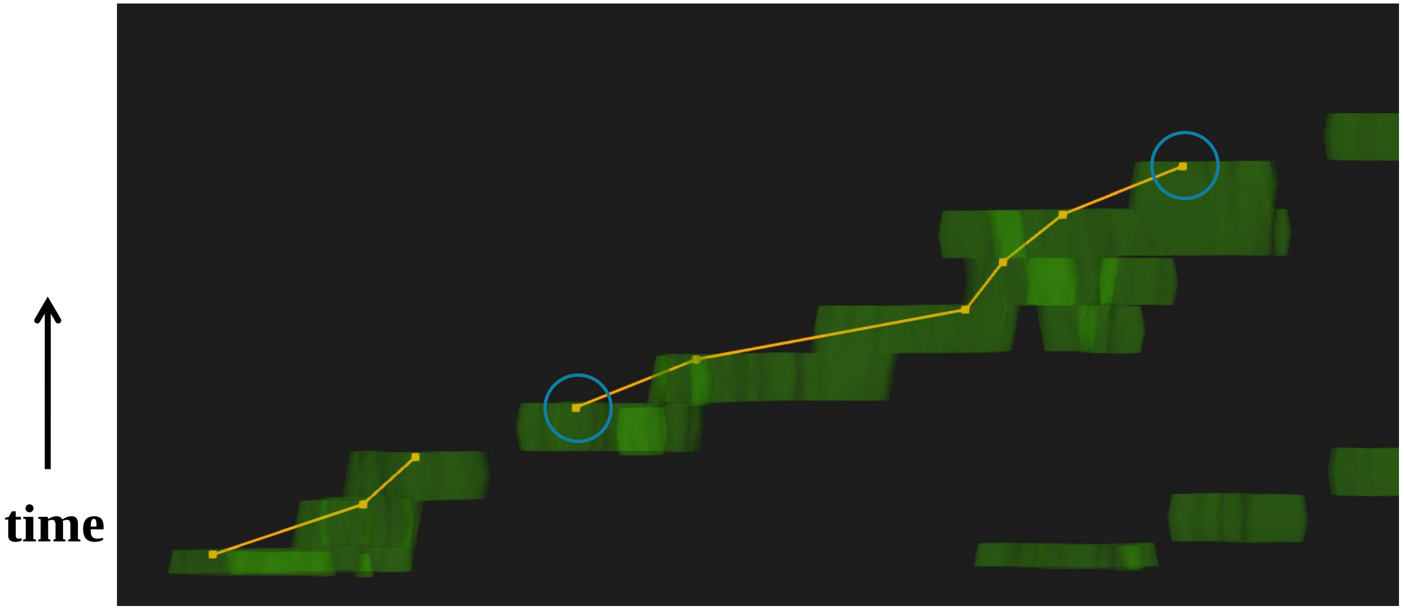
Cell tracking
- Connection of partial trajectories

We assume that the reason for non-overlapping cells is that their ***movement is relatively fast***.

→ ***non-overlapping cells keep their direction of movement*.**

Cell tracking
- Connection of partial trajectories

The tangent is computed by third order accuracy using finite difference approximation

