Image Analysis in Cell and Developmental Biology

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EMBL Heidelberg, Germany

20120807 Biocity, Turku

Experiment → Image Acquisition → Processing → Analysis

Aug 6, 2012		
9:00	Lecture Image Analysis: an overview	
10:30	Lecture: BioimageXD, Image Analysis Software	
13:00	Lecture: Microscopy	
Aug. 7, 2012		
9:00 - 12:00	Practical: Microscopy / ImageJ (sub-groups)	1.5 hrs
13:00 – 14:30	Practical : BioimageXD	
14:30 – 17:00	Practical: ImageJ	2.5 hrs
Aug. 8, 2012		
9:00 – 11:30	Lecture: Microscopy	
13:00 – 15:00	Practical: Microscopy / BioImageXD (sub-groups)	
15:00 – 17:00	Practical: ImageJ	2 hrs
Aug. 9, 2012		
9:00 – 12:00	Practical: ImageJ Macro programming	3 hrs
13:00 – 14:30	Practical: BioImageXD	
14:30 – 17:00	Practical: Microscopy	

EMBL Heidelberg

Rainer Pepperkok (CBB, ALMF)
Heiko Runz (Uni. Heidelberg)
Fatima Verissimo (CBB)
Francois Pouthas (became an Artist)
Emmanuel Reynaud (now in Dublin)
Cihan Cetin (now in Pasadena)
Clemens Grabher (now in Karlsruhe)
Adam Cliffe (now in Singapore)
Tina Diamantara (DB)
Stefano De Lenzis (DB)
Darren Gilmour (DB)
Gulcin Cakan (now in Boston)
Petra Haas (Heidelberg)
Asifa Akhtar (now in Freiburg)
Ritsuko Suyama (now in Kyoto)
Janina Karres
Steve Cohen (Singapore)

EMBL ALMF

Jens Rietdorf (now in Munich)
Timo Zimmermann (now in Barcelona)
Stefan Terjung (ALMF)

Univ. Munich (LMU)

Florian Siegert
Charles David

Osaka Univ

Satoshi Ogihara

cmci.embl.de

Daily Life

Images (pictures, movies)

→ interpretation (“Fantastic!”)

Images in Science

Daily Life

Images (pictures, movies)

→ interpretation (“Fantastic!”)

Scientific Research

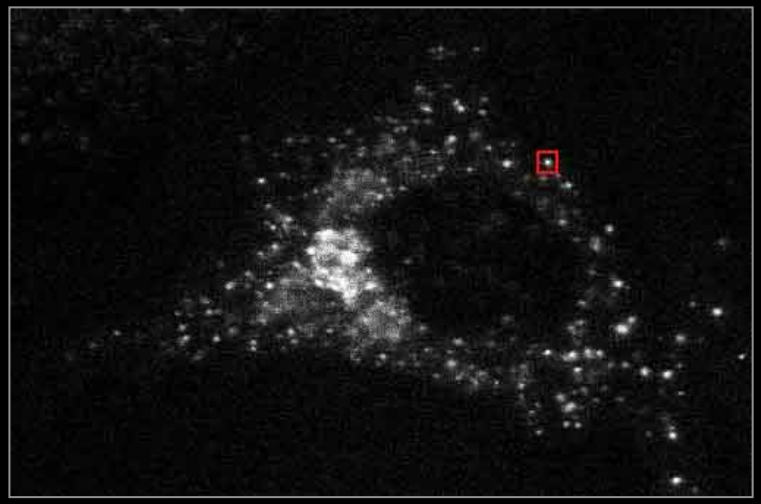
Images (static, dynamic)

→ extract numerical parameters (Results)

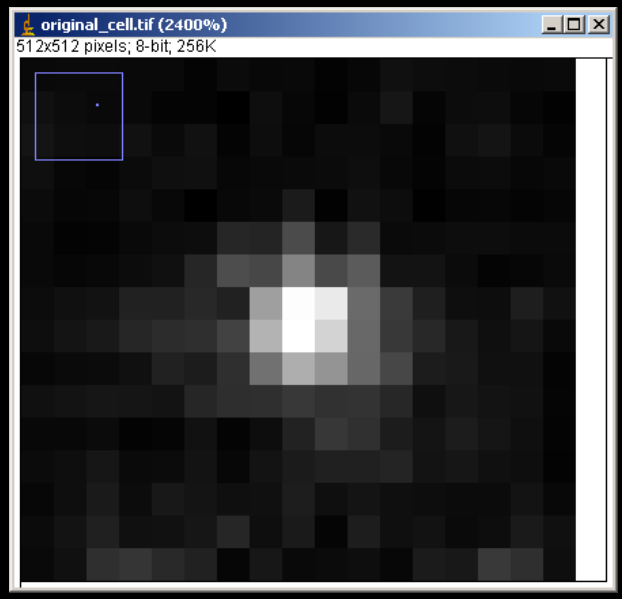
→ interpretation (Discussion)

See Rossner and Yamada (2004; pdf) for more about ‘**manipulation**’

Numerical Nature of Digital Images



Fatima Verissimo



Pixel intensity

↓

Protein density

original_cell_c1_roi_crop.txt - WordPad

File Edit View Insert Format Help

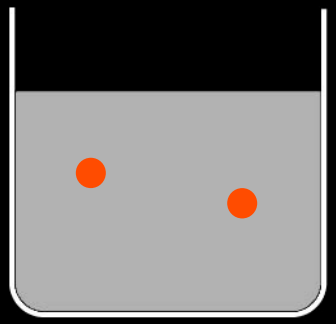
19	22	25	19	0	0	10	37	0	15	20
29	16	8	11	16	16	13	12	0	13	17
14	5	1	11	29	36	23	5	5	11	13
0	5	19	33	54	70	62	43	19	12	13
12	29	46	70	115	152	140	103	38	19	18
58	56	57	92	176	238	210	140	61	30	28
63	58	50	82	175	249	217	135	82	42	38
34	42	40	61	138	208	186	115	95	50	45
17	38	51	51	60	78	78	64	42	29	29
8	21	22	15	26	53	67	63	49	37	37
7	18	17	6	14	37	49	43	42	29	29
13	31	38	30	31	40	34	17	25	12	12

For Help, press F1 NUM

Images in Cell and Developmental Biology

Biochemistry（solution）

$$[E]+[S] \underset{k2}{\overset{k1}{\rightleftarrows}} [ES] \xrightarrow{k3} [P]$$

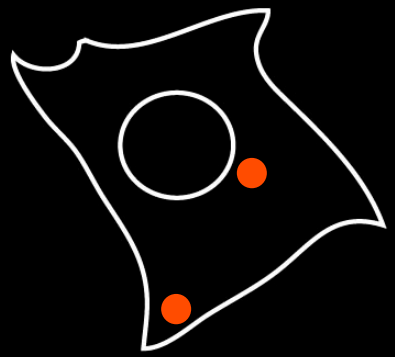


B([E], [S], [ES], [P], t)

Topo-biochemistry

(in vivo)

B([E], [S], [ES], [P], t, x, y, z)



Extracting Parameters out of Images: Overview

Experiment → Image Acquisition → Processing → Analysis

Extract numbers and interpret

Static images

- Intensity Measurement
- Morphometry
- Distribution
- Colocalization

Image sequences (dynamics)

- Intensity changes
- Shape changes
- Position changes:
  - kymograph
  - tracking
- Distribution changes
- Optical flow estimation

Static Images: Intensity Measurement

Static images

- Intensity Measurement → Intensity Difference, Intensity Profile
- Morphometry
- Distribution
- Colocalization

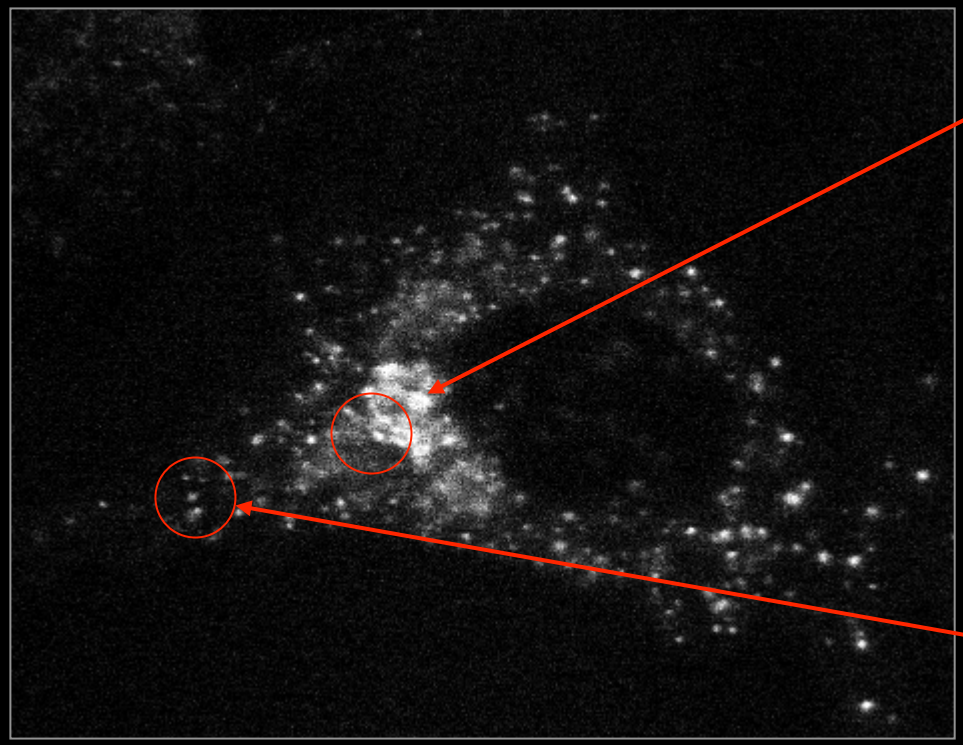
Image sequences

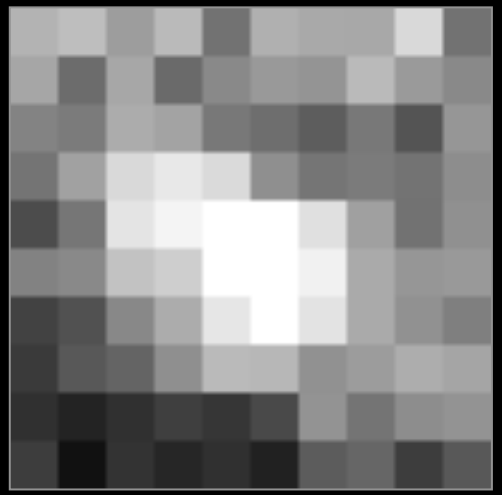
- Intensity dynamics
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation

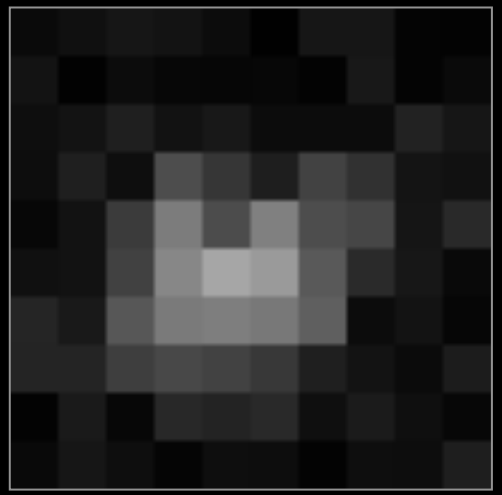
Static Images: Intensity Measurement

-Intensity Difference

-Intensity Profile







Fatima Verissimo

Static Images: Intensity Measurement

dot1.txt

File Edit Font Macros

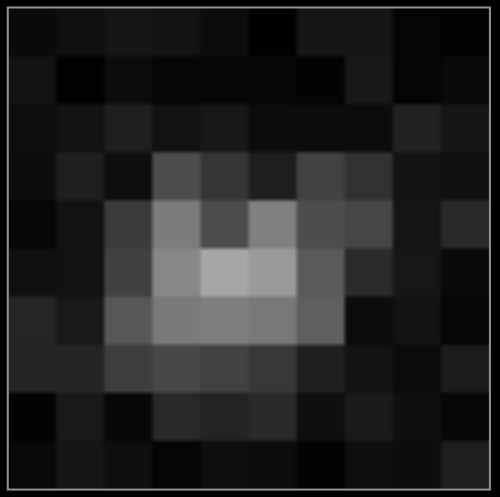
179	190	157	186	114	176	169	168	217	114
166	108	167	106	137	153	148	186	154	137
131	123	172	163	120	110	93	120	84	150
116	161	217	232	218	143	117	123	115	141
76	118	228	244	255	255	224	160	114	144
130	137	194	206	255	255	241	170	150	153
66	81	136	172	230	255	227	170	145	127
58	88	100	143	186	183	145	156	173	165
48	35	48	63	54	73	147	116	141	147
61	17	51	38	48	33	92	102	61	88

Mean Pixel Intensity: 141.6 ± 58.0 (saturated)

dot2.txt

File Edit Font Macros

10	16	22	19	12	1	22	22	4	3
19	2	12	7	6	7	3	24	4	10
14	19	31	18	24	12	12	12	34	22
13	31	14	77	54	30	66	49	20	17
7	18	59	124	76	128	77	70	21	41
16	18	65	135	166	154	89	42	23	9
37	25	87	122	126	120	95	12	19	6
36	36	62	72	66	56	31	19	11	28
3	26	7	40	35	41	15	27	15	7
9	22	14	5	14	13	3	14	13	30

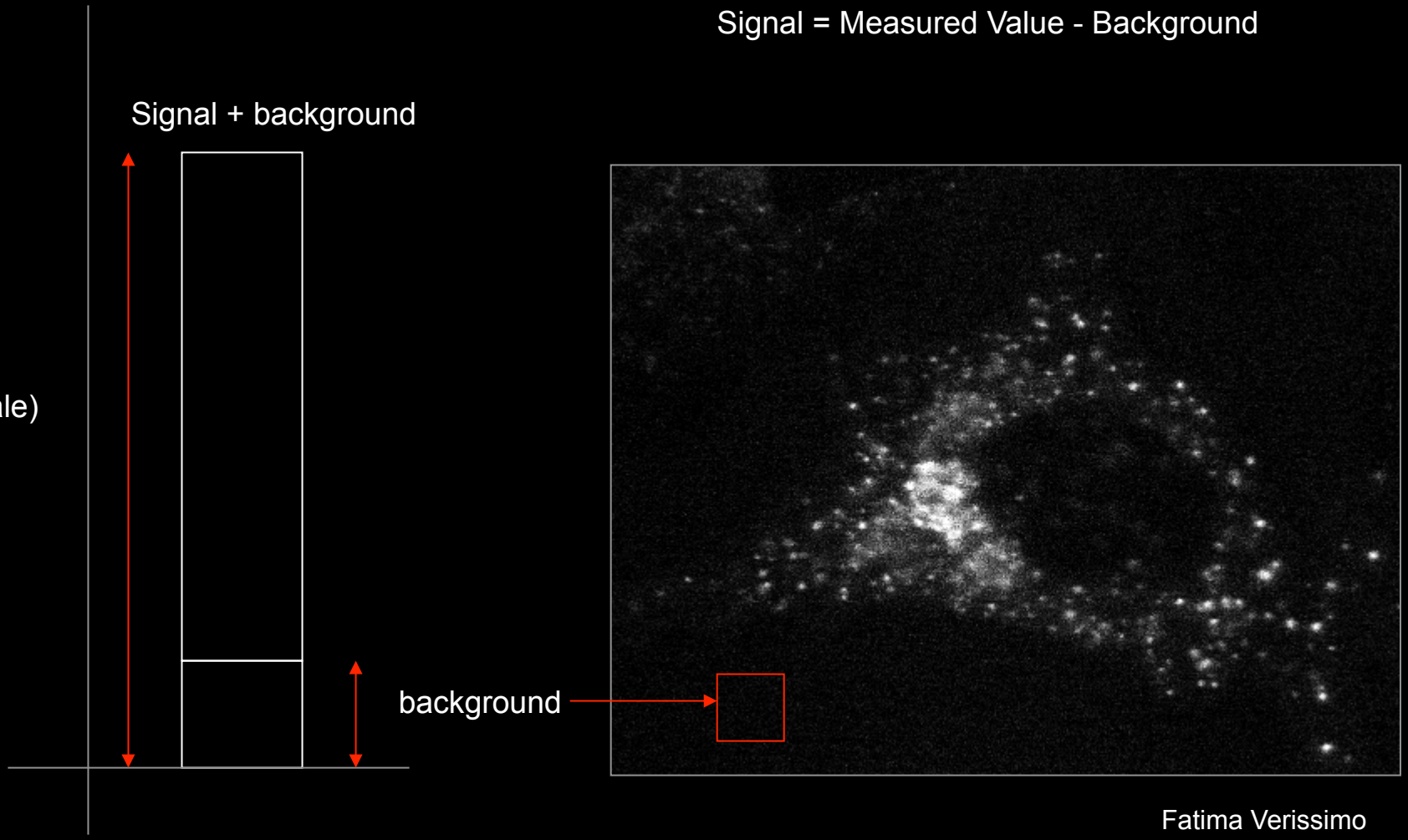


Mean Pixel Intensity: 35.5 ± 36.8

Static Images: Intensity Measurement

Background Subtraction

Signal = Measured Value - Background



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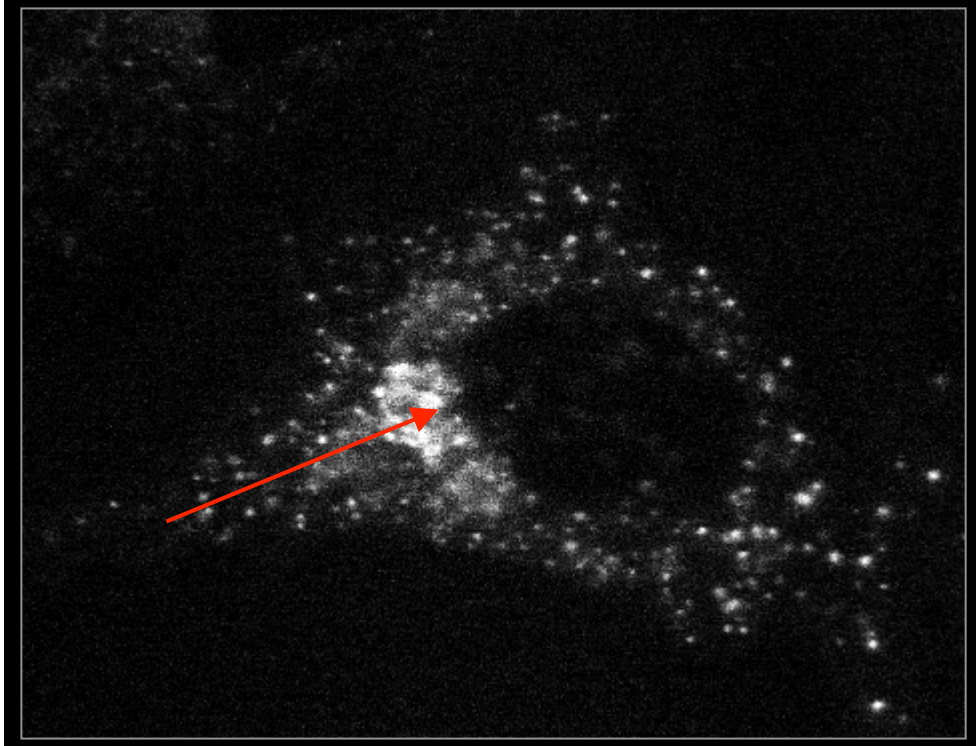
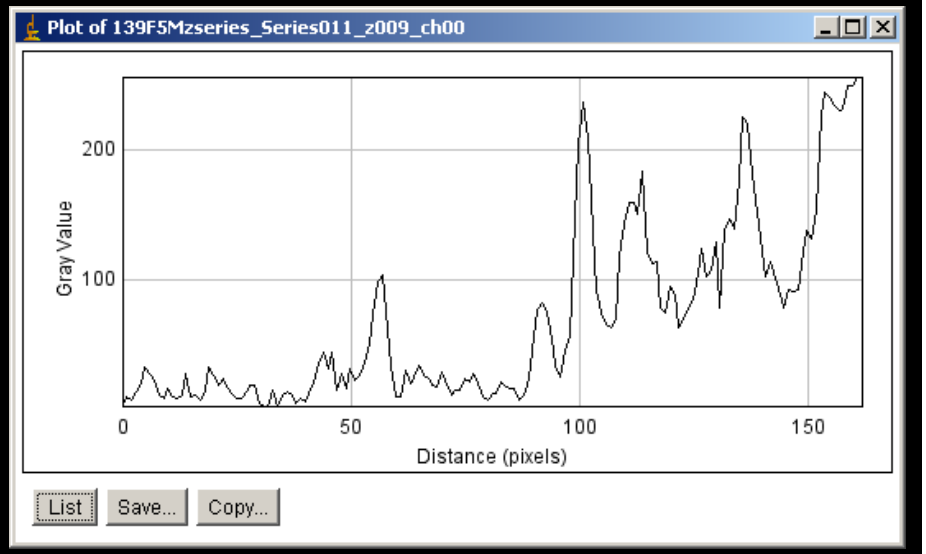
Static Images: Intensity Measurement

-Intensity Difference

-Intensity Profile





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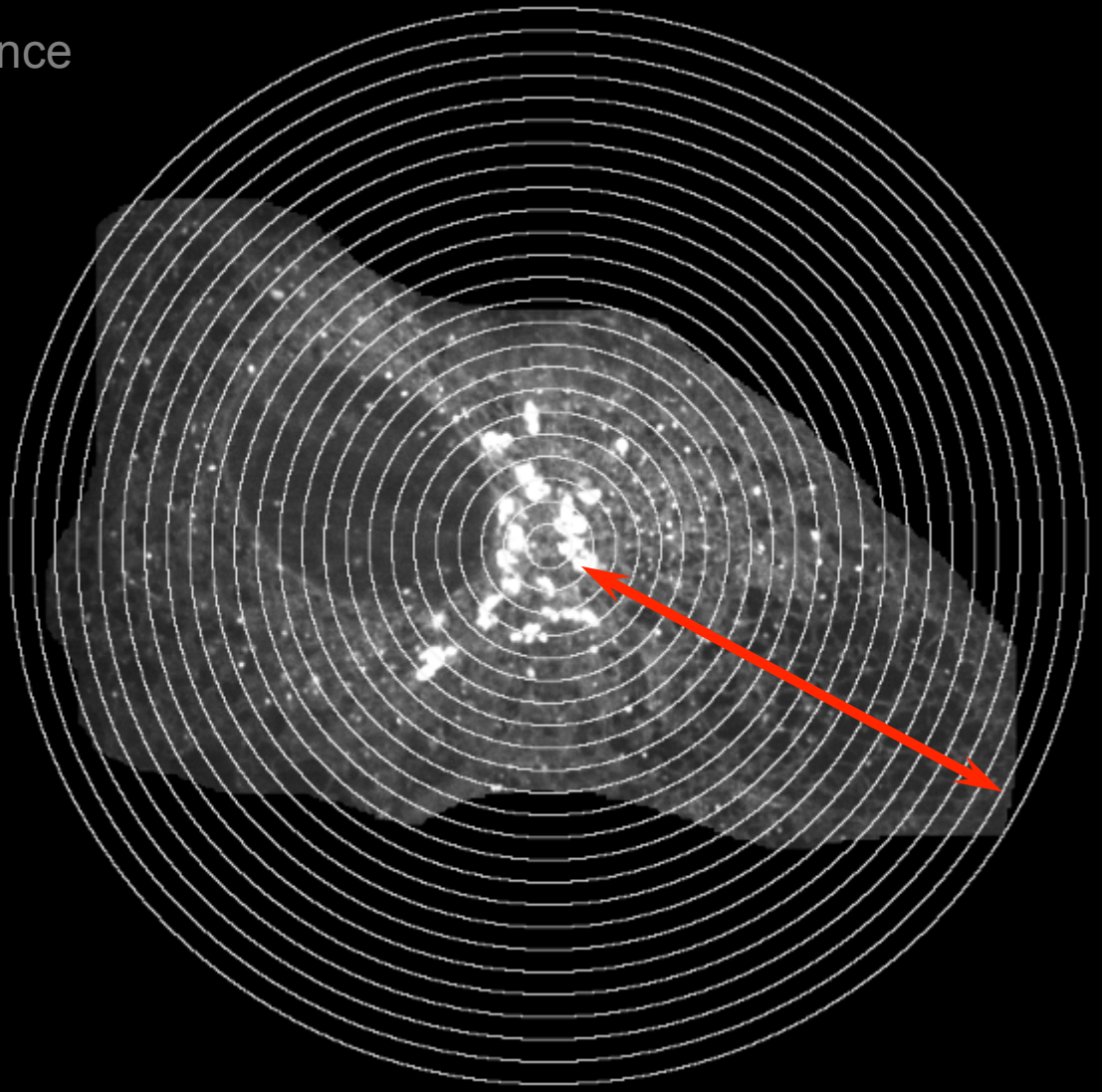
Static Images: Intensity Measurement An Example