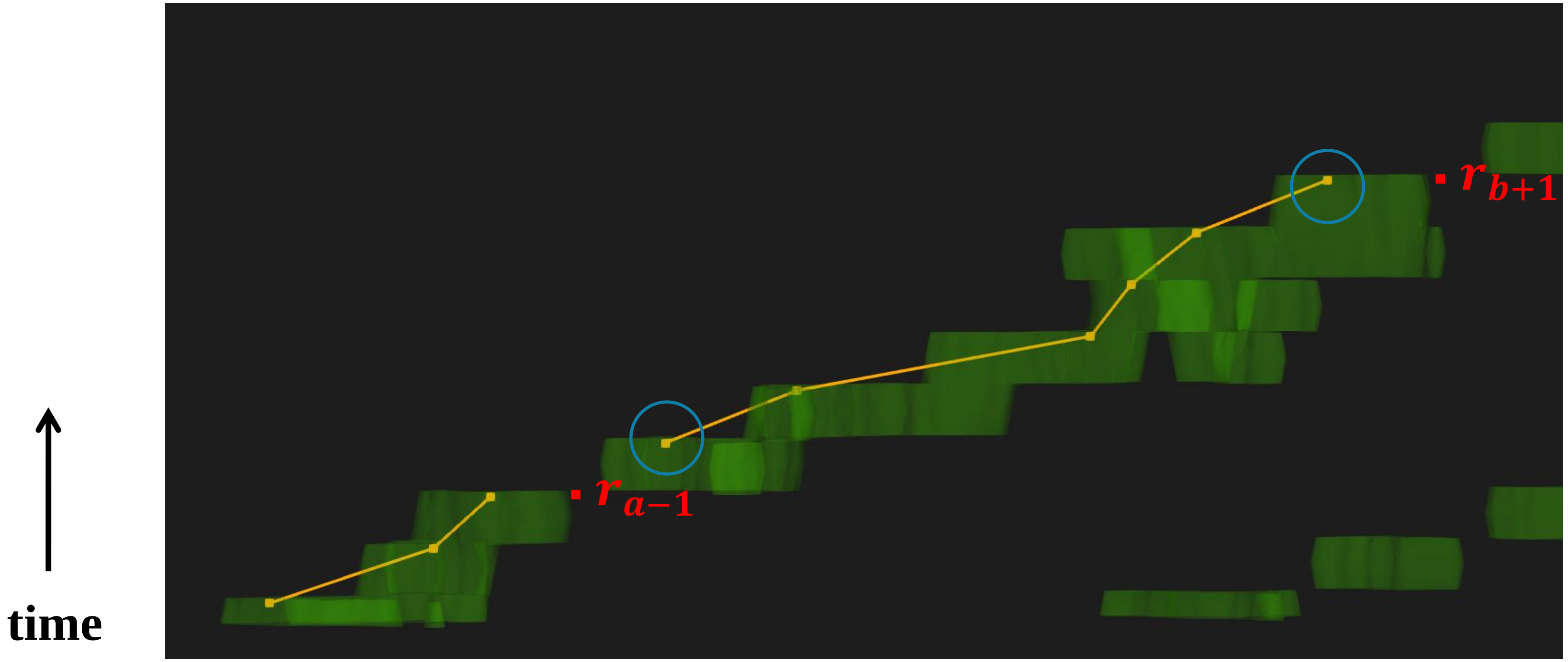


$$V^{b}(\mathbf{r}_{\theta}) = \frac{1}{\Delta\theta}\left(\frac{11}{6}\mathbf{r}_{\theta} - 3\mathbf{r}_{\theta-1} + \frac{3}{2}\mathbf{r}_{\theta-2} - \frac{1}{3}\mathbf{r}_{\theta-3}\right), \quad V^{f}(\mathbf{r}_{\theta}) = \frac{1}{\Delta\theta}\left(-\frac{11}{6}\mathbf{r}_{\theta} + 3\mathbf{r}_{\theta+1} - \frac{3}{2}\mathbf{r}_{\theta+2} + \frac{1}{3}\mathbf{r}_{\theta+3}\right)$$

$\mathbf{r}_{\theta} = (x_{\theta}, y_{\theta})$ point of the partial trajectory at θ

Cell tracking

- Connection of partial trajectories



Under the assumption, $V^f(\mathbf{r}_{a-1}) = V^f(\mathbf{r}_a)$

$$\mathbf{r}_{a-1} = -\frac{6}{11}V^f(\mathbf{r}_a)\cdot\Delta\theta + \frac{18}{11}\mathbf{r}_a - \frac{9}{11}\mathbf{r}_{a+1} + \frac{2}{11}\mathbf{r}_{a+2},$$