-Intensity Difference

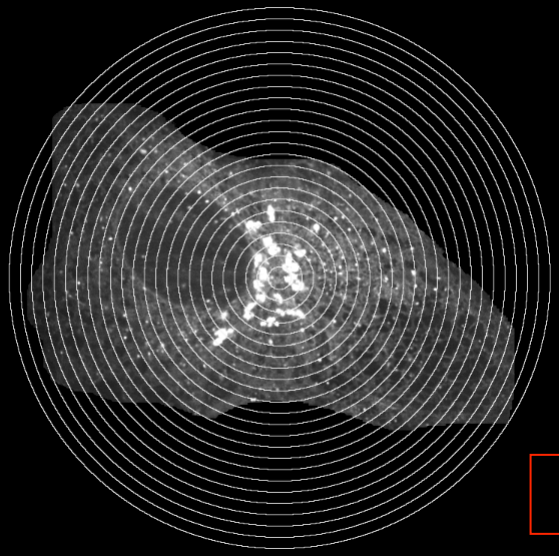
-Intensity Profile



Static Images: Intensity Measurement An Example

-Intensity Difference

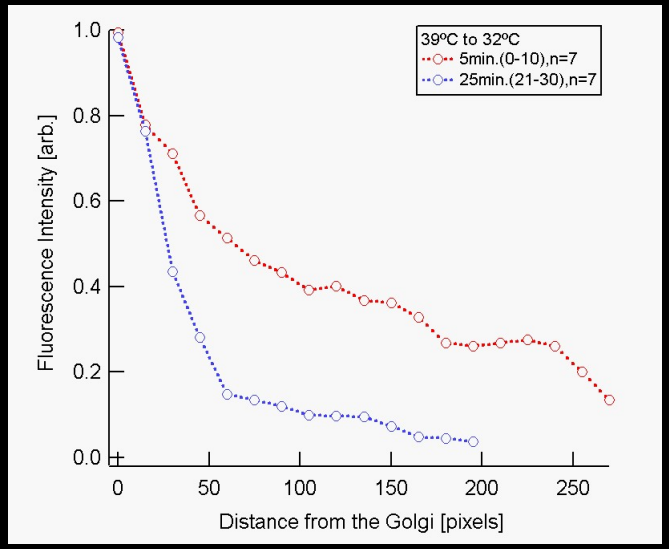
-Intensity Profile

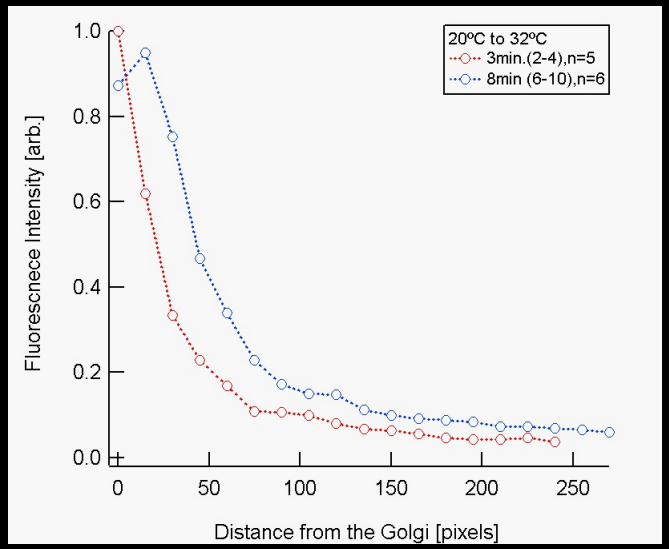


ER → Golgi

Golgi → PM

Radial Intensity Distribution





Static Images: Morphometry

Static images

- Intensity Measurement
- Morphometry → Length, Area, Perimeter, Circularity
- Distribution
- Colocalization

Image sequences

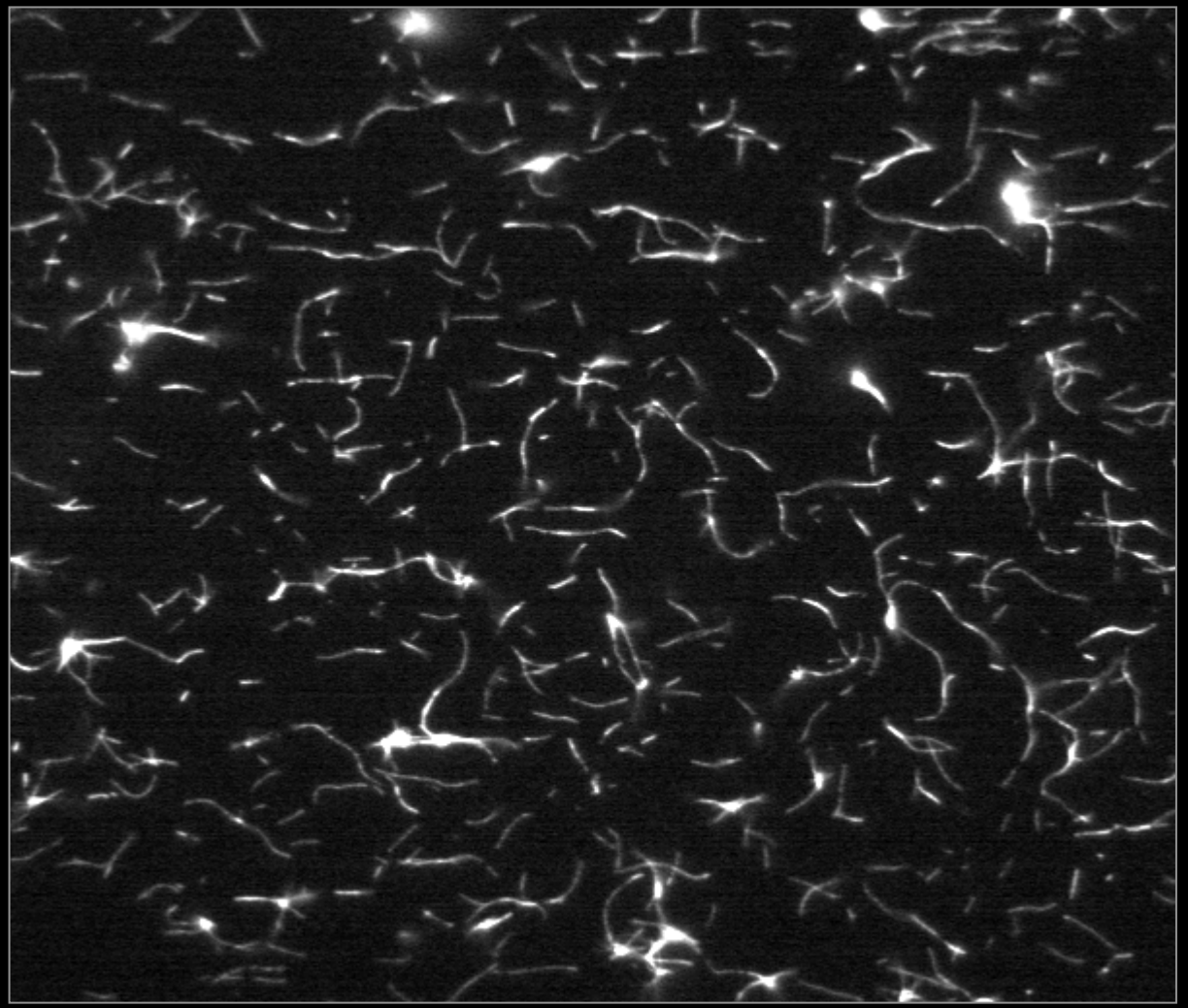
- Intensity dynamics
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation

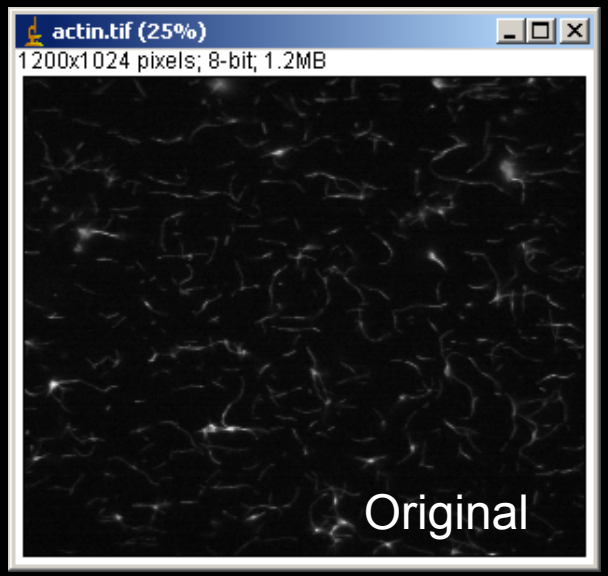
Static Images: Morphometry

Length

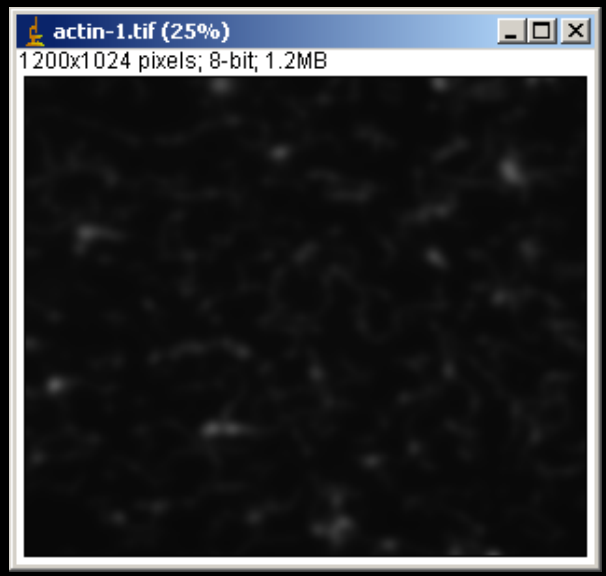
straight: (x1, y1) to (x2, x2)
curves

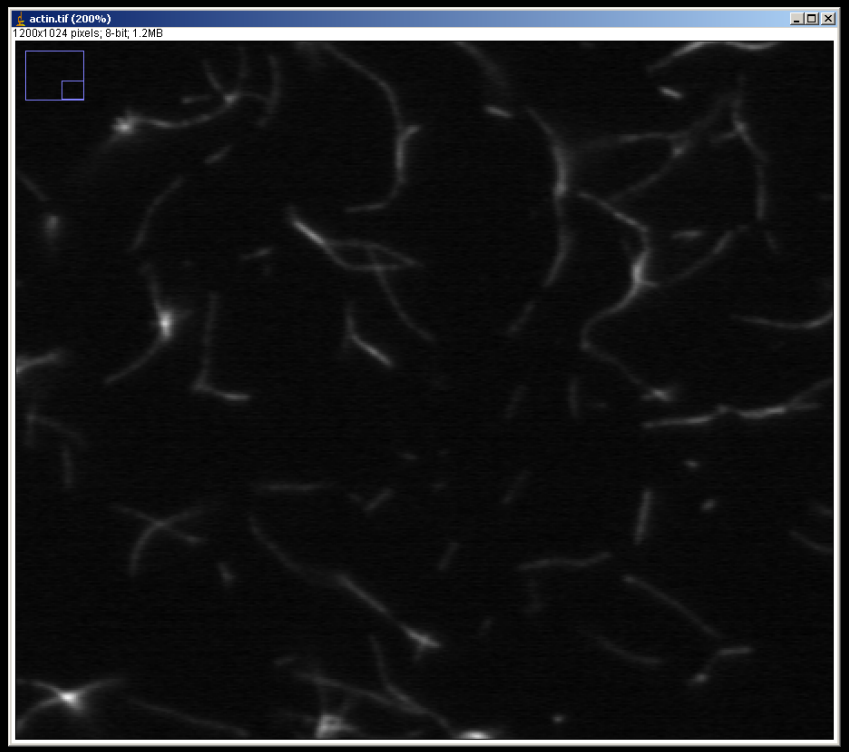
Area
Perimeter
Circularity

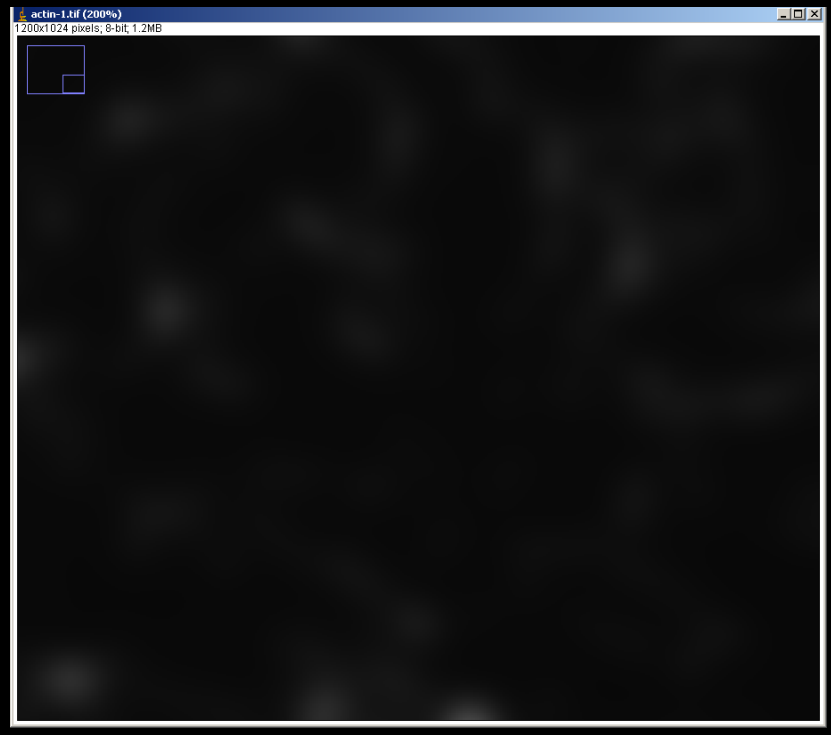




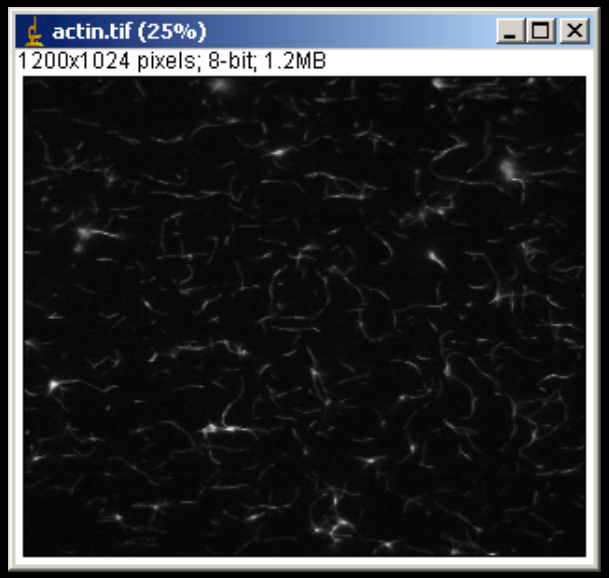
Gaussian Blur (10)







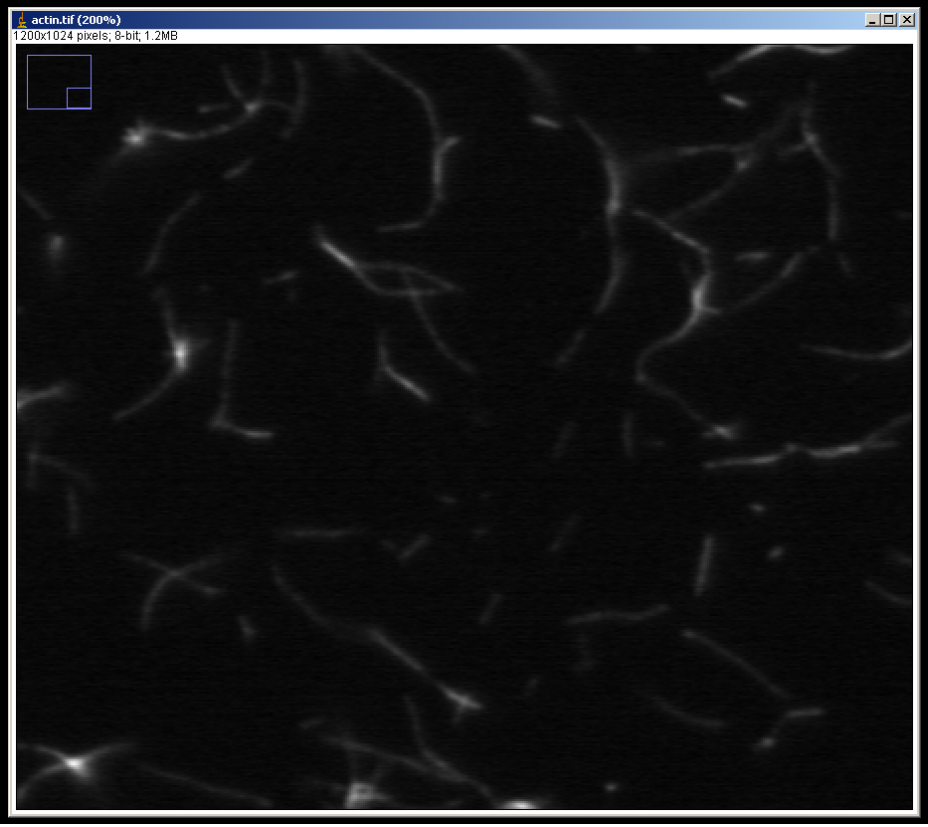
Remove local difference

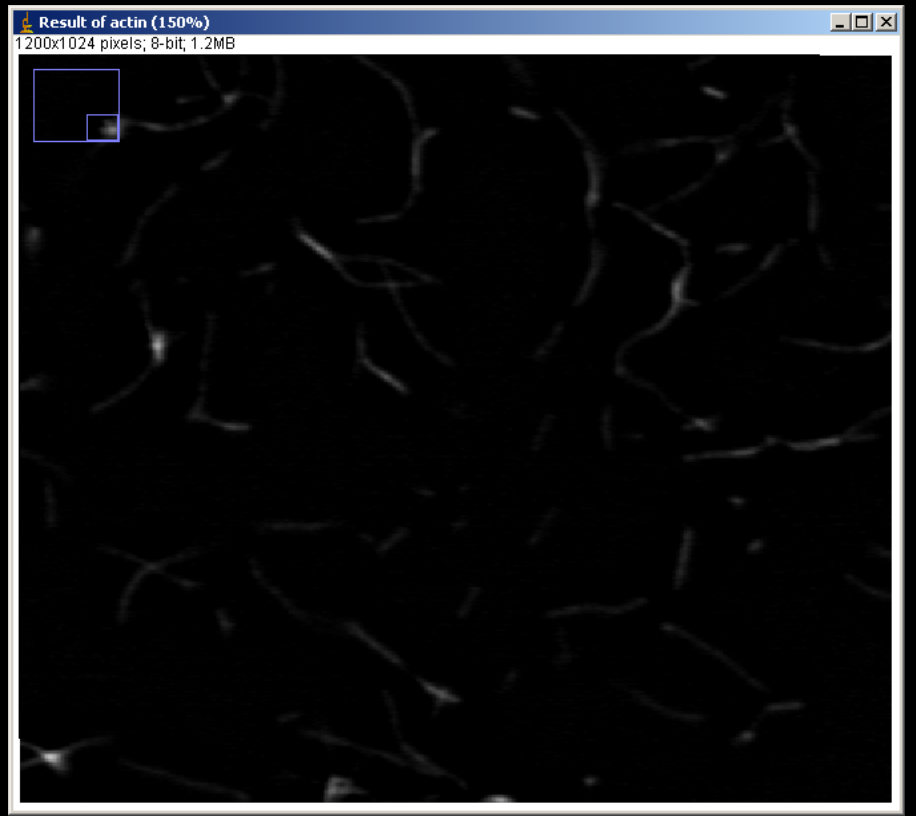


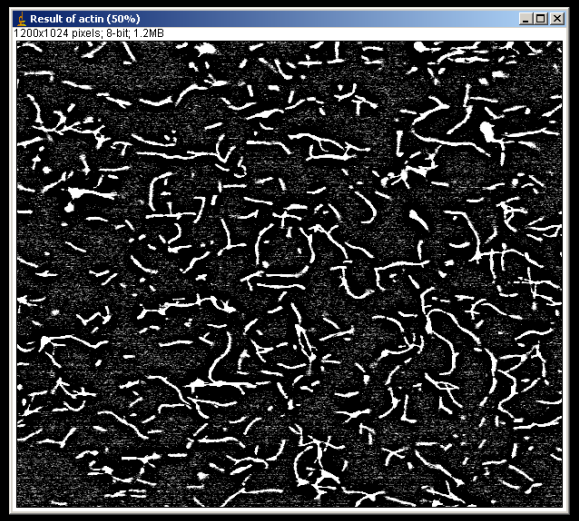
Original

Subtract Blurred

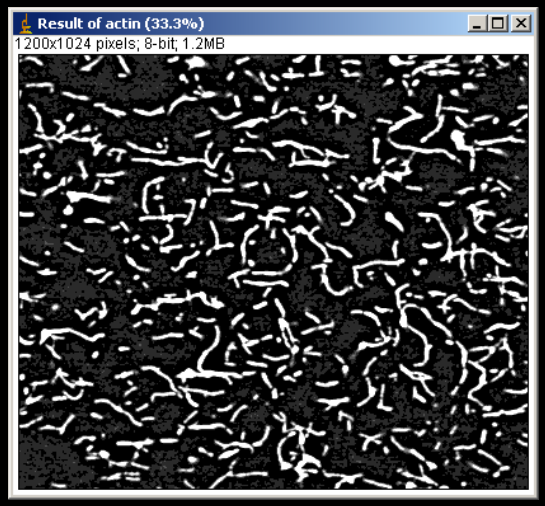
Local Difference Suppressed

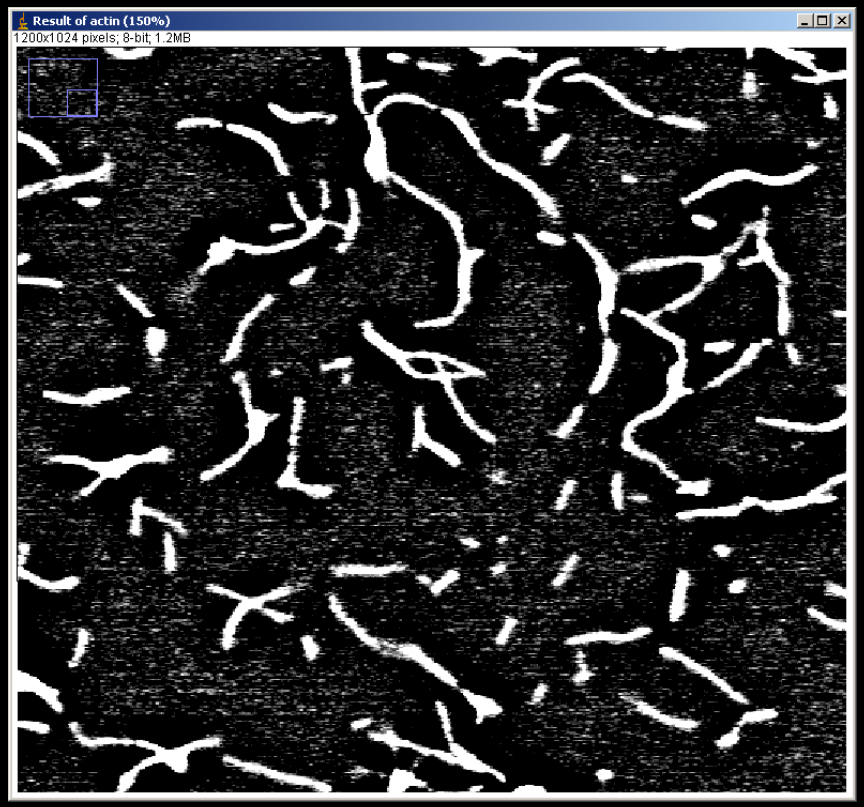


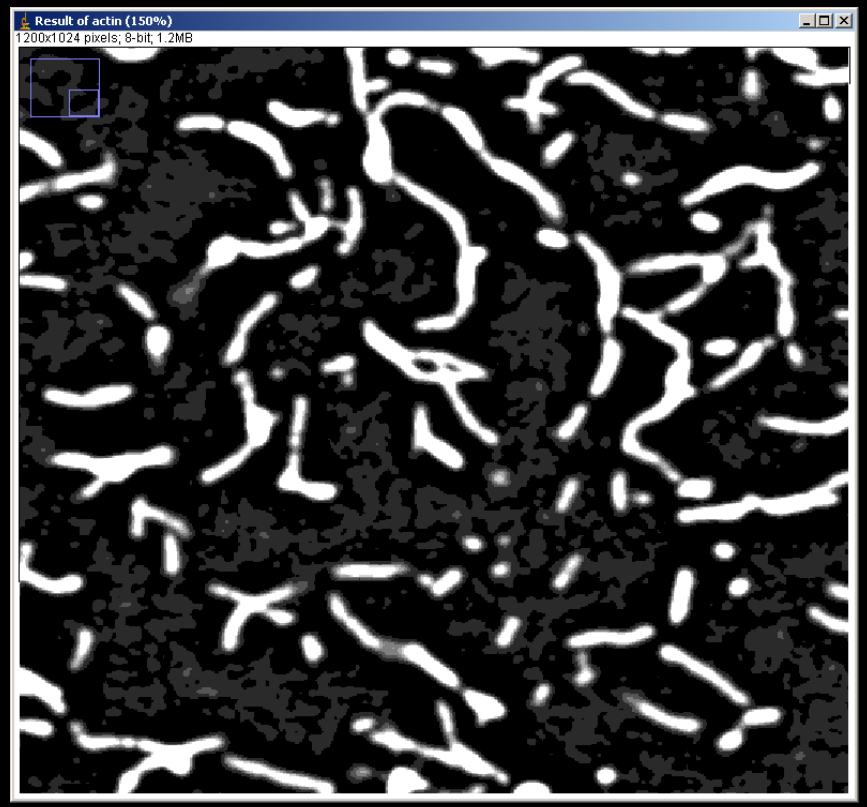


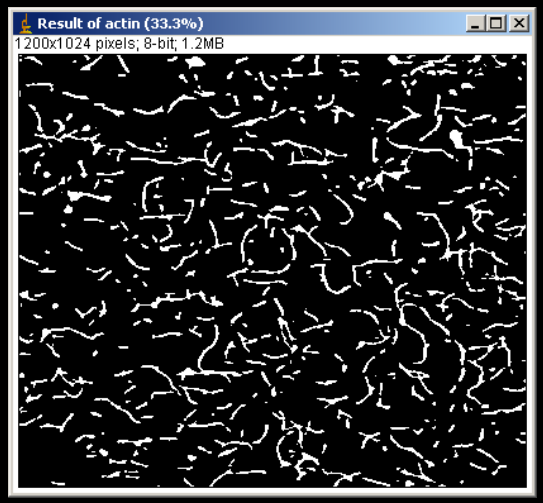


Gaussian Blurr (2)
Remove noise



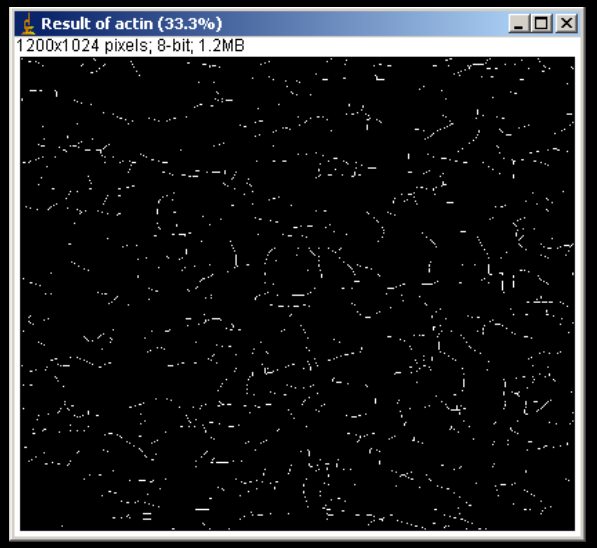


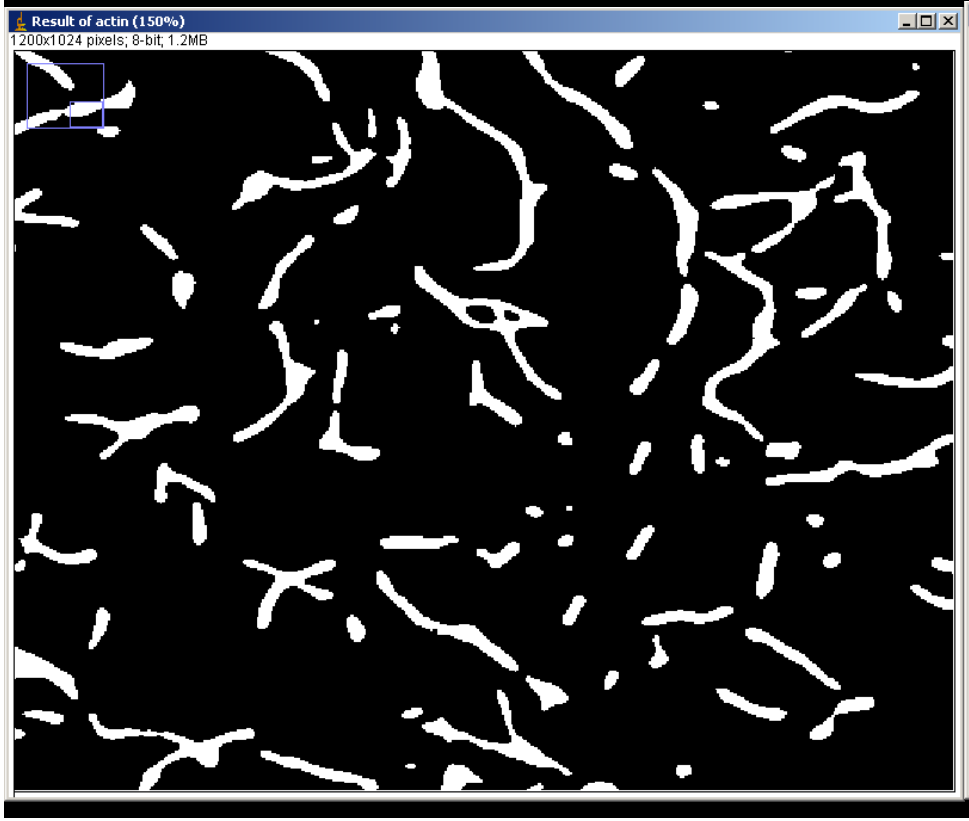


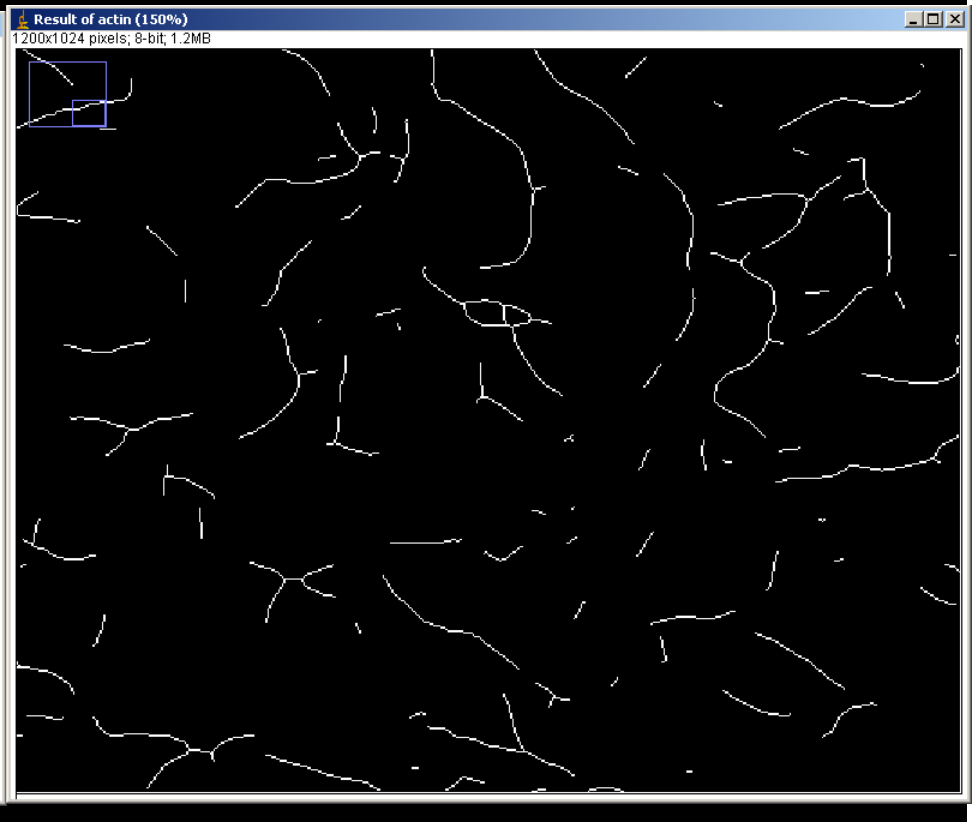


Binarized
(thresholding)

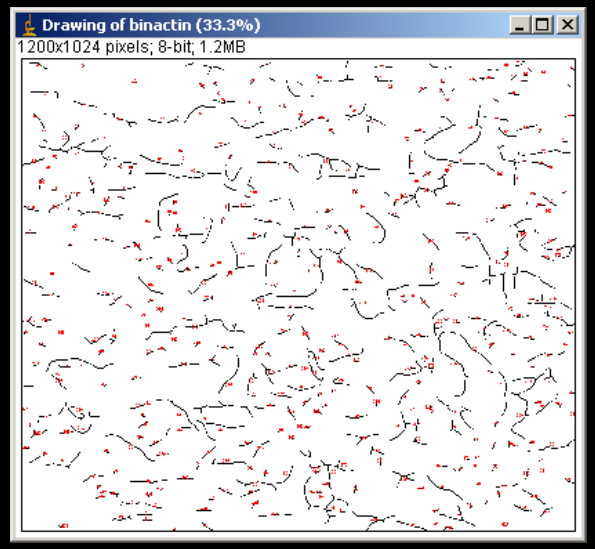
Skeltonize
(erosion)

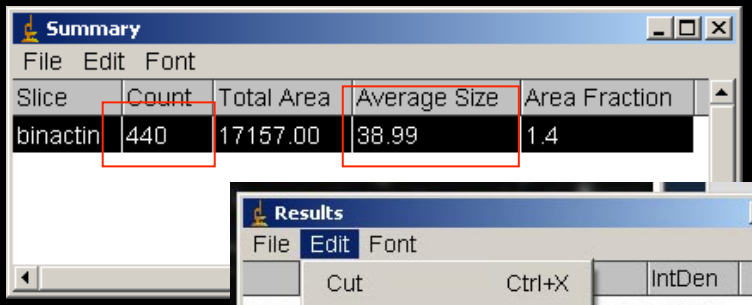


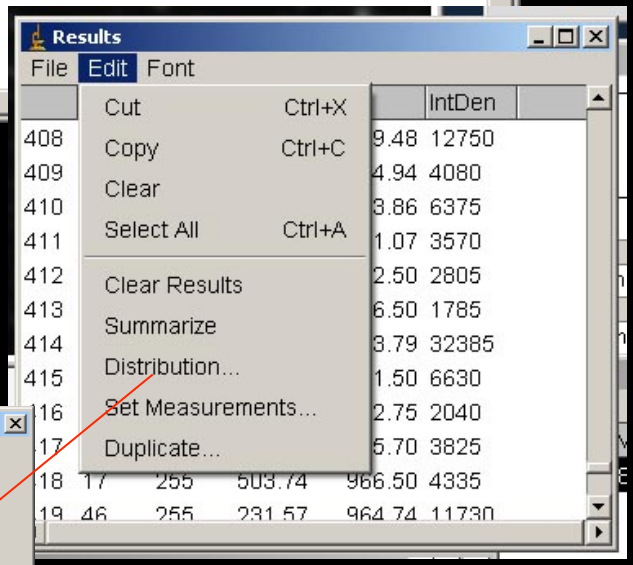


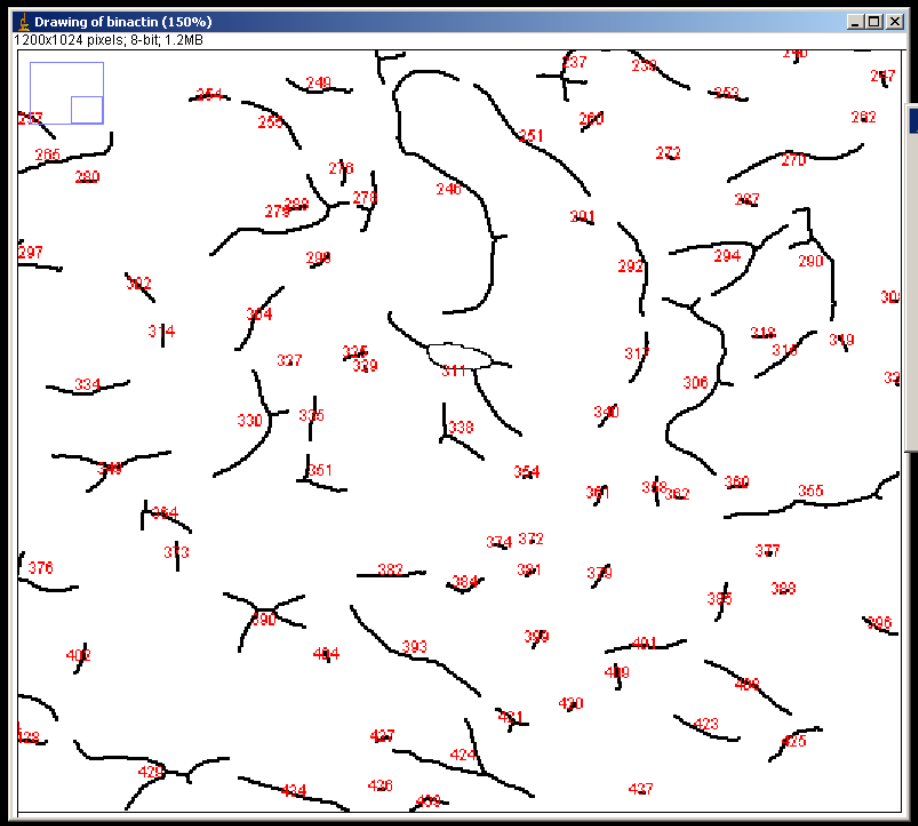


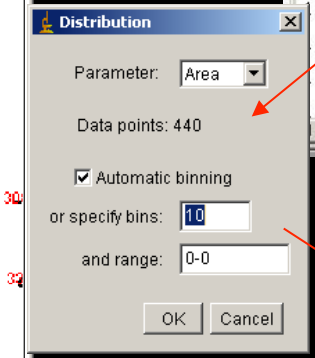
Threshold and Particle Count…

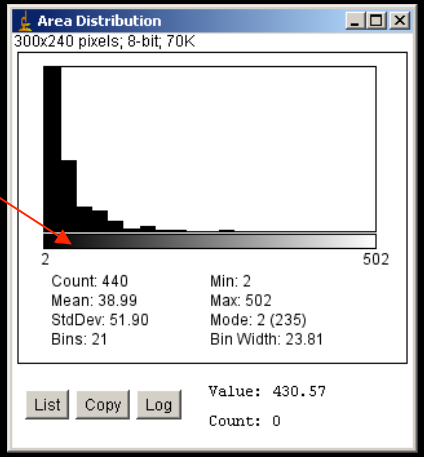












Static Images: Morphometry

Length

straight: (x1, y1) to (x2, x2)

curves

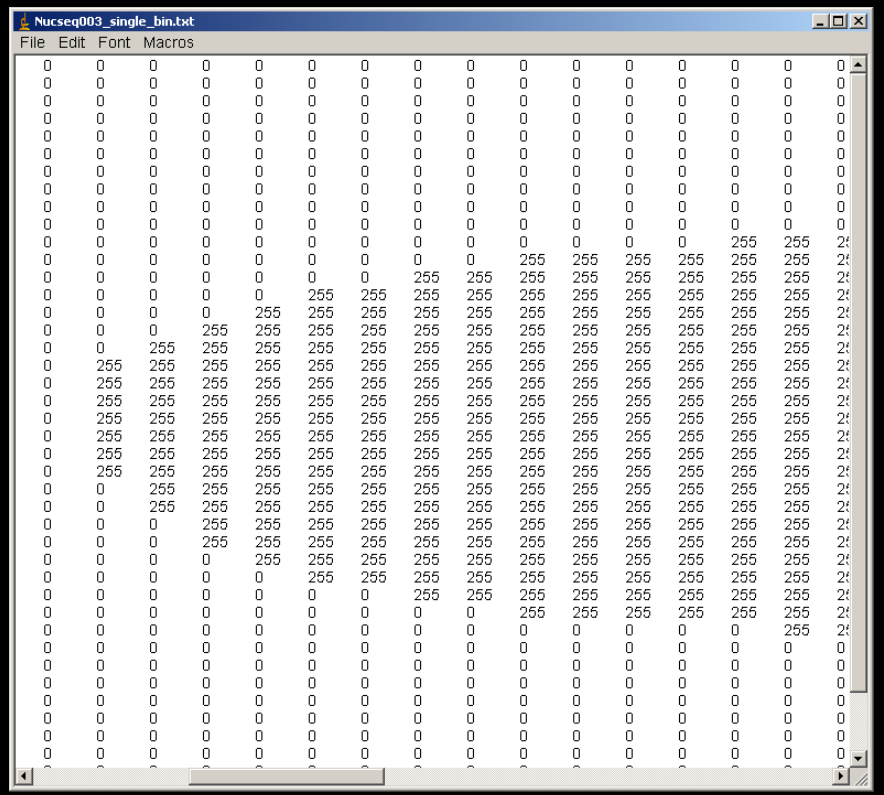
Area

Perimeter

Circularity

number of pixels with value 255 = Area

“segmentation”



Static Images: Morphometry

Length

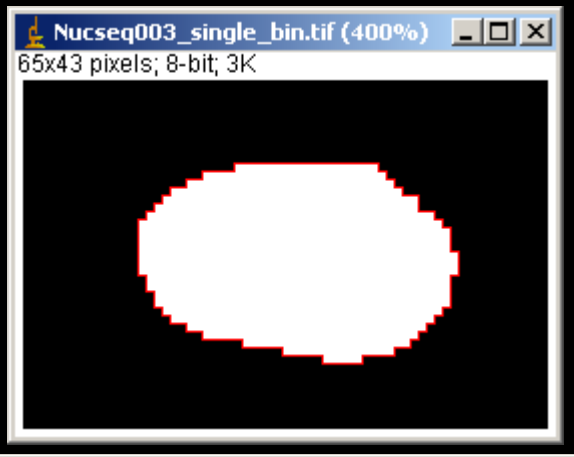
straight: (x1, y1) to (x2, x2)

curves

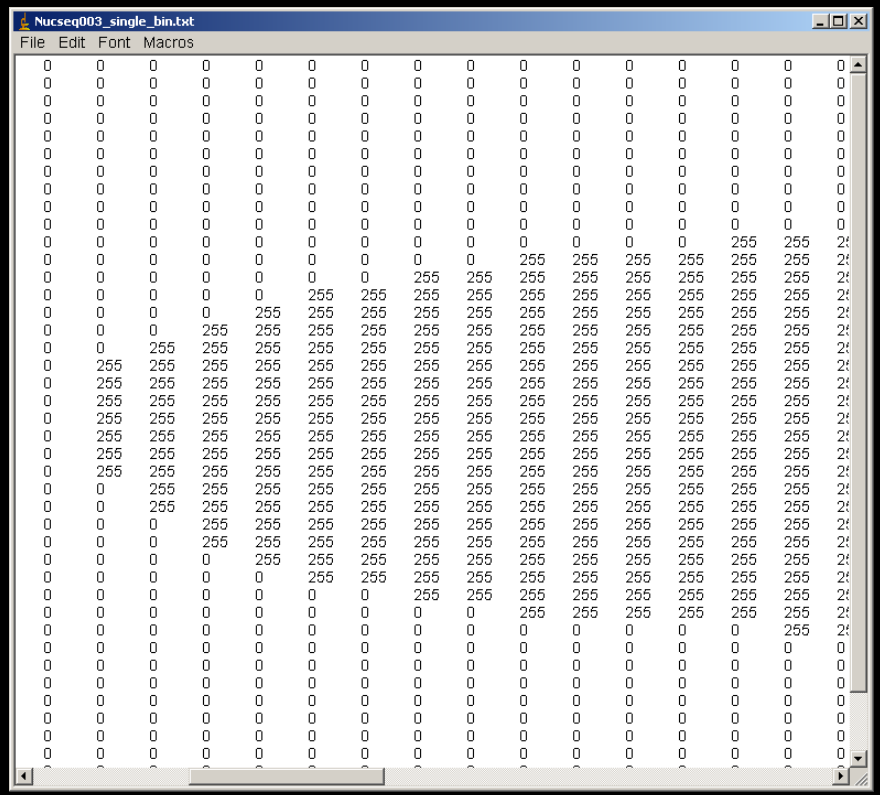
Area

Perimeter

Circularity



"segmentation"



Static Images: Morphometry

Length

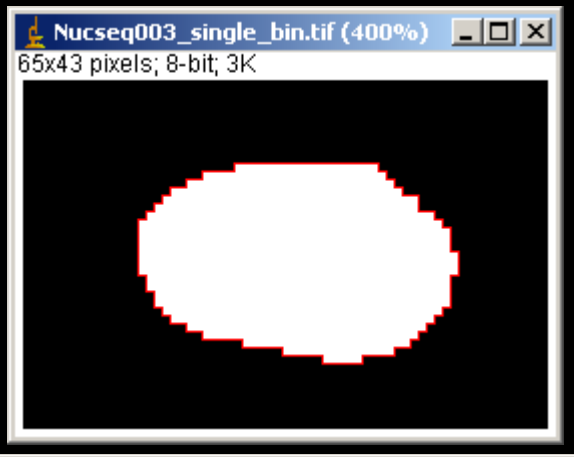
straight: (x1, y1) to (x2, x2)

curves

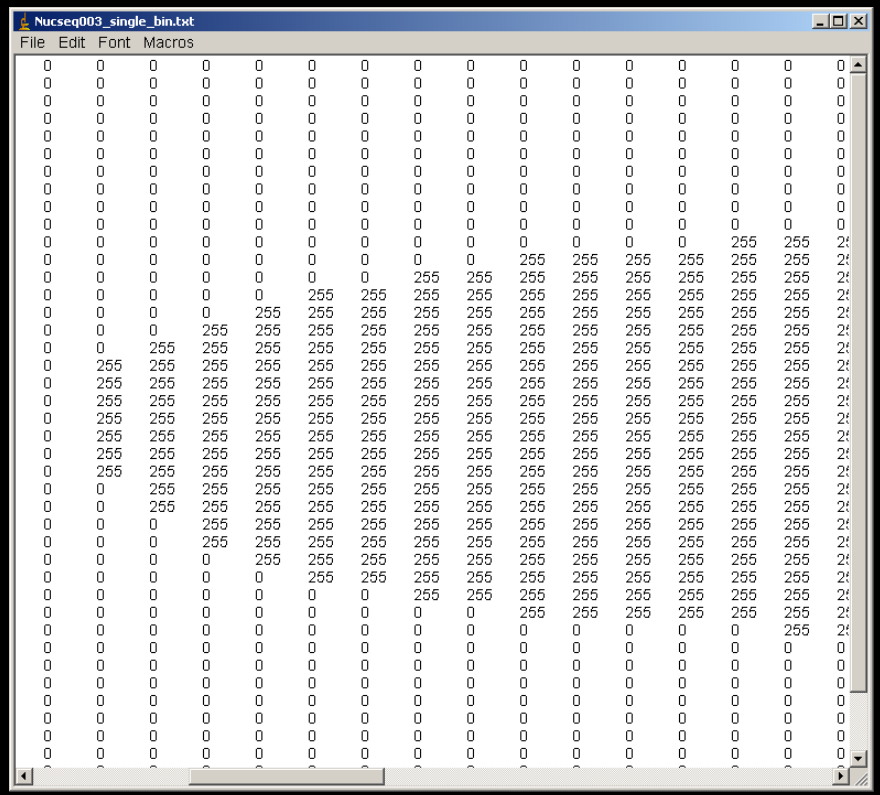
Area

Perimeter

Circularity



"segmentation"



Static Images: Morphometry

Length

straight: (x1, y1) to (x2, x2)

curves

Area

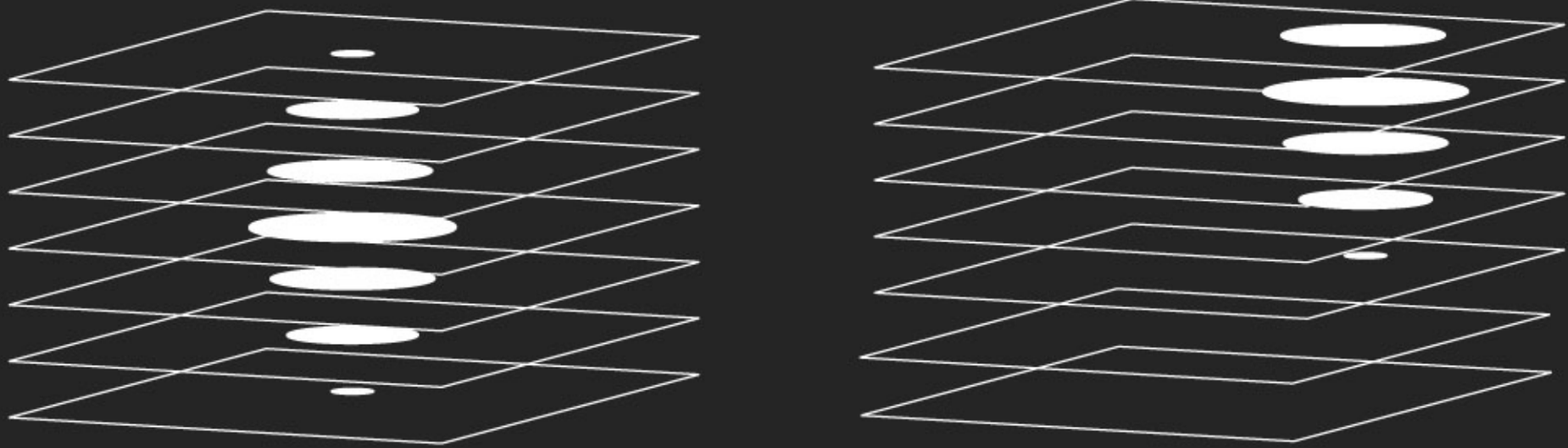
Perimeter

Circularity

$$\text{Circularity} = 4\pi(\text{area} / \text{perimeter}^2)$$

Circularity	0.906	0.843	0.348

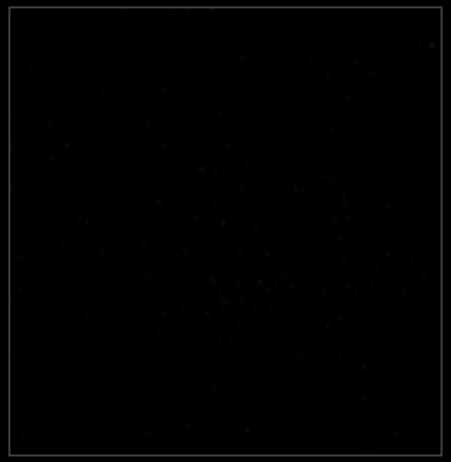
Static Images: Morphometry of three dimensional objects



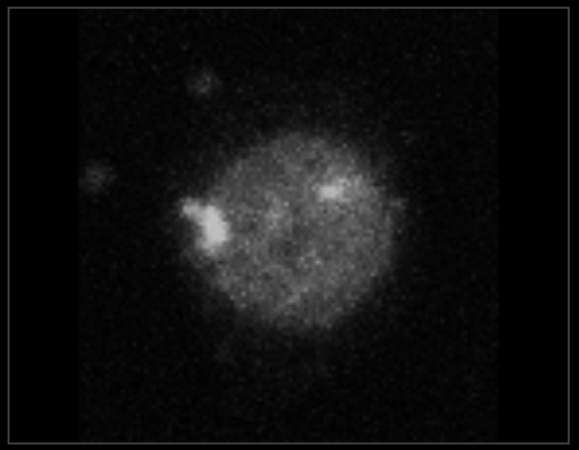
3D data sets (x-y-z): stack of optical sections