Under the assumption, $V^b(\mathbf{r}_{b+1}) = V^b(\mathbf{r}_b)$

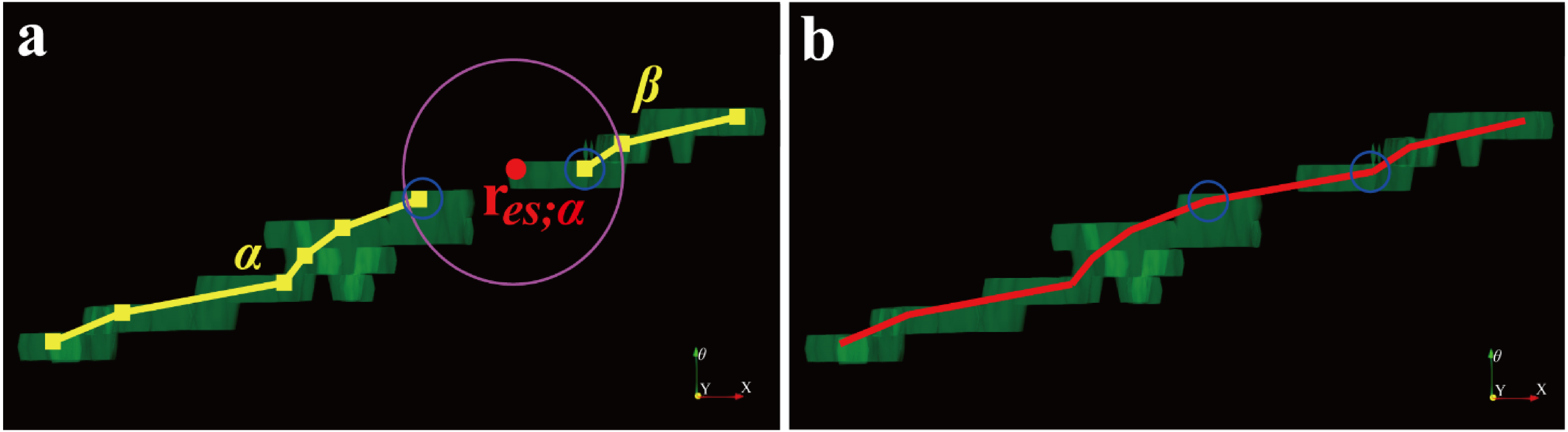
$$\mathbf{r}_{b+1} = \frac{6}{11}V^b(\mathbf{r}_b)\cdot\Delta\theta + \frac{18}{11}\mathbf{r}_b - \frac{9}{11}\mathbf{r}_{b-1} + \frac{2}{11}\mathbf{r}_{b-2}$$

Cell tracking

- Connection of partial trajectories

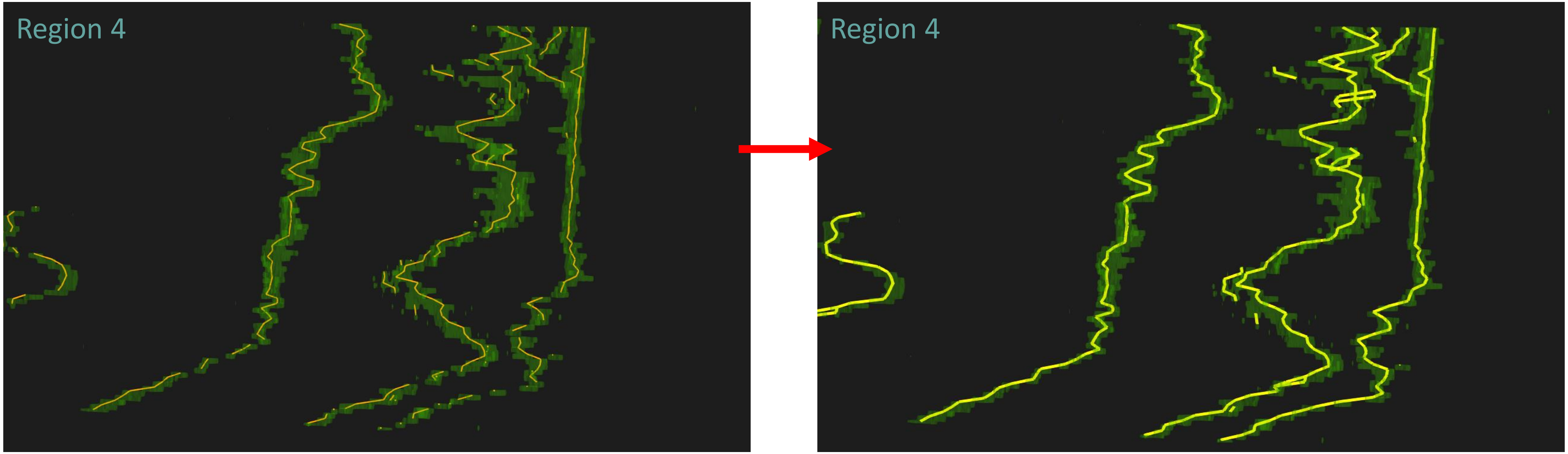
If there is a trajectory near the estimated point $\mathbf{r}_{es}$, i.e., $|\mathbf{r}_{es} - \mathbf{r}_i| < \Delta \mathbf{r}$