Static Images: Morphometry of three dimensional objects

XY stack



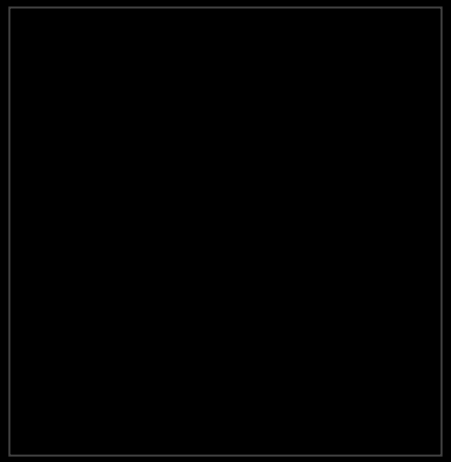
3D projection

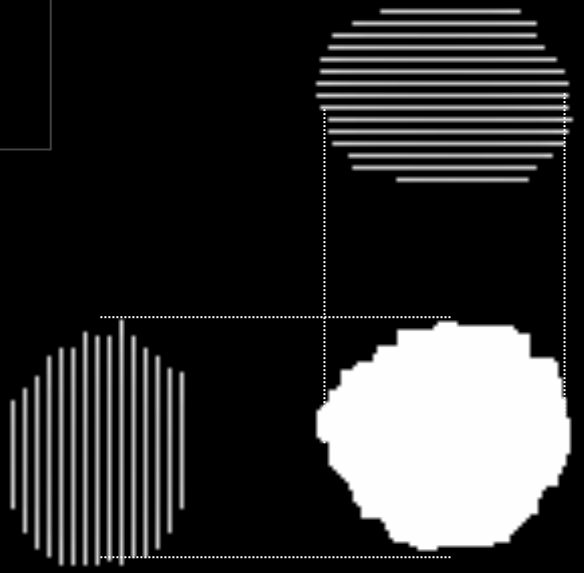


Volume =

Sigma(Area(slice) * thickness)

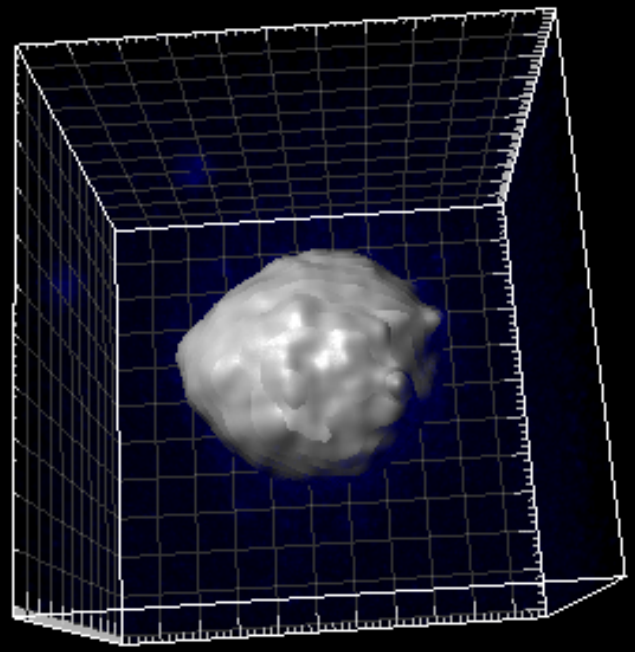
Segmented Image





Static Images: Morphometry of three dimensional objects

Software:
Imaris (Bitplane)



Visualization of
3D object

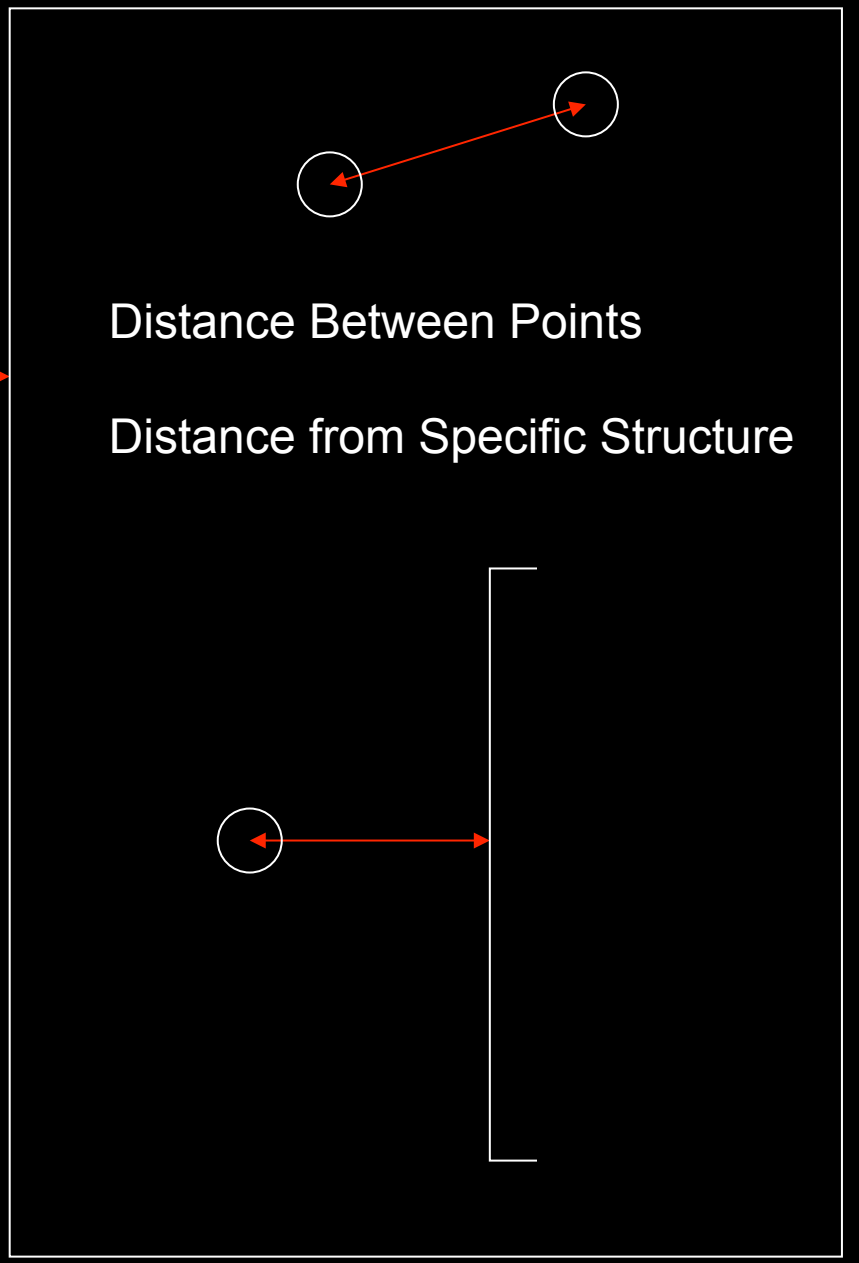
Static Images: Distribution

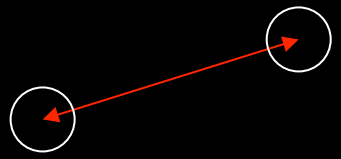
Static images

- Intensity Measurement
- Morphometry
- Distribution
- Colocalization

Image sequences

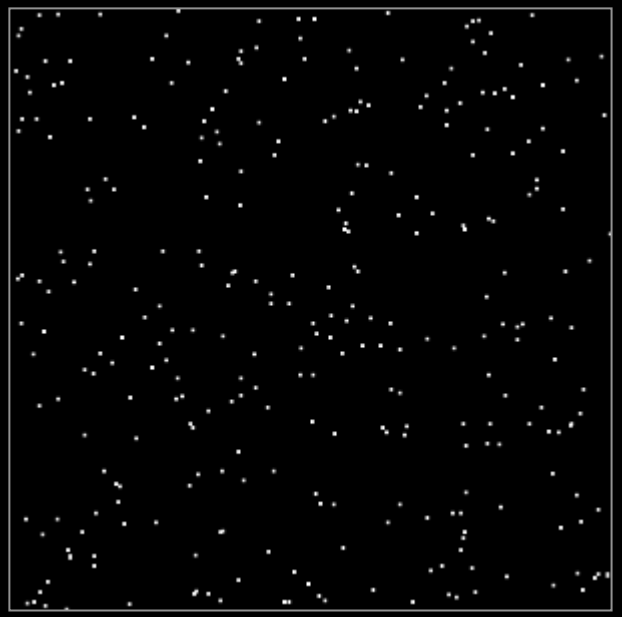
- Intensity dynamics
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation



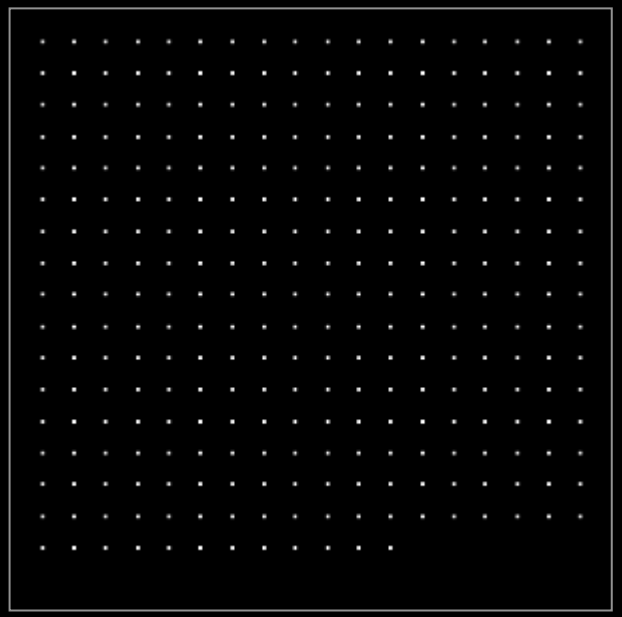


Distance Between Points

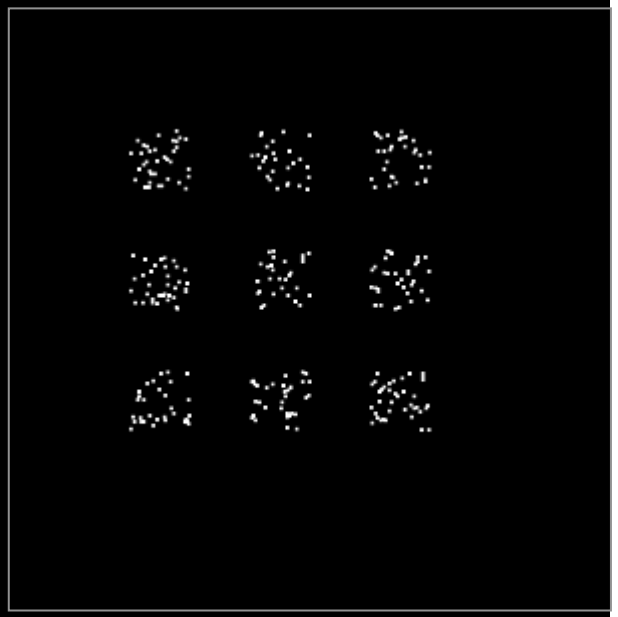
Static Images: Distance Between Points Quantification of Particle Distribution



random

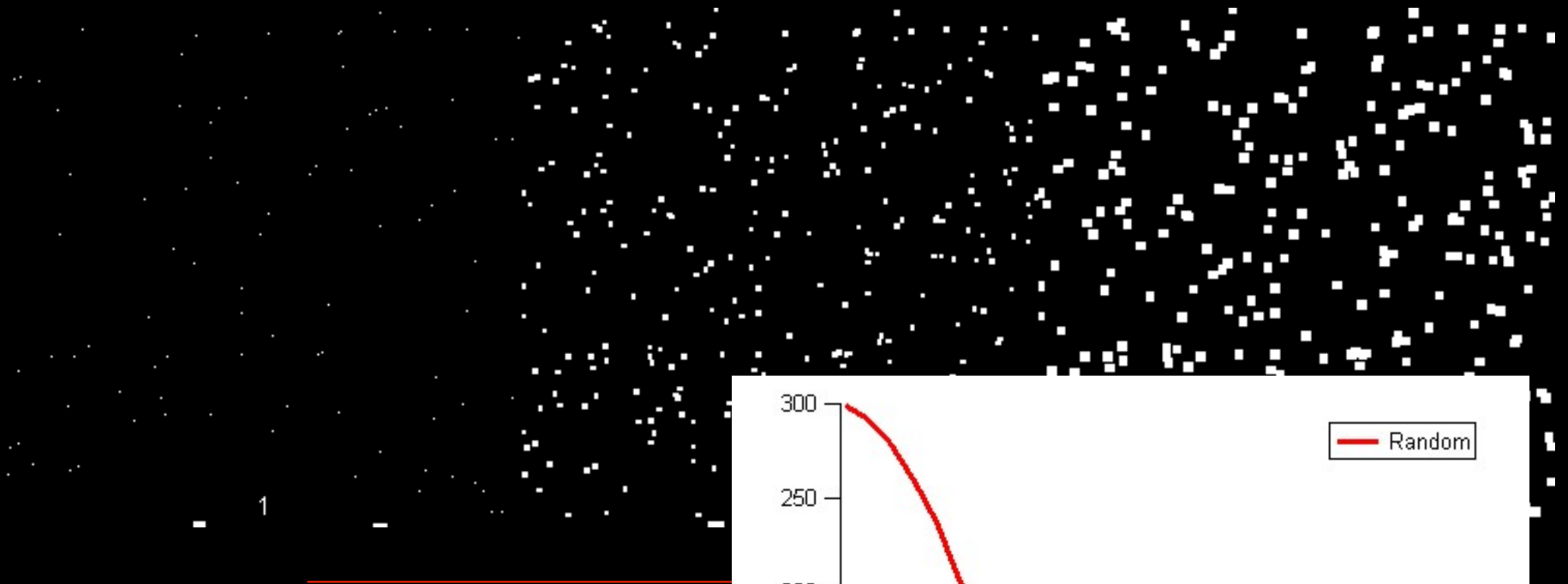


grid

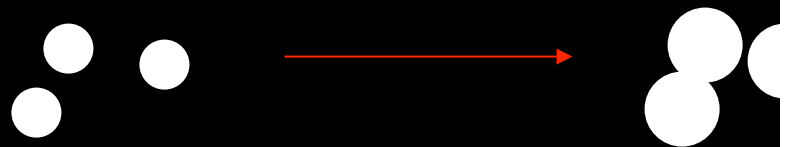


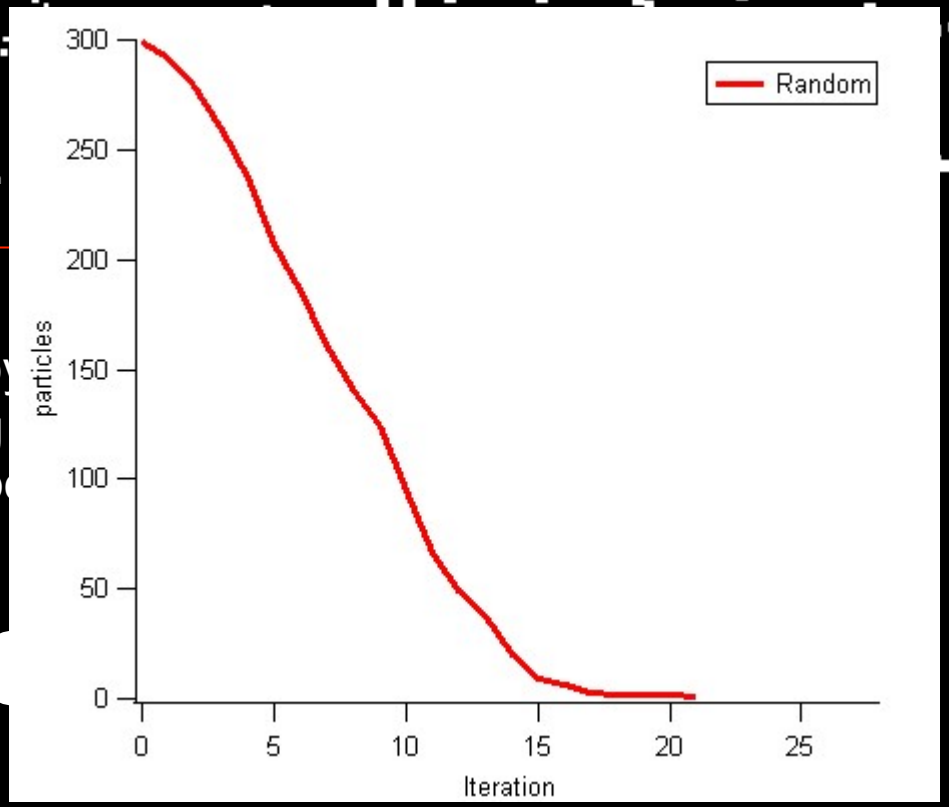
clusters

Static Images: Distance Between Points

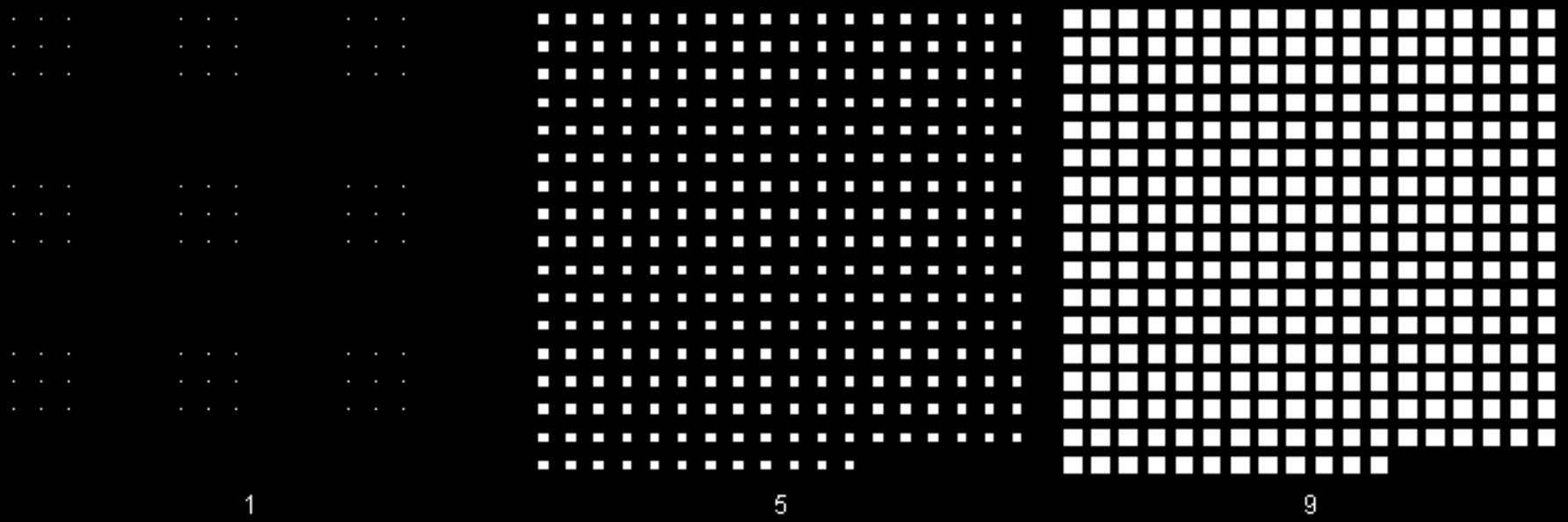


Dilating signals step-by
→ increasing
→ less numb





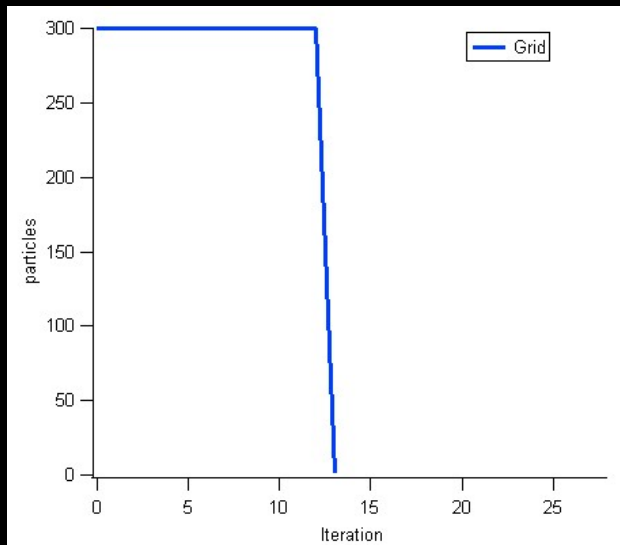
Static Images: Distance Between Points



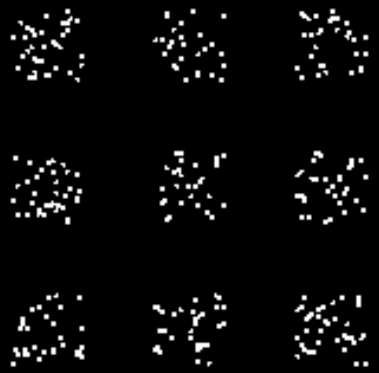
Dilating the signal

→ increasing connections between particle

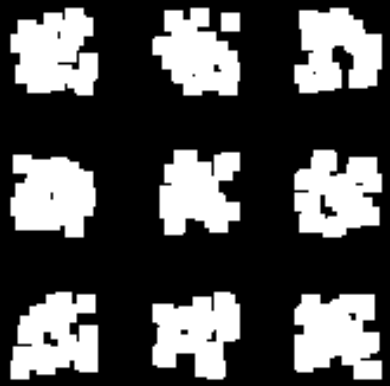
→ less number of “particles”



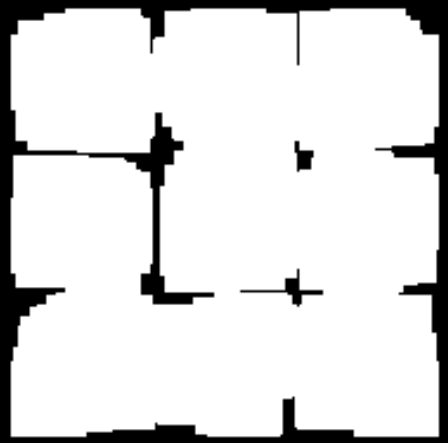
Static Images: Distance Between Points



Many



9 clusters

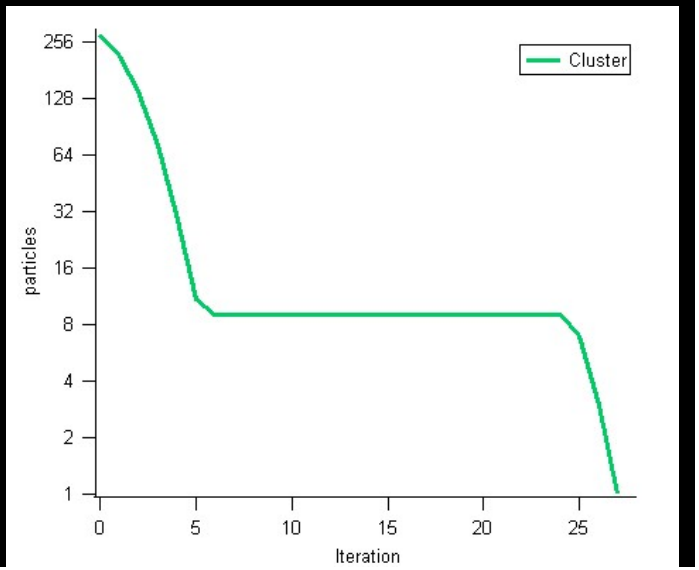


Single Cluster

Dilating the signal

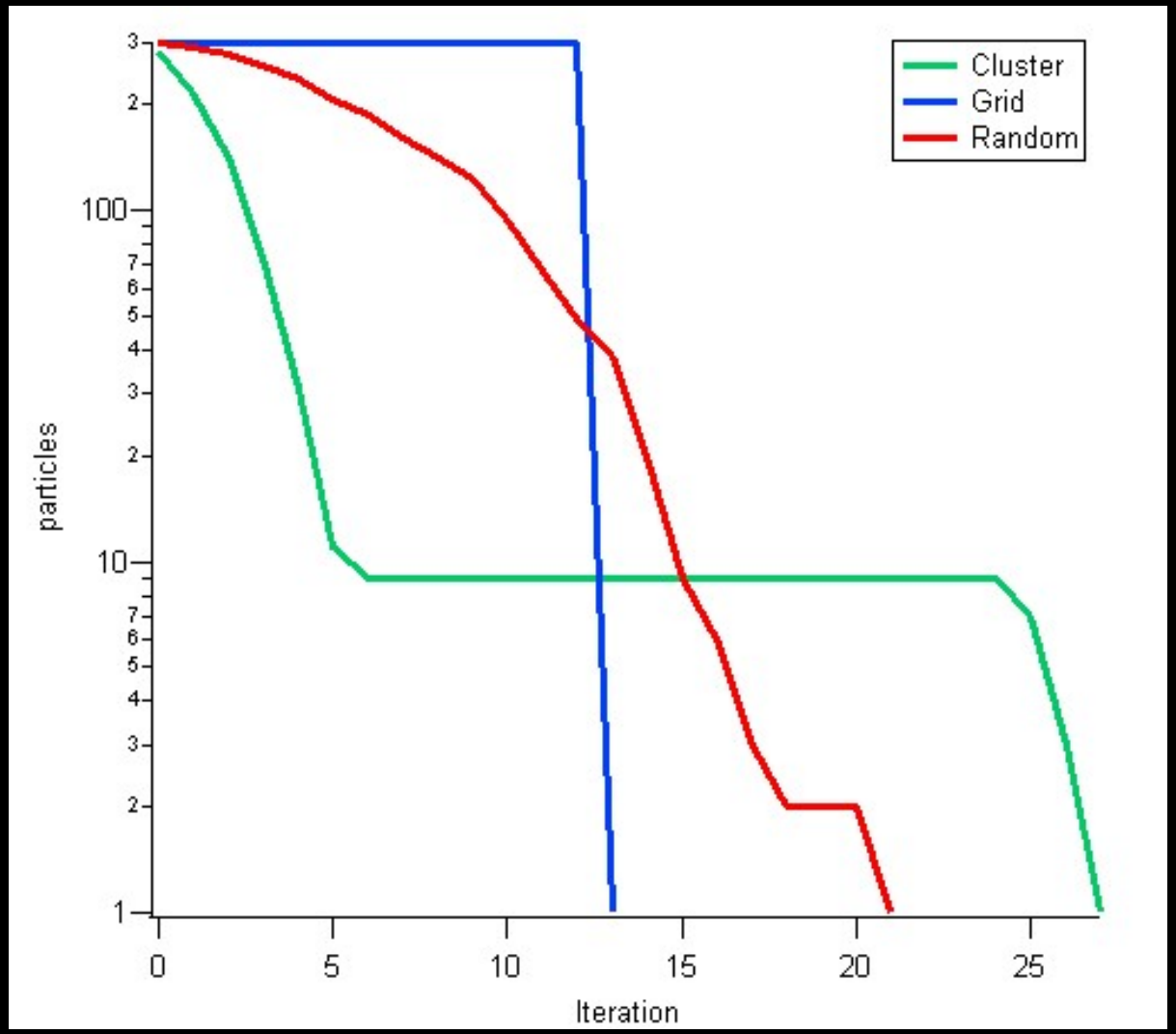
→ increasing connections between particle

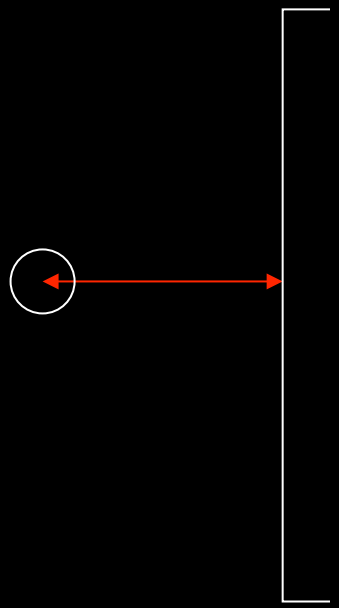
→ less number of “particles”



Static Images: Distance Between Points

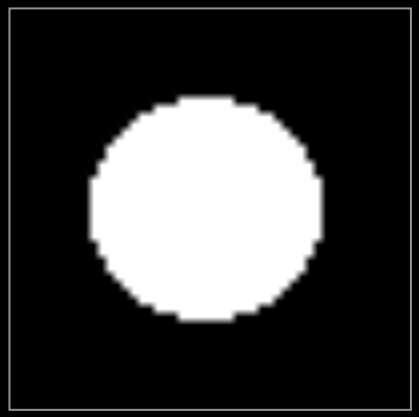
Indicator of particle distribution → Connected Region Statistics



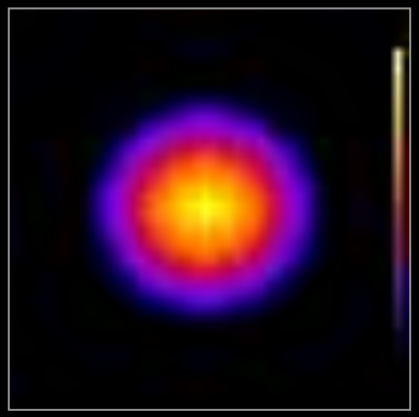


Distance from Specific Structure

2. Measurement of Distance



Distance Map (2D)



2. Measurement of Distance

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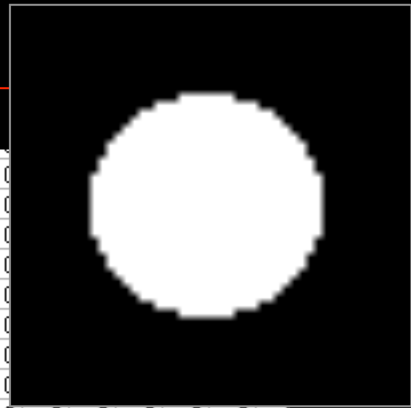
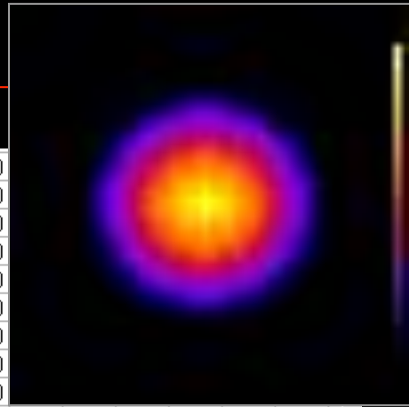


2. Measurement of Distance



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0	0	0	0	0	1	1	2	3	4	4	4	5	5	6	6	6	6	5	5	4	4	4	3	2	1	1	0	0	0	0	0
0	0	0	0	0	0	1	1	2	3	3	4	4	4	5	5	5	5	4	4	4	3	3	2	1	1	0	0	0	0	0	0
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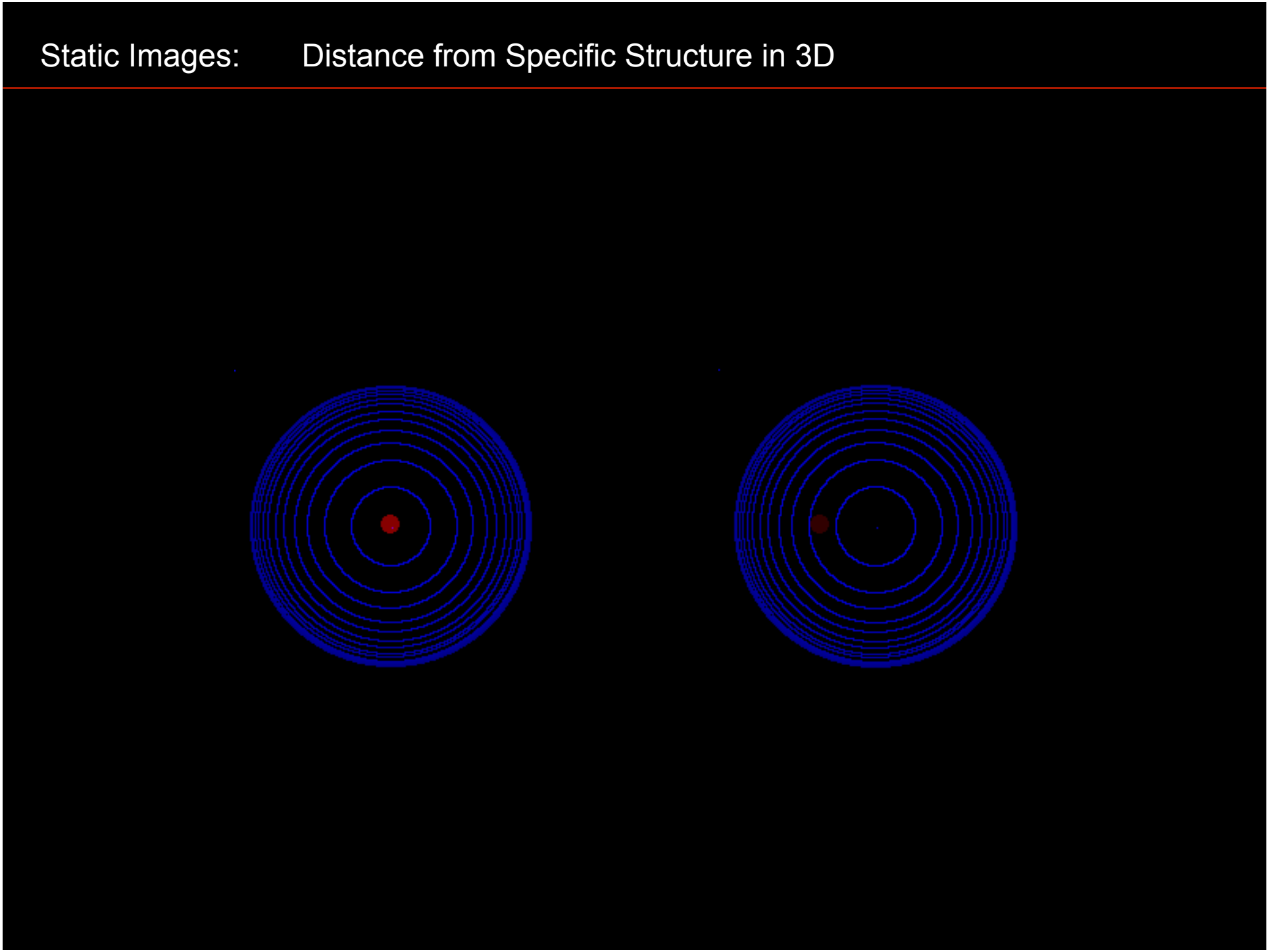
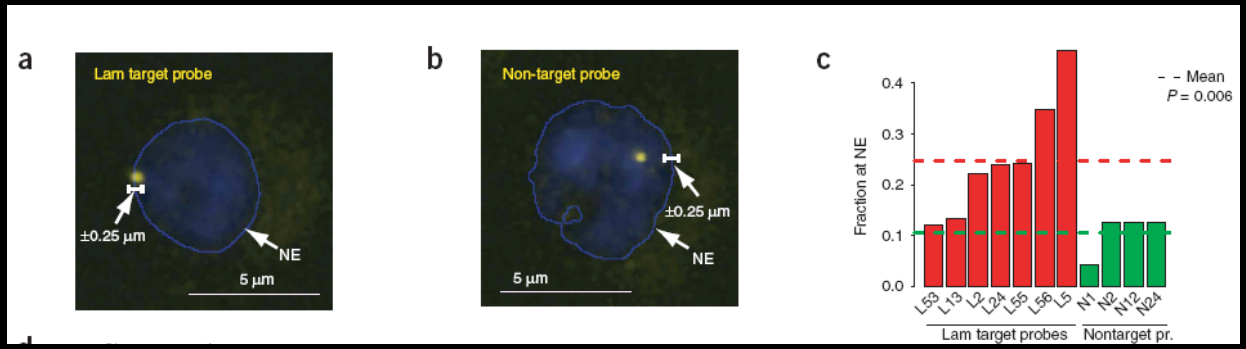
Static Images: Distance from Specific Structure in 3D

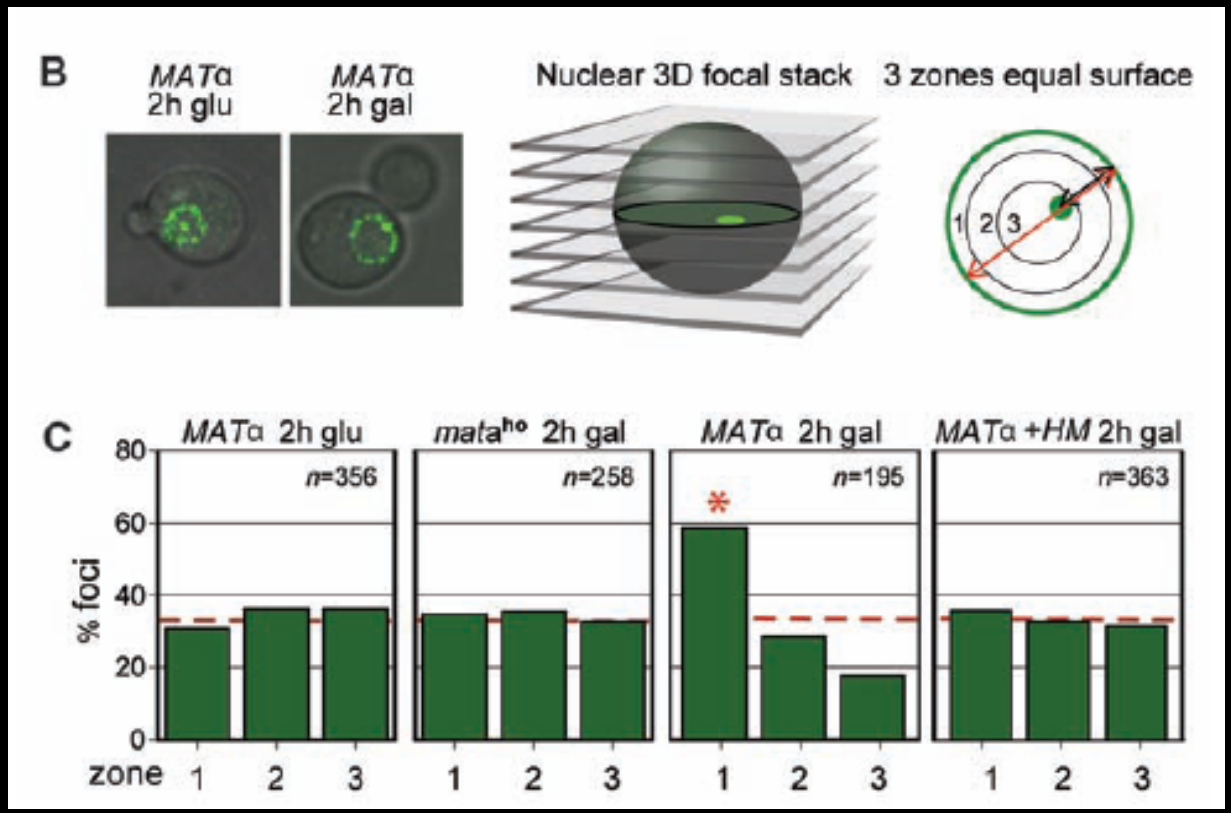


3D Localization of Chromosome signal: Conventional Method

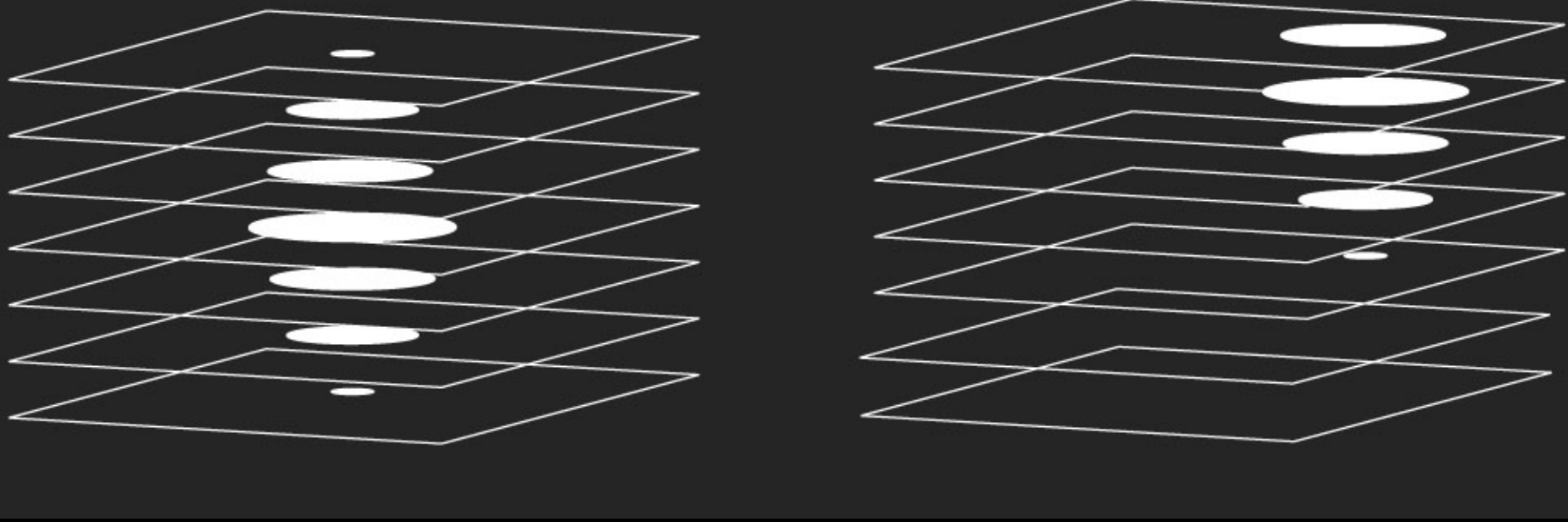
Pickersgill et al. (2006)
Nature Genetics 38 p1005



Nagai et al. (2008)
Science 332 p597



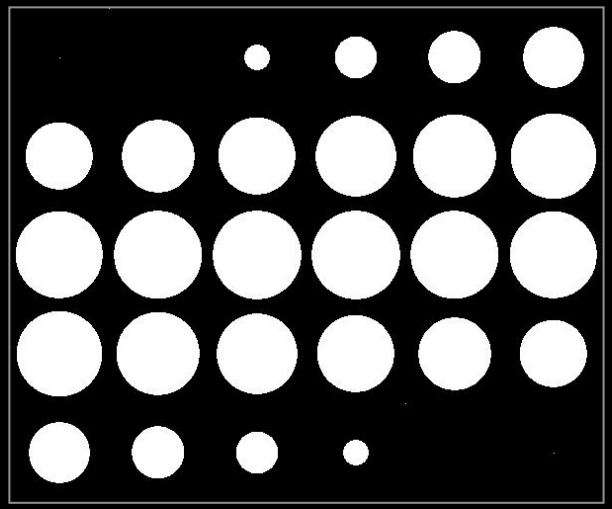
Static Images: Three dimensional data



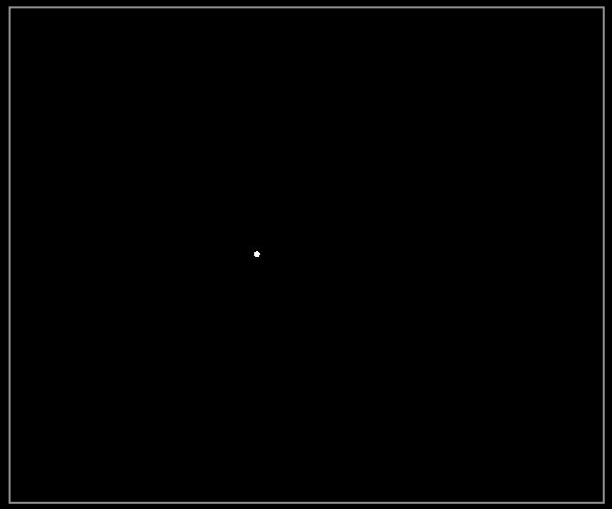
3D data sets (x-y-z)

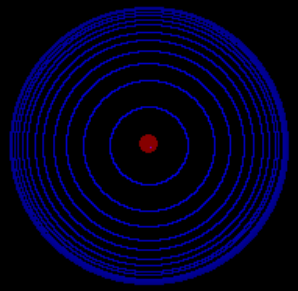
Static Images: Distance from Specific Structure

Nucleus



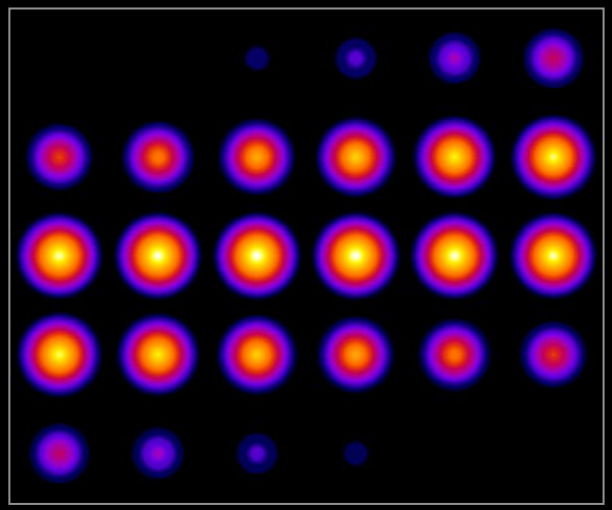
chromosome

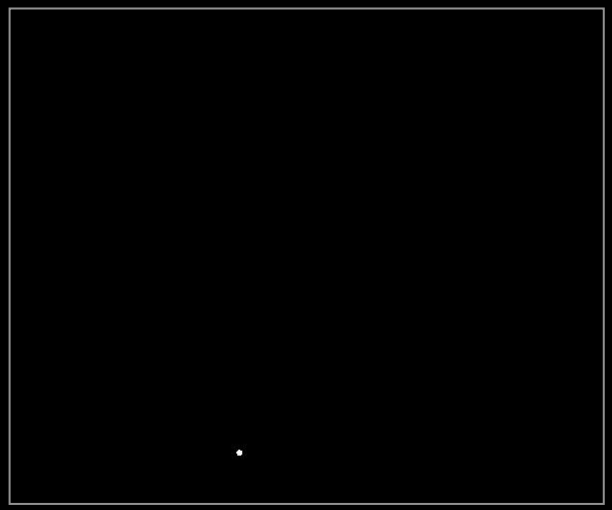


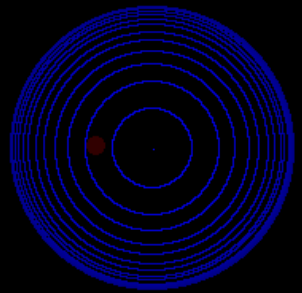


Center

Nucleus 3D distance map



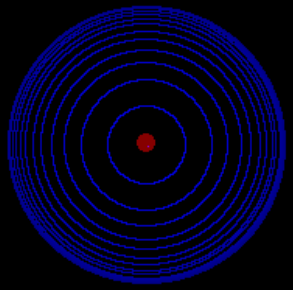


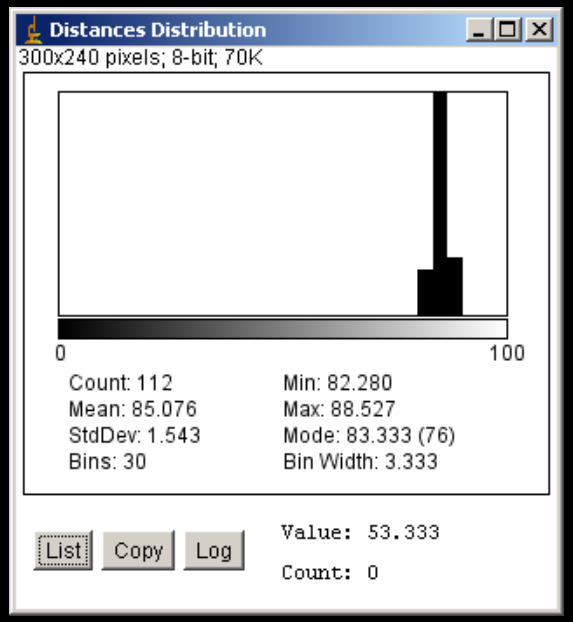


Periphery

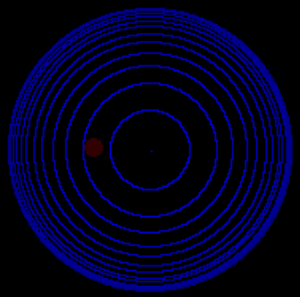
Static Images: Distance from Specific Structure