The trajectory is linked to the trajectory containing point r_i



Cell tracking

- Connection of partial trajectories

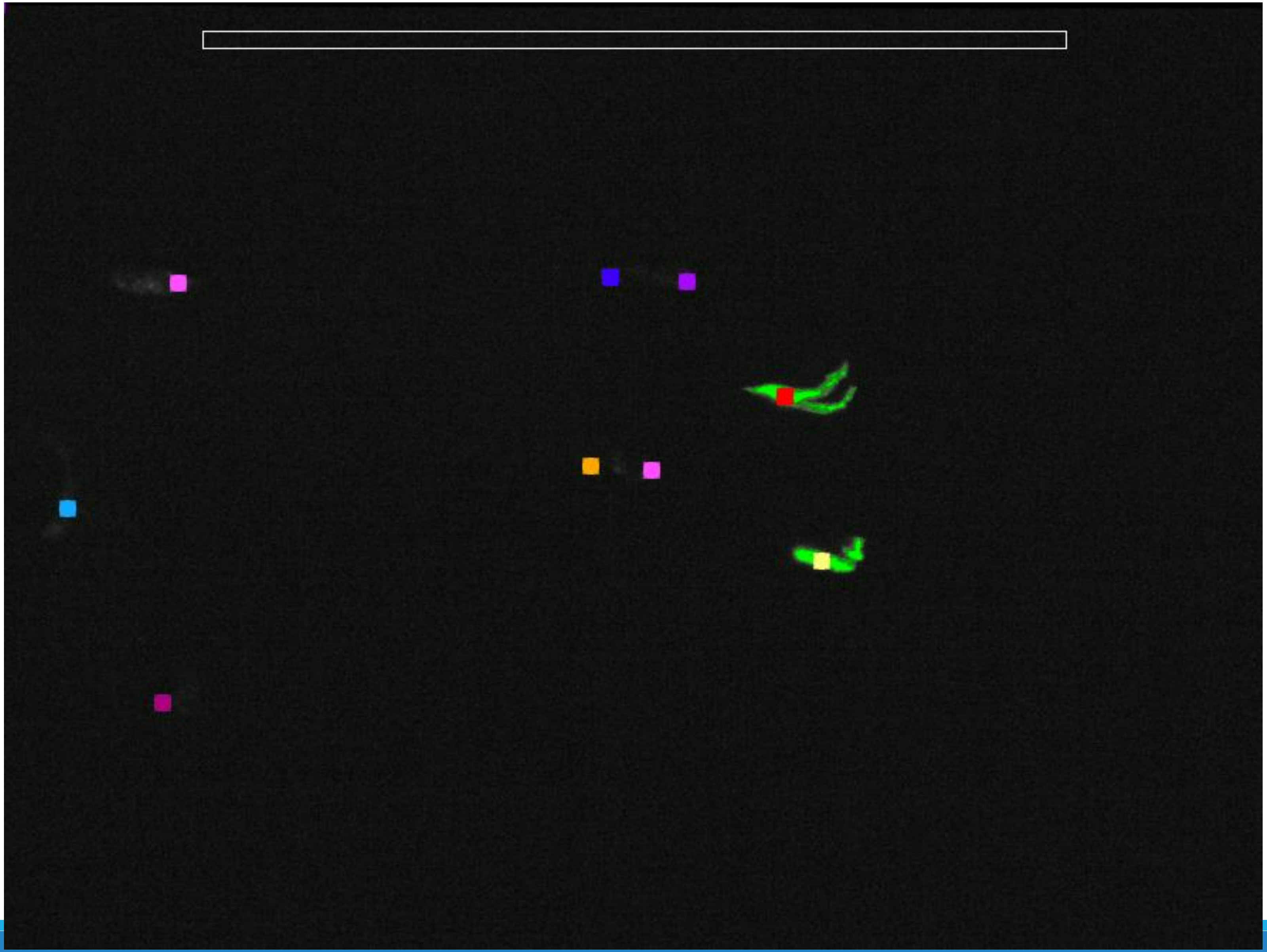


Result - 1st dataset of macrophages

$$accuracy = \frac{\# \ of \ correct \ links}{\# \ of \ total \ links}$$

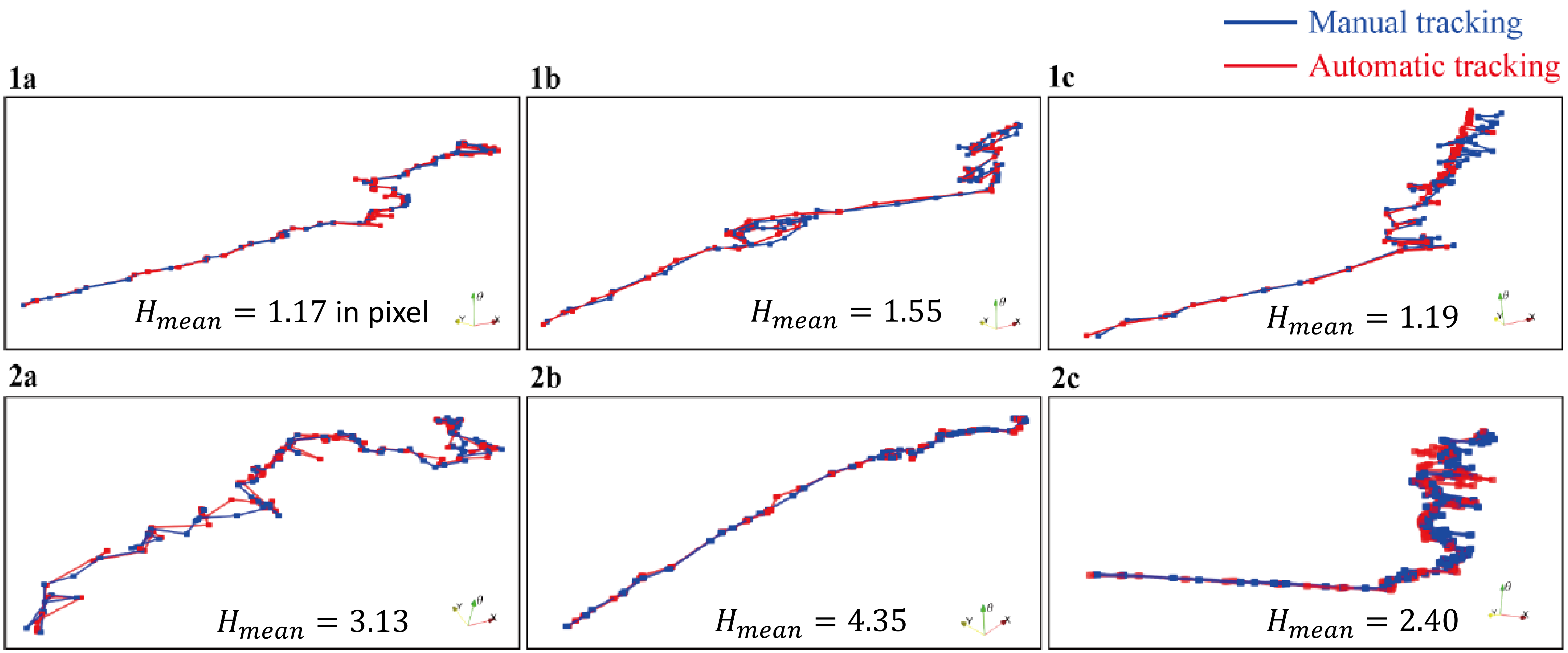
$$Mean \ accuracy \approx 0.975$$

Result - 2^{nd} dataset of macrophages



$Mean\ accuracy \approx 0.974$

Cell tracking- quantitative analysis by using the mean Hausdorff distance



$$H_{mean}(A,M) = \max\left(h_{mean}(A,M), h_{mean}(M,A)\right), \quad h(A,M) = \frac{1}{N_{\theta_F}} \sum_{a \in A} \min_{m \in M} \|a - m\|$$

1 pixel=0.326μm

Conclusion

- Combination of space-time filtering, local Otsu's, and SUBSURF method **segmented all macrophages** having high variability of the image intensity
- The proposed cell tracking method traces the movement of cells by considering **overlap of cell bodies** in the temporal direction and **the approximate direction** of the movement.

Acknowledgement

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UNIVERSITÉ DE MONTPELLIER

Thank you for your attention