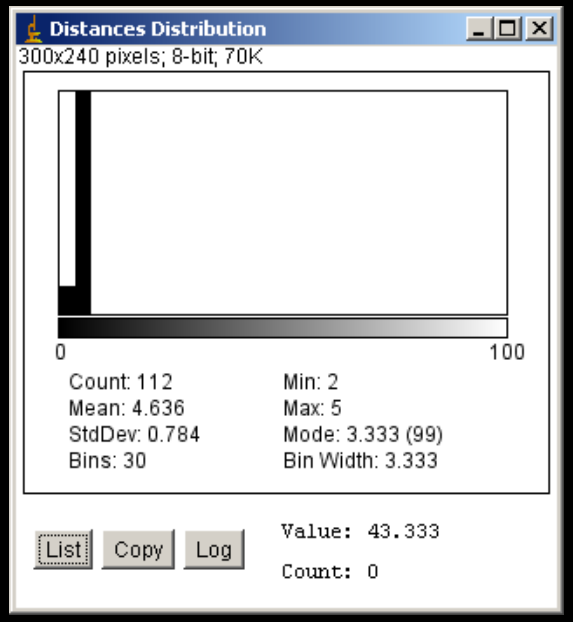
Center



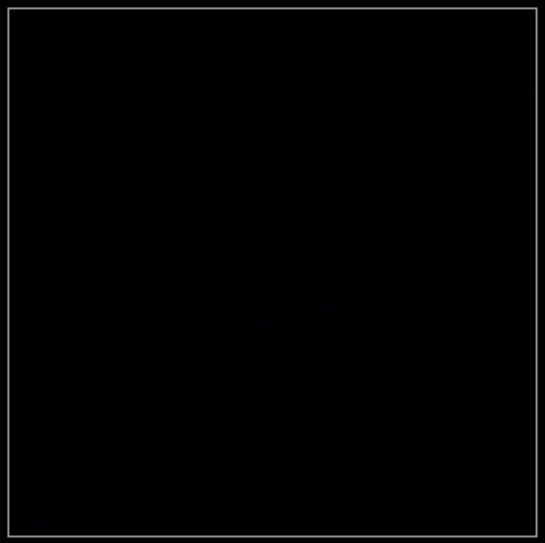


Periphery

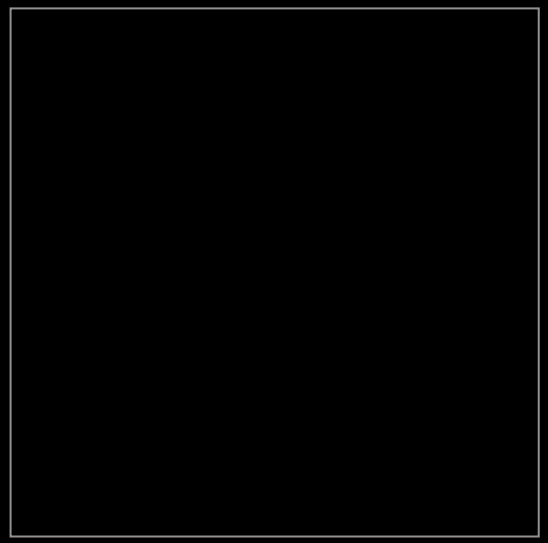




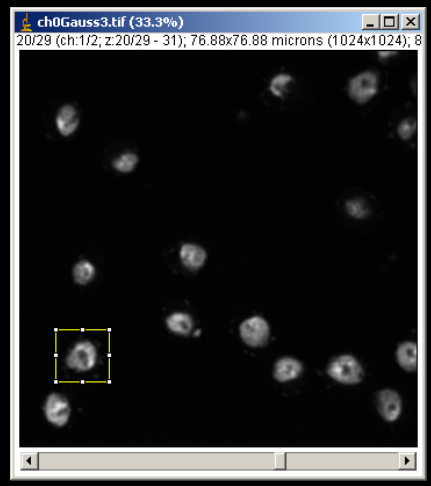
1. Segmentation of DAPI and FISH

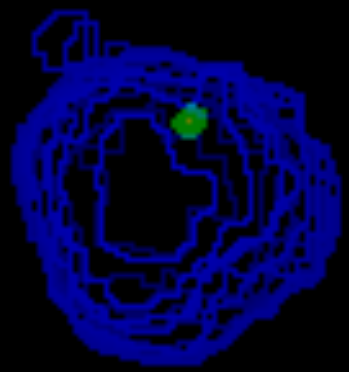


DAPI image

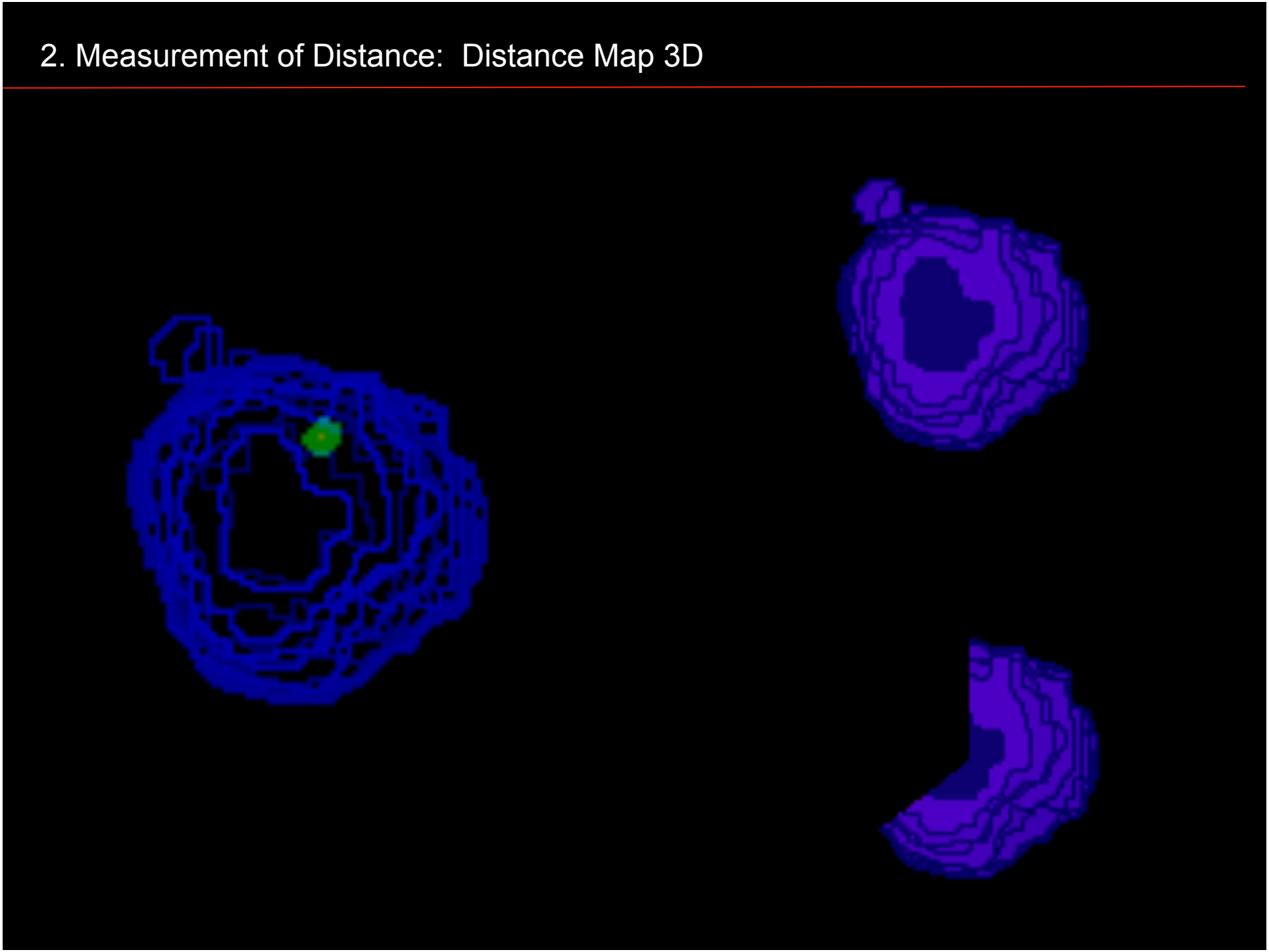


FISH image





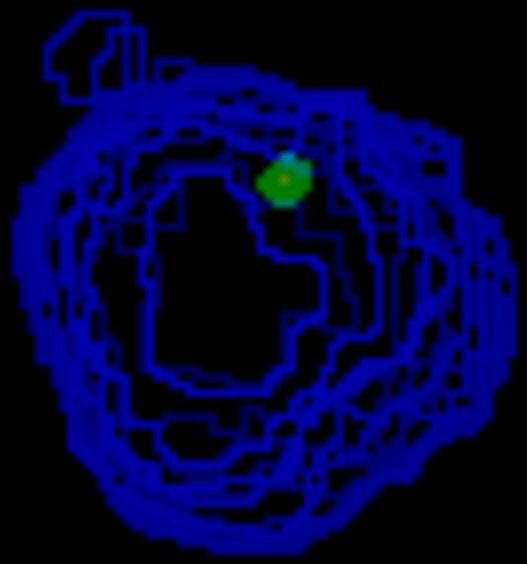
2. Measurement of Distance: Distance Map 3D

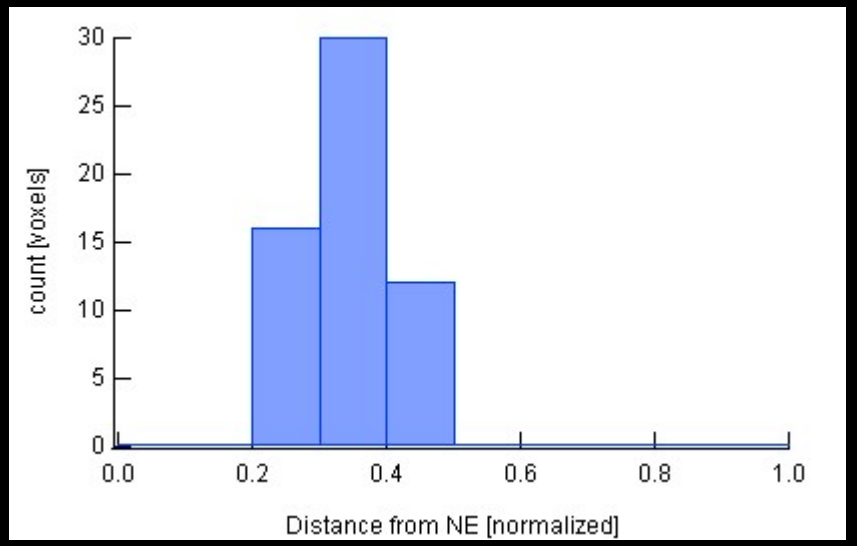


2. Measurement of Distance: Distance Map 3D

S

-> Leaves only FISH voxels with distances





1. Vaquerizas et al. Nuclear pore proteins nup153 and megator define transcriptionally active regions in the Drosophila genome. PLoS genetics. 2010;6 (2):e1000846

Static Images: Colocalization

Static images

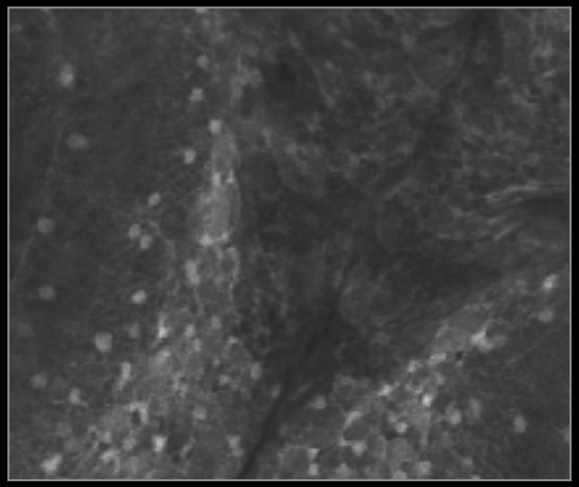
- Intensity Measurement
- Morphometry
- Distribution
- Colocalization → Qualitative, Quantitative

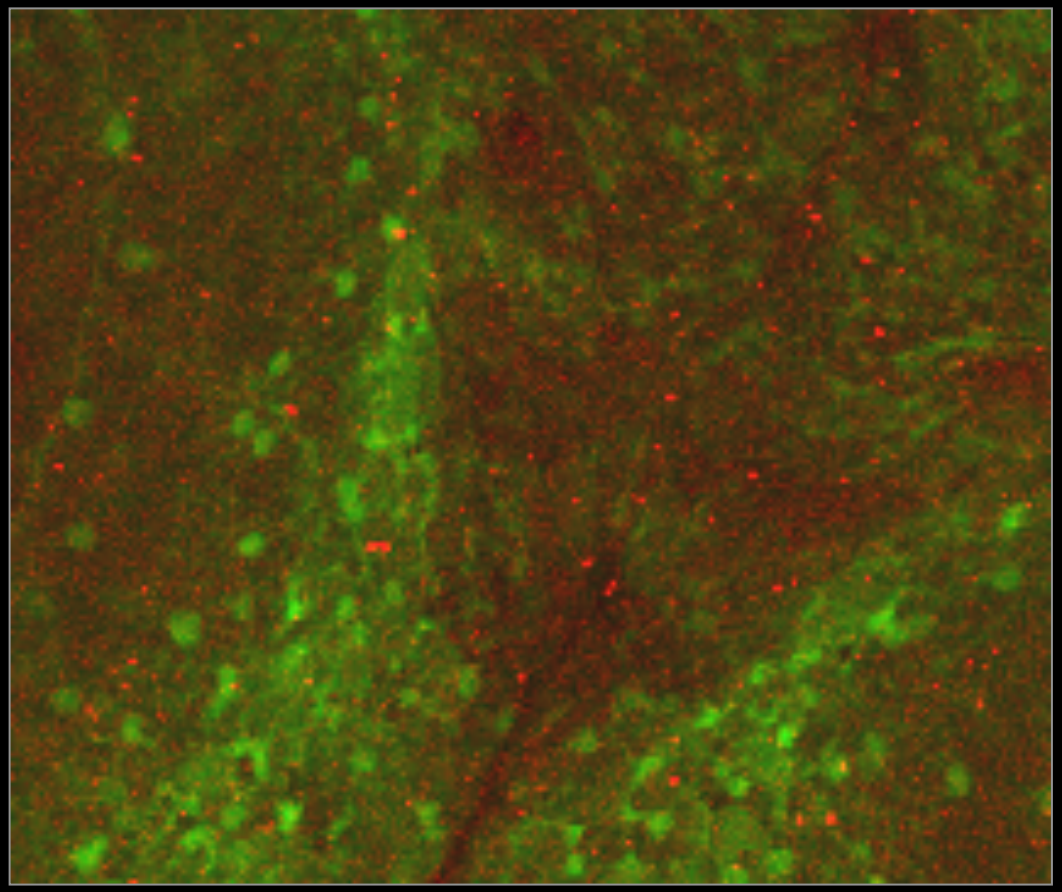
Image sequences

- Intensity dynamics
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation

Static Images: Co-localization

Qualitative

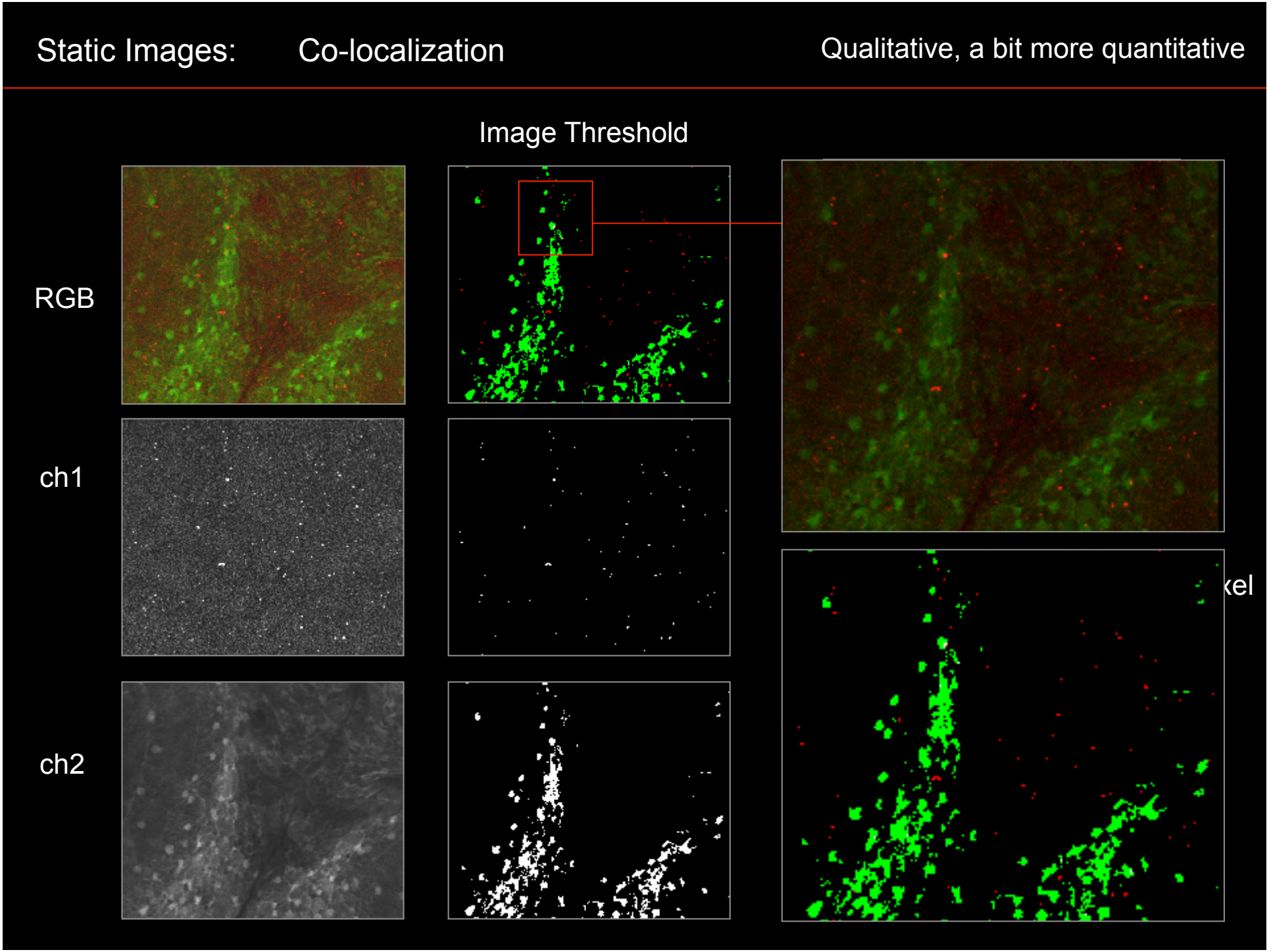




“see, they are colocalized!”

Static Images: Co-localization

Qualitative, a bit more quantitative

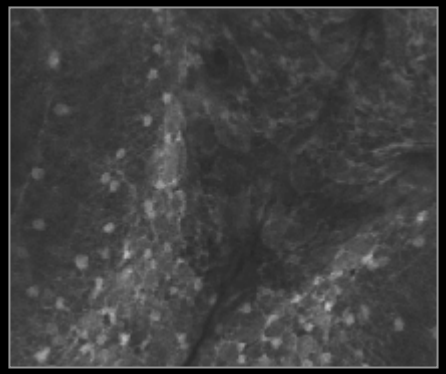


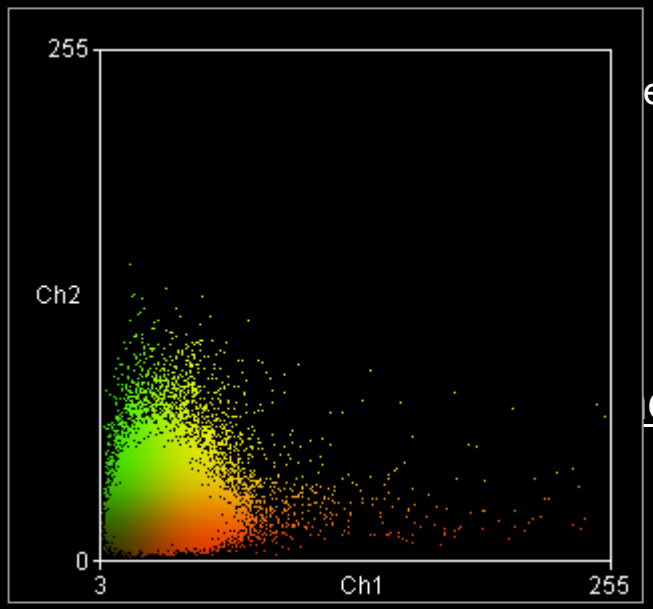
Static Images: Co-localization

Quantitative

ch1

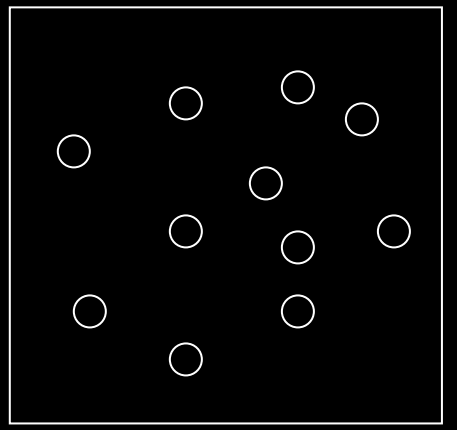
ch2





ensity

dent



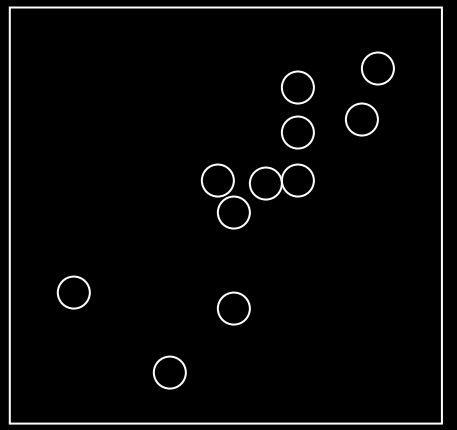
Ch1 intensity

Ch2 intensity

- Plot a point at (ch1 intensity, ch2 intensity)

- Color code the number of pixels for each position.

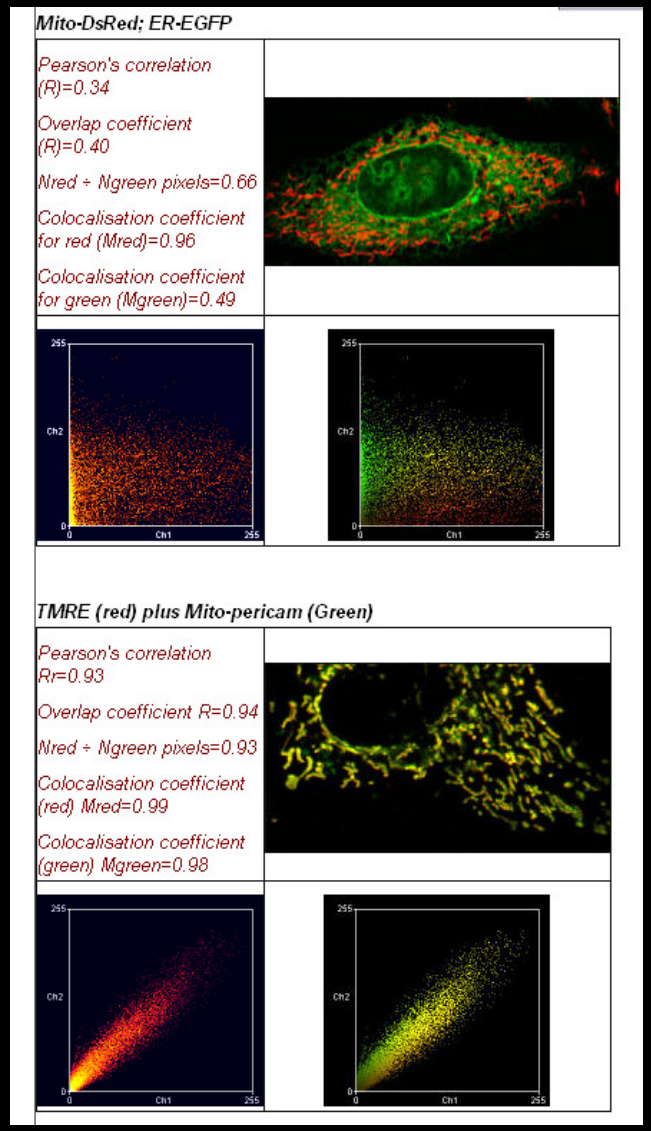
colocalized



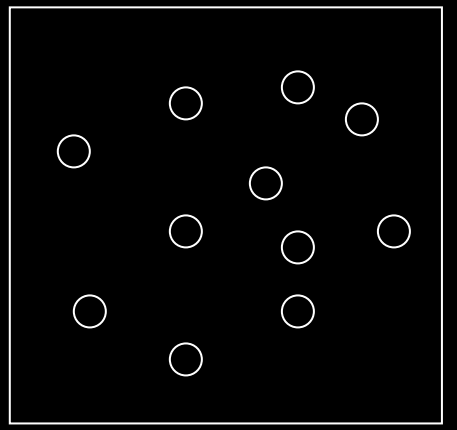
Ch1 intensity

Static Images: Co-localization

Quantitative



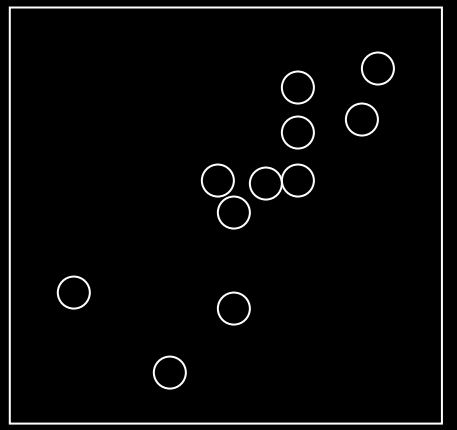
Ch2 intensity



<u>independent</u>

Ch1 intensity

Ch2 intensity



<u>colocalized</u>

Ch1 intensity

http://www.uhnresearch.ca/facilities/wcif/imagej/colour_analysis.htm

Static Images: Co-localization

Quantitative

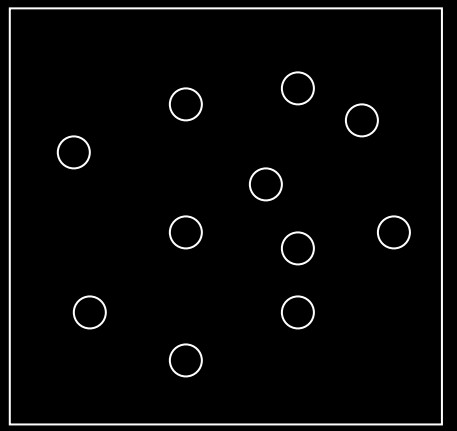
Statistics:

van Steensels test
(J.Cell Sci. 109:787)

Costes test
(Costes et al. 2004. Biophys. J 86:3993)

Fay's test
(Fay et al. 1997 Exp. Cell Res. 231: 27)

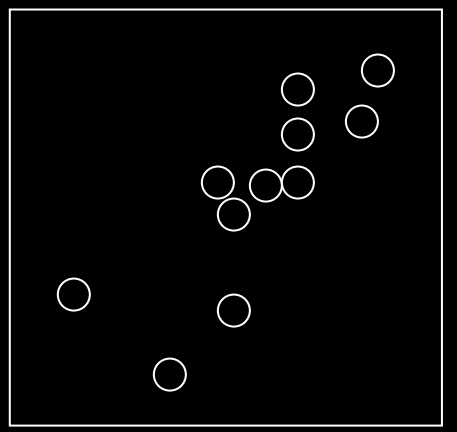
Manders' coefficients test
(Manders et al. 1993. J. Microscopy. 169:375)



independent

Review

Bolte, S. & Cordelieres, F.P. (2006) J. Microsc. 224, 213.
A guided tour into subcellular colocalization analysis in light microscopy.



colocalized

Dynamics: Image Series

Static images

- Intensity Comparison
- Morphometry
- Distribution
- Colocalization

Image sequences

- Intensity dynamics
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
  - optical flow
- Distribution dynamics

Image Sequences: Overview

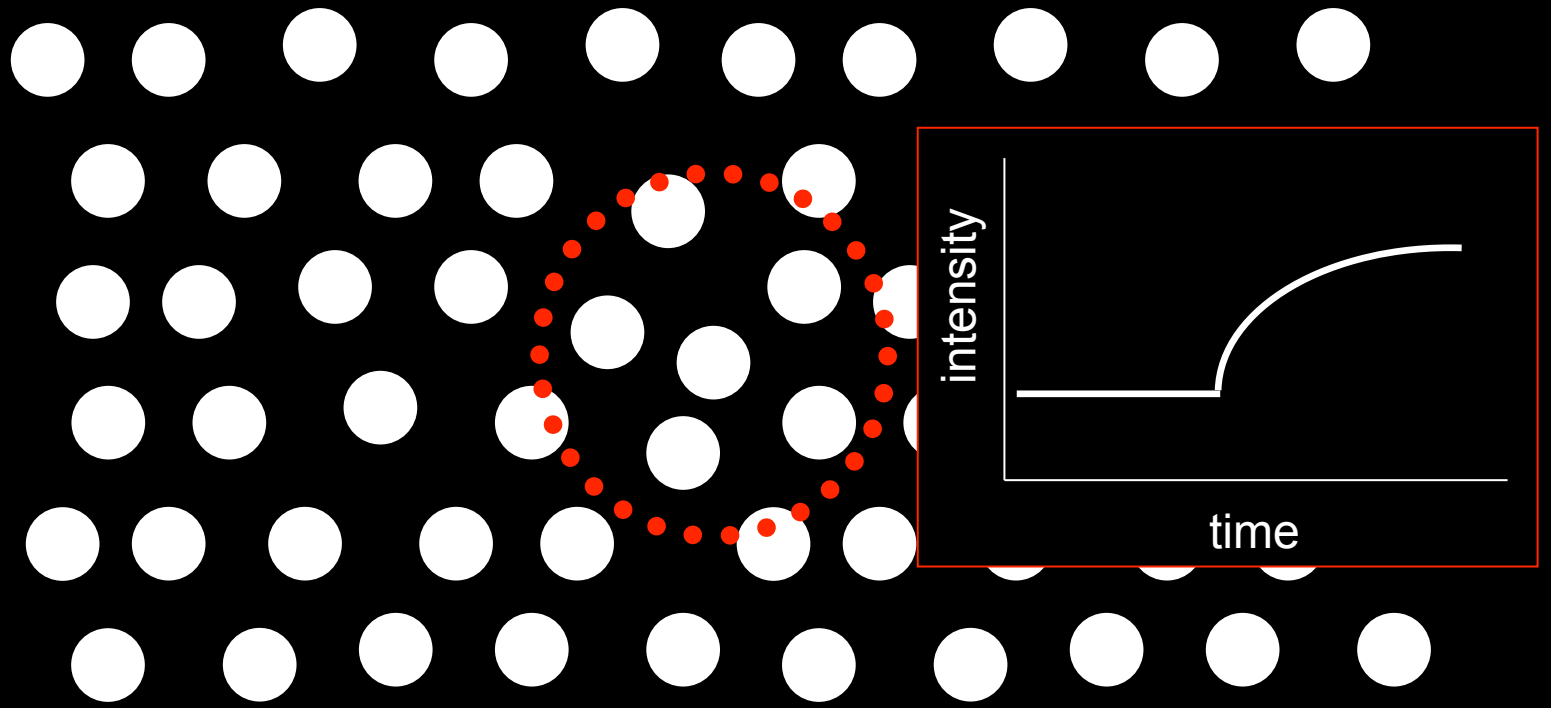
Static images

- Intensity Comparison
- Morphometry
- Distribution
- Colocalization

Image sequences

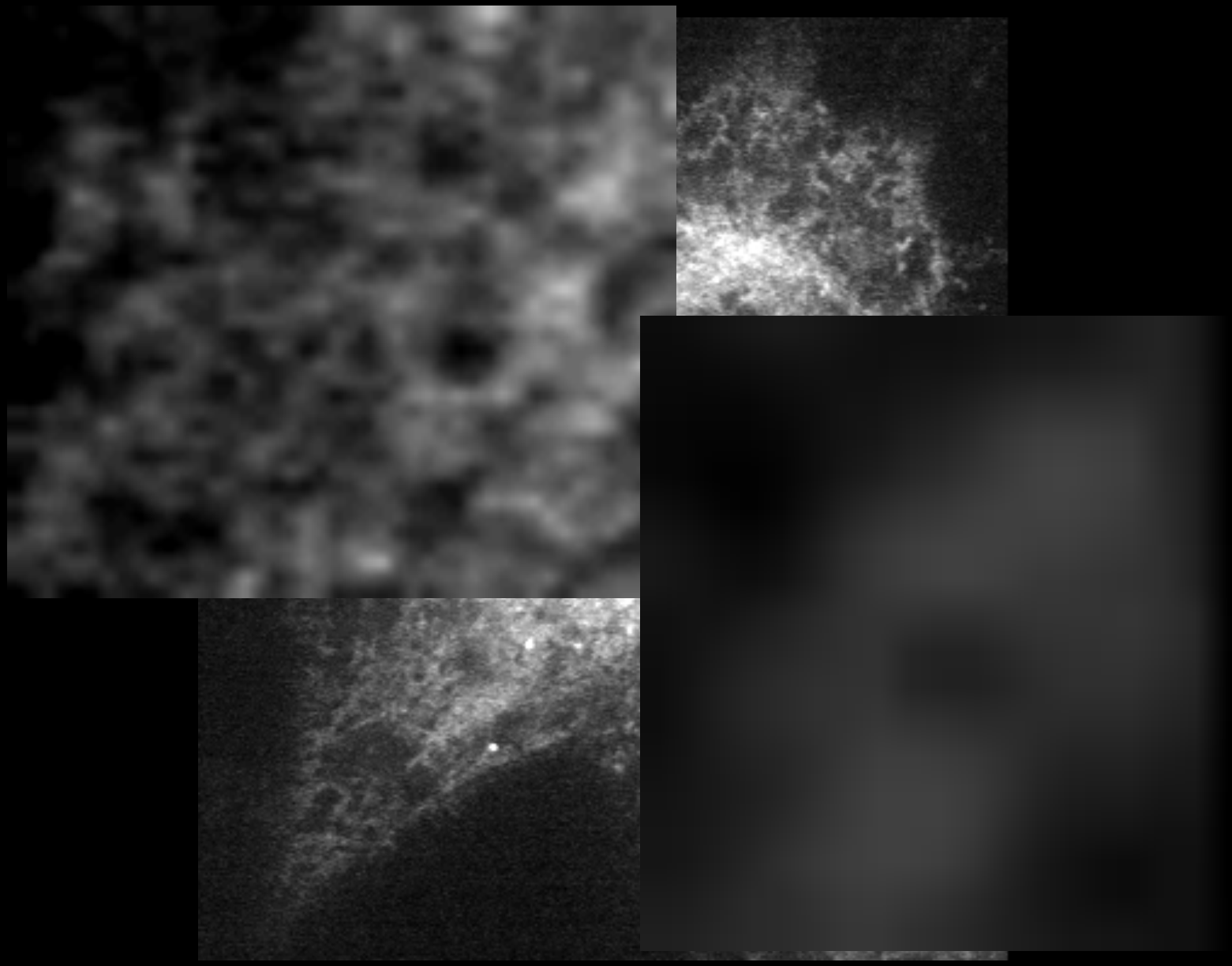
- Intensity dynamics → Kinetics, FRAP, FLIP …
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation

Intensity Dynamics



Intensity Dynamics

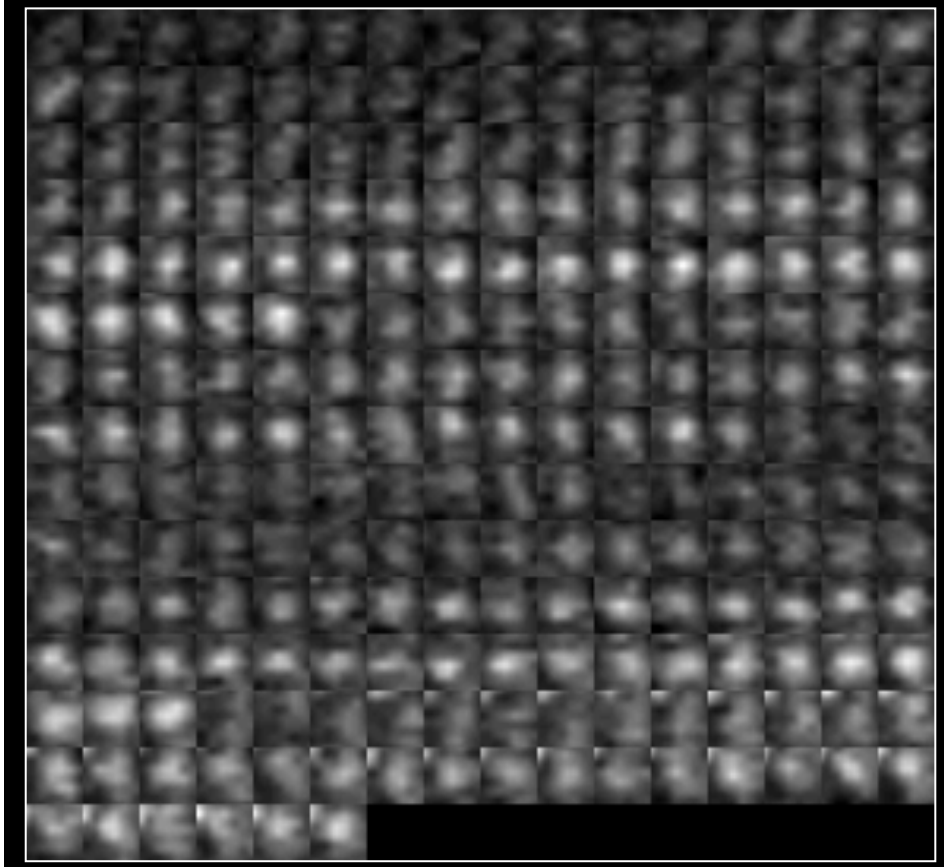
Accumulation of VSVG protein at the exit sites

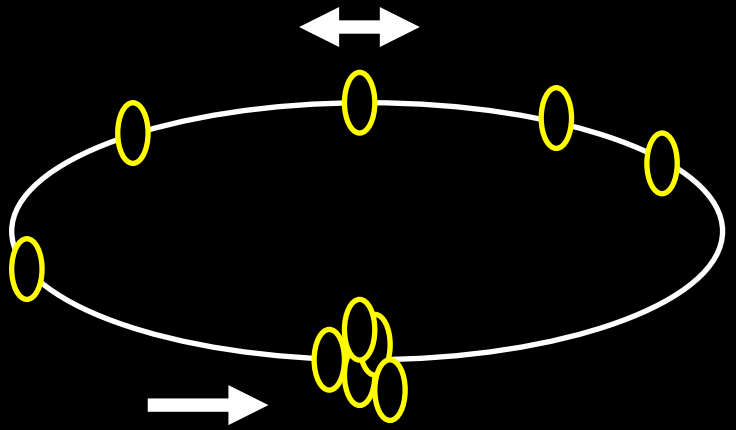


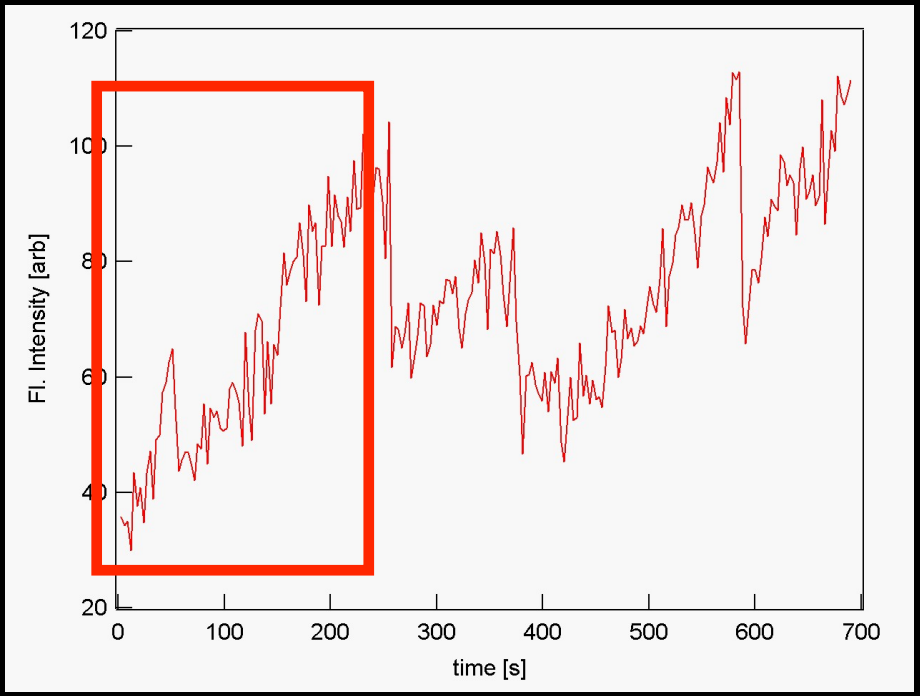
Runz et al. (2006) EMBO J. 12;25(13):2953-65

Intensity Dynamics

Accumulation of VSVG protein at the exit sites





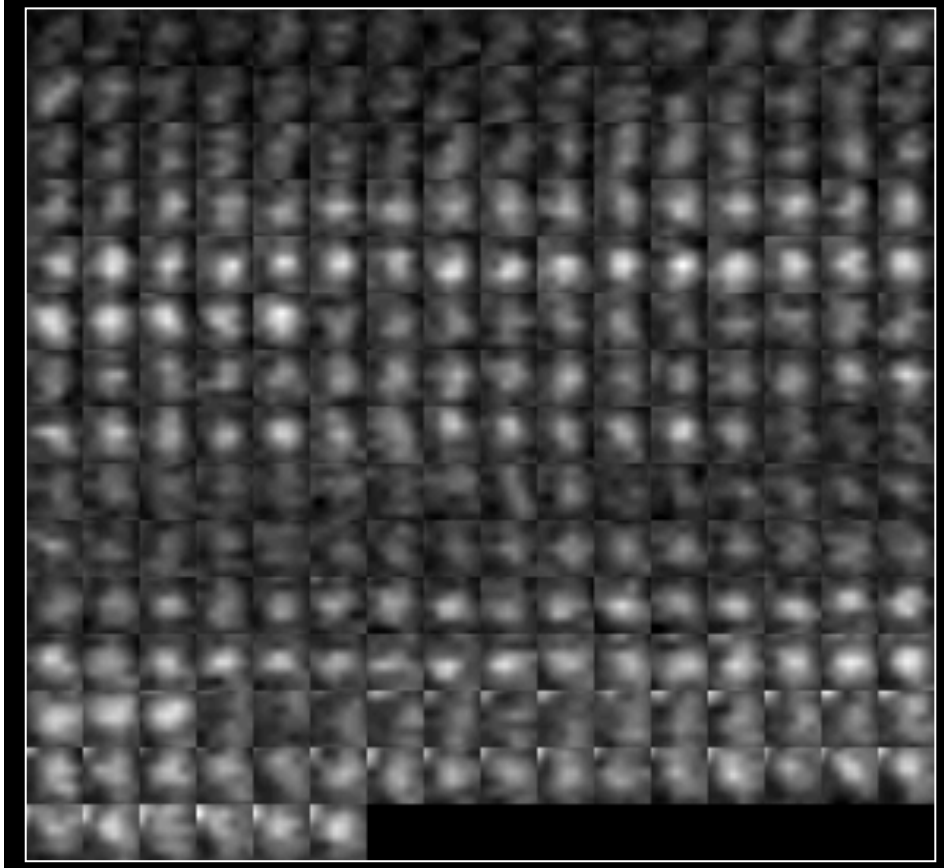


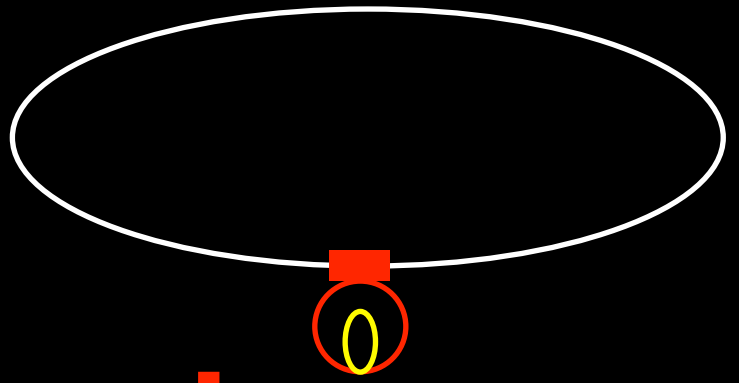
→ Binding constants of VSVG protein to the ER exit site can be measured directly.

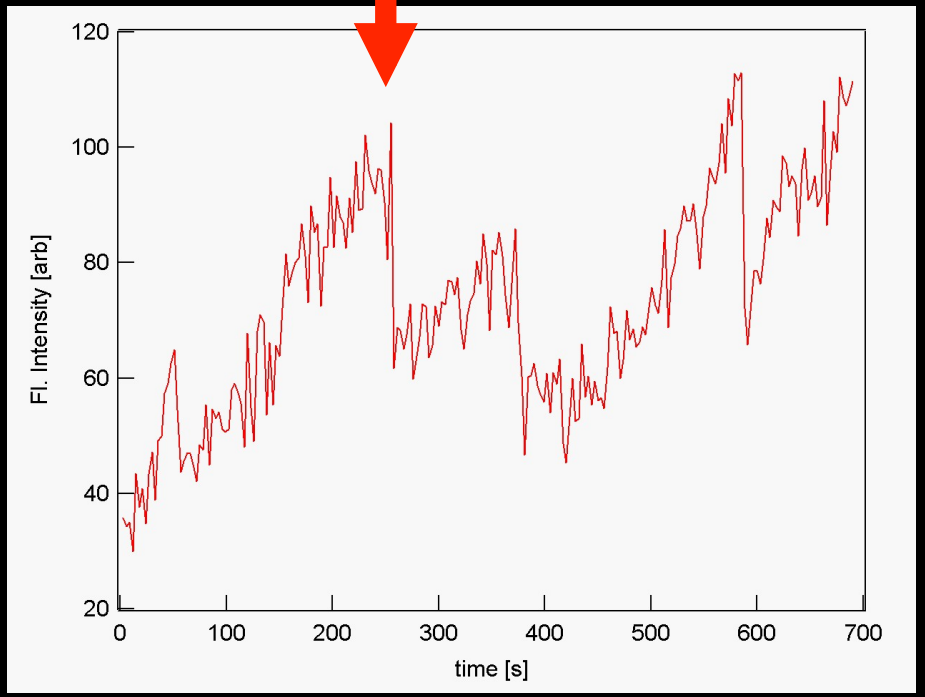
Runz et al. (2006) EMBO J. 12;25(13):2953-65

Intensity Dynamics

Accumulation of VSVG protein at the exit sites







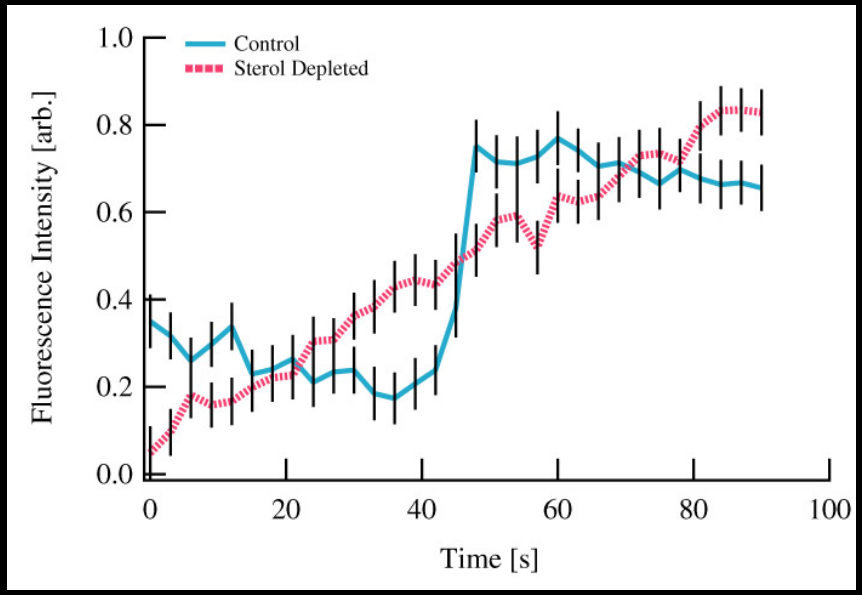
→ Binding constants of VSVG protein to the ER exit site can be measured directly.

Runz et al. (2006) EMBO J. 12;25(13):2953-65

Intensity Dynamics

Accumulation of VSVG protein at the exit sites

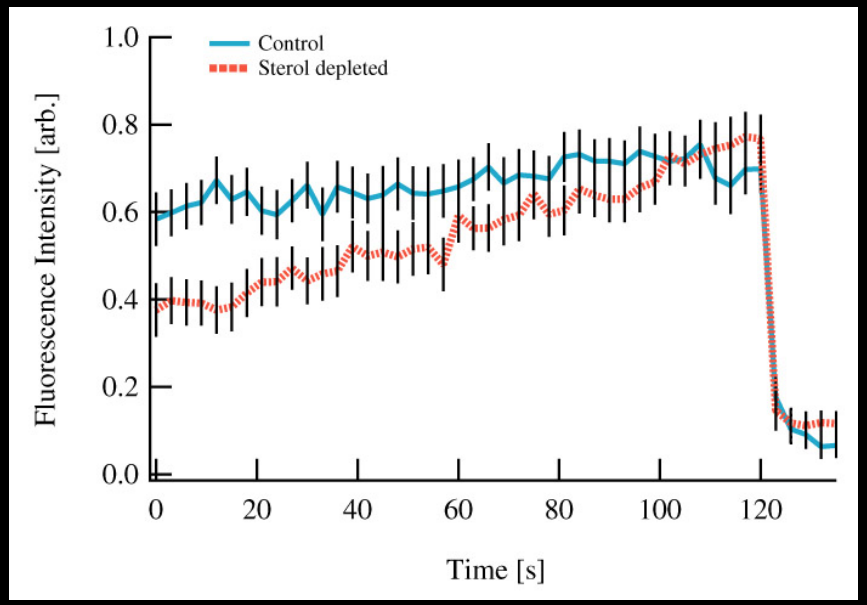
Loading



$$\frac{d[tsO45G_{ERES}]}{dt} = K_{on}[tsO45G_{free}] - K_{off}[tsO45G_{ERES}]$$

$$\left.\frac{d[tsO45G_{ERES}]}{dt}\right|_{initial} = K_{on}[tsO45G_{free}]$$

Budding



Runz et al. (2006) EMBO J. 12;25(13):2953-65

Intensity Dynamics

→This strategy does not work under steady state…
→ FRAP

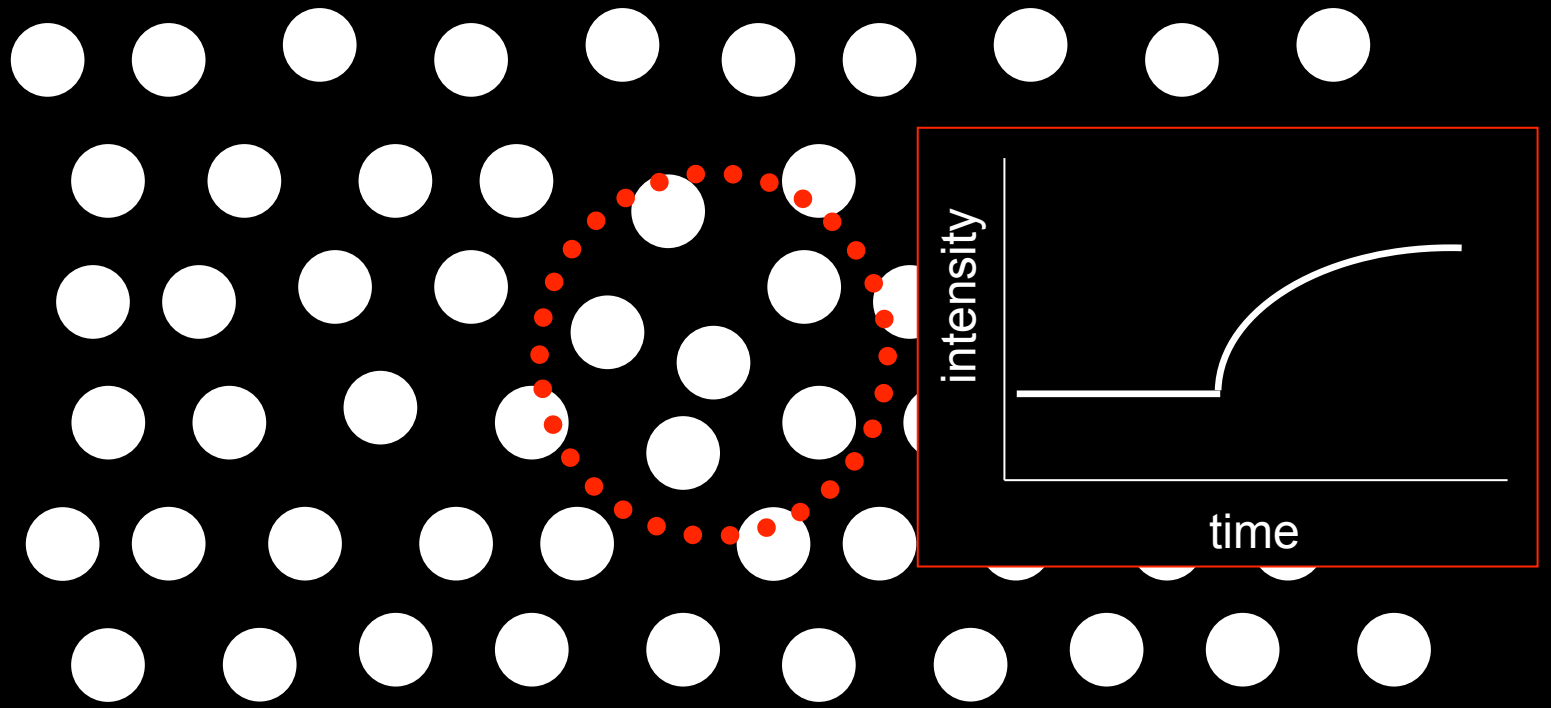


Image Sequences: Overview

Static images

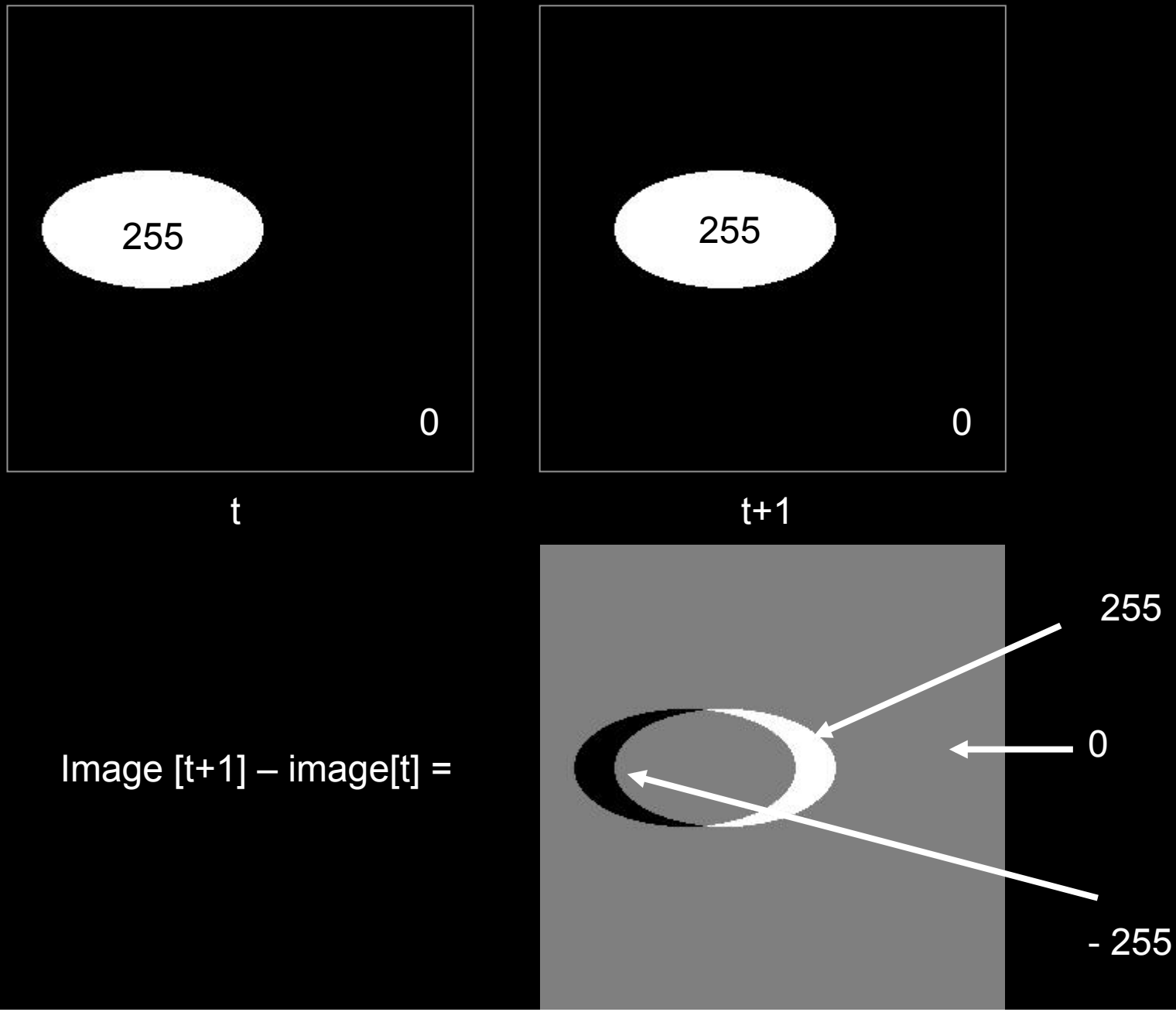
- Intensity Comparison
- Morphometry
- Distribution
- Colocalization

Image sequences

- Intensity dynamics
- Shape dynamics → Length, Area dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation

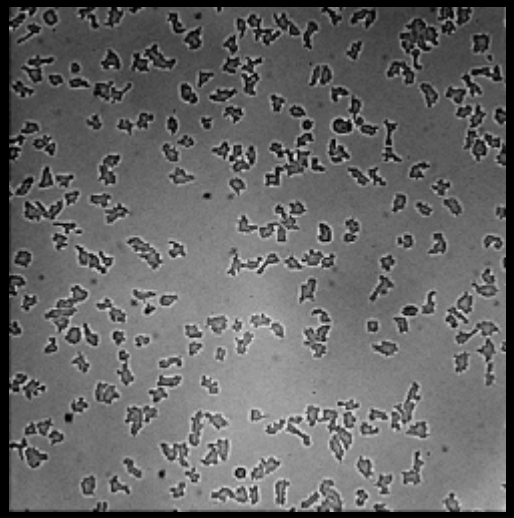
Measurement of Shape Changes: Area Dynamics

$D_j\,(x,y) = I_{t+1}(x,y) - I_t(x,y)$



Measurement of Shape Changes: Area Dynamics

Light increases signaling waves

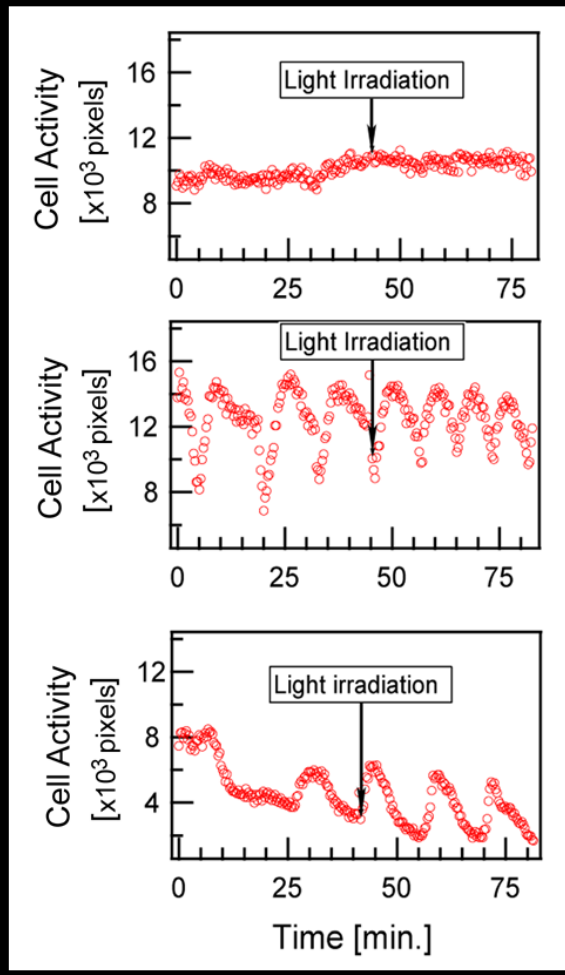


Starvation:

2hr

4hr

6.5hr



Miura & Siegert (2000) PNAS

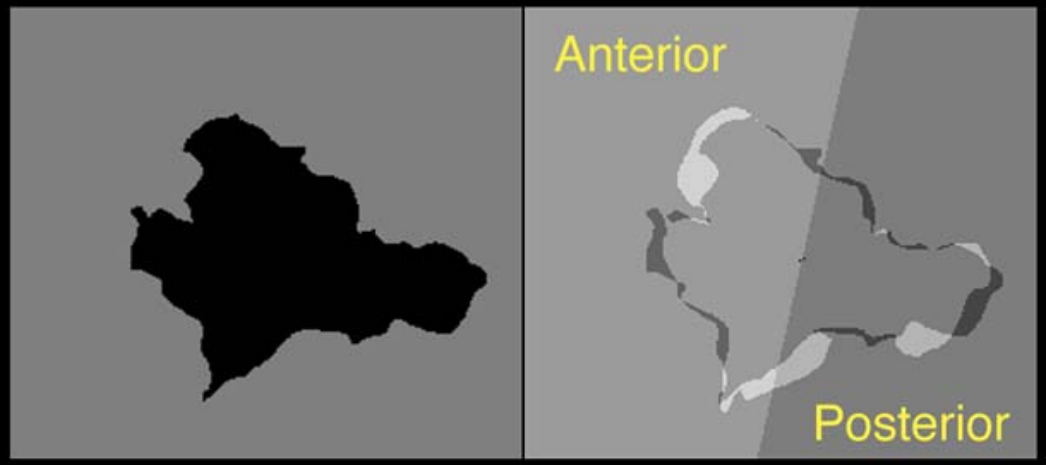
Measurement of Shape Changes: Area Dynamics

Single Cell Movement

Miura and Ogihara (1996)

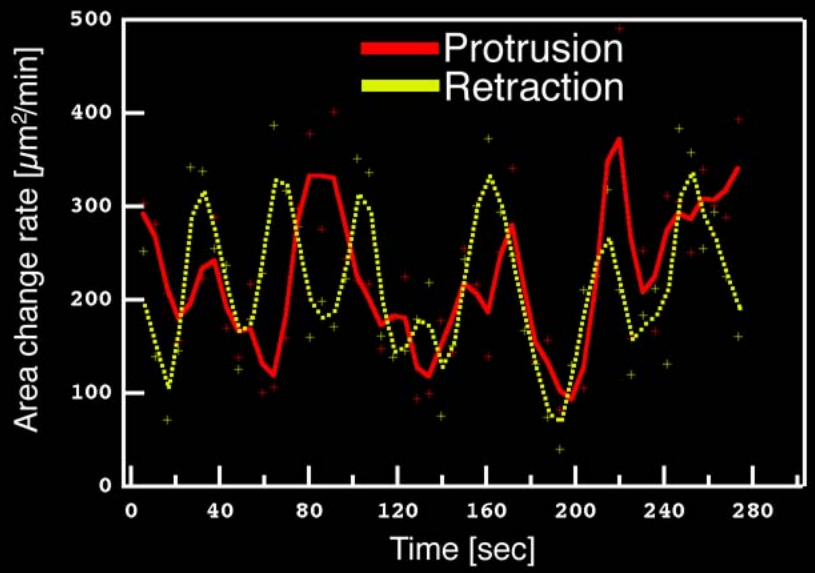
Measurement of Shape Changes: Area Dynamics

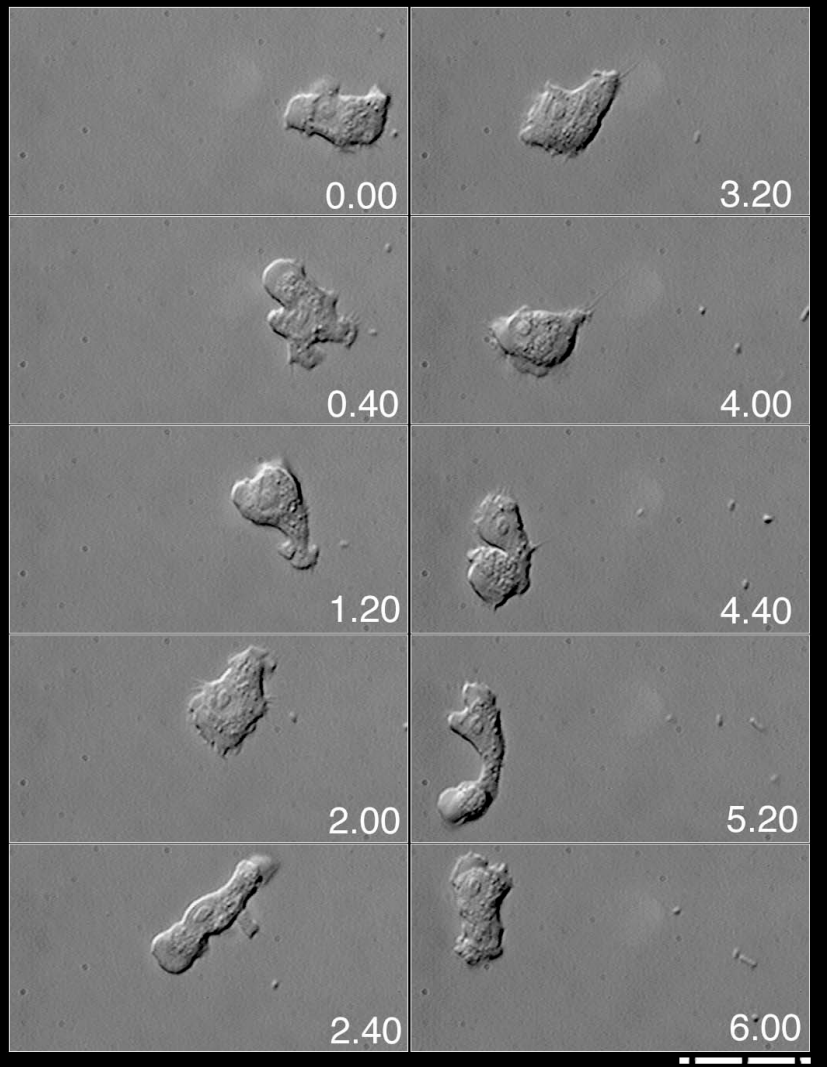
Single Cell Movement



Area changes in anterior half and posterior half.

Cell movement on a plane

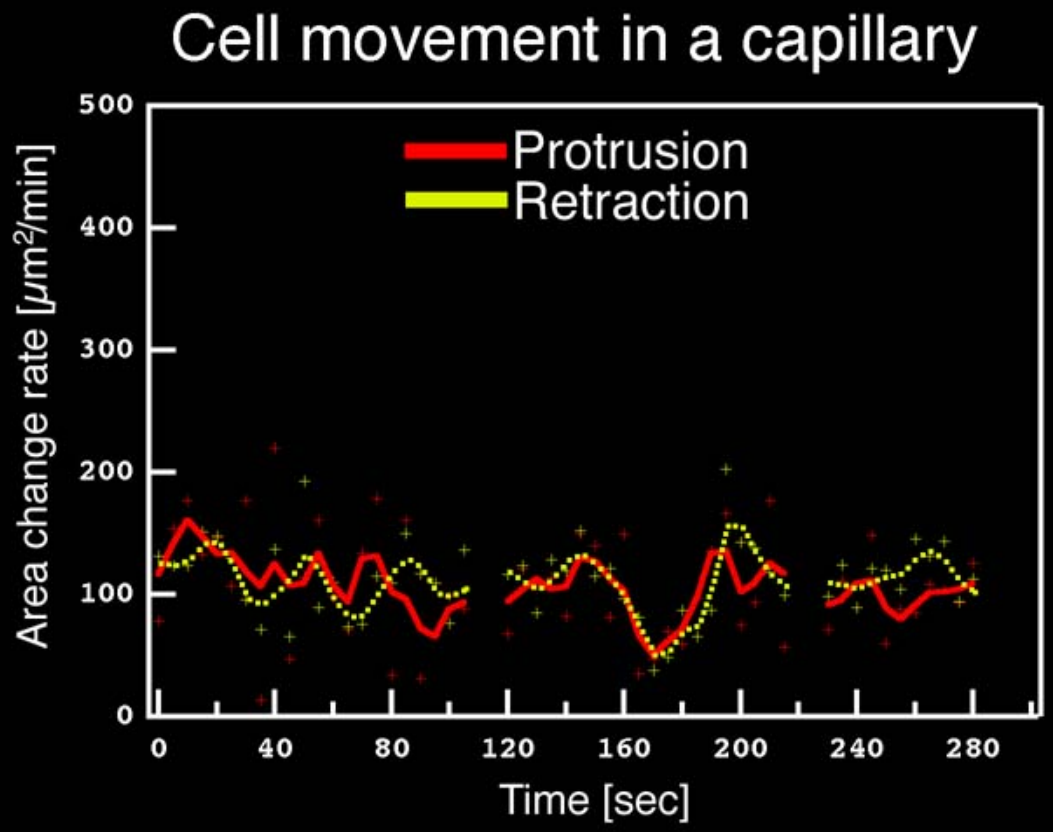


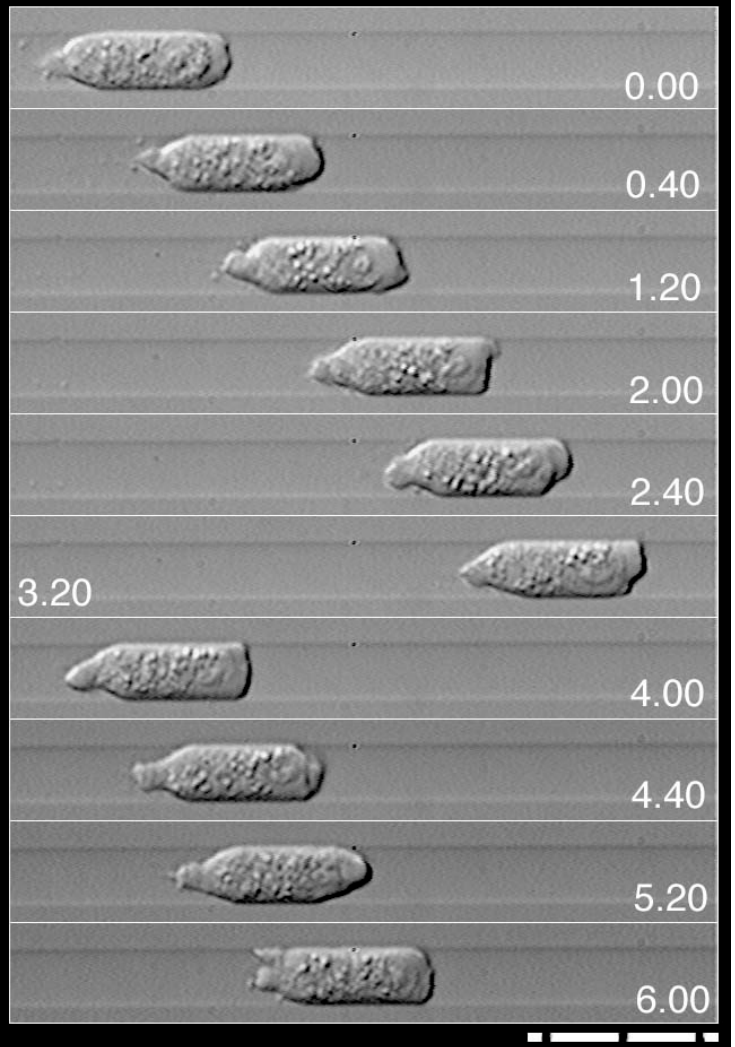


Miura and Ogihara (1996)

Measurement of Shape Changes: Area Dynamics

Cell Migration in agar capillary

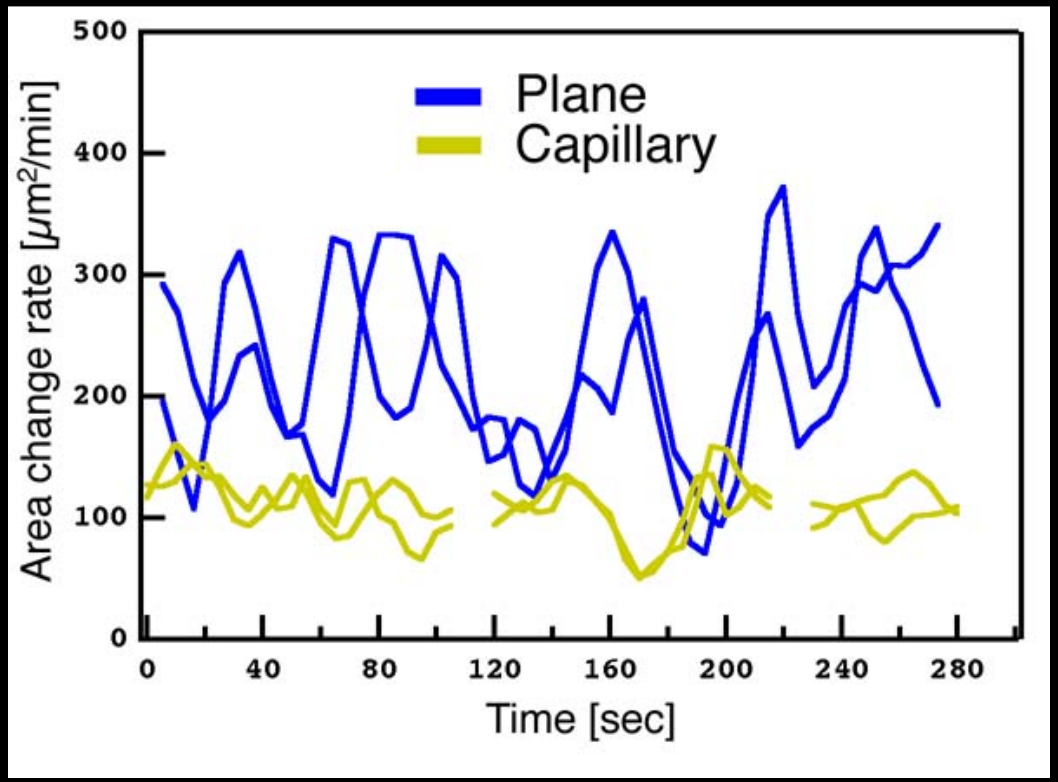


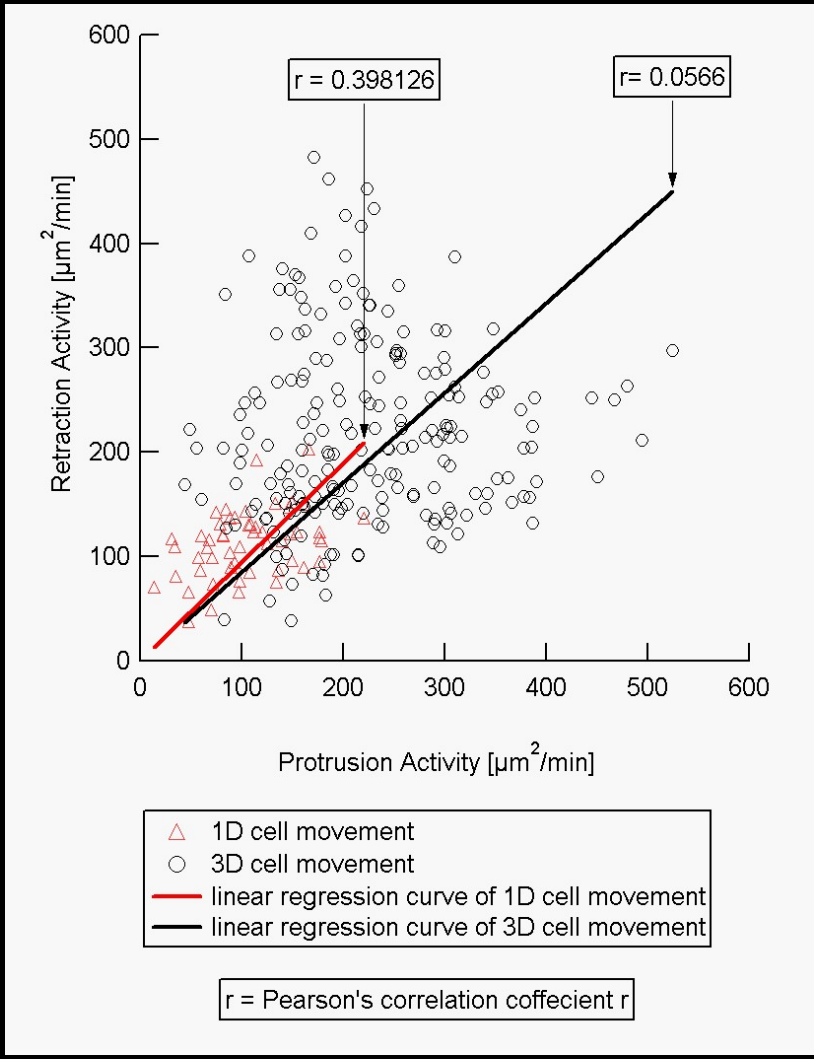


Miura and Ogihara (1996)

Measurement of Shape Changes: Area Dynamics

Protrusion / retraction coupling





Miura and Ogihara (1996)

Image Sequences: Overview

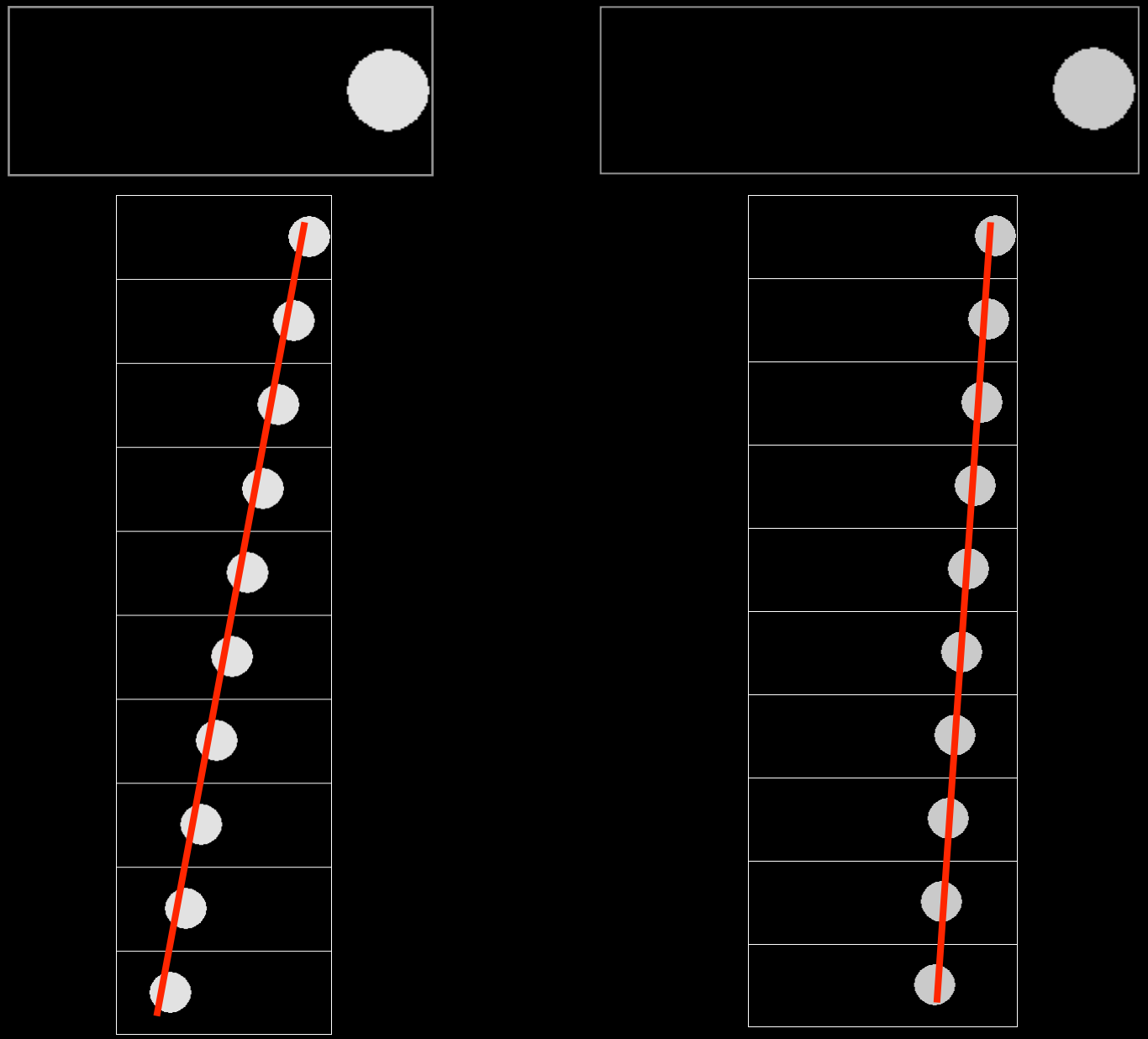
Static images

- Intensity Comparison
- Morphometry
- Distribution
- Colocalization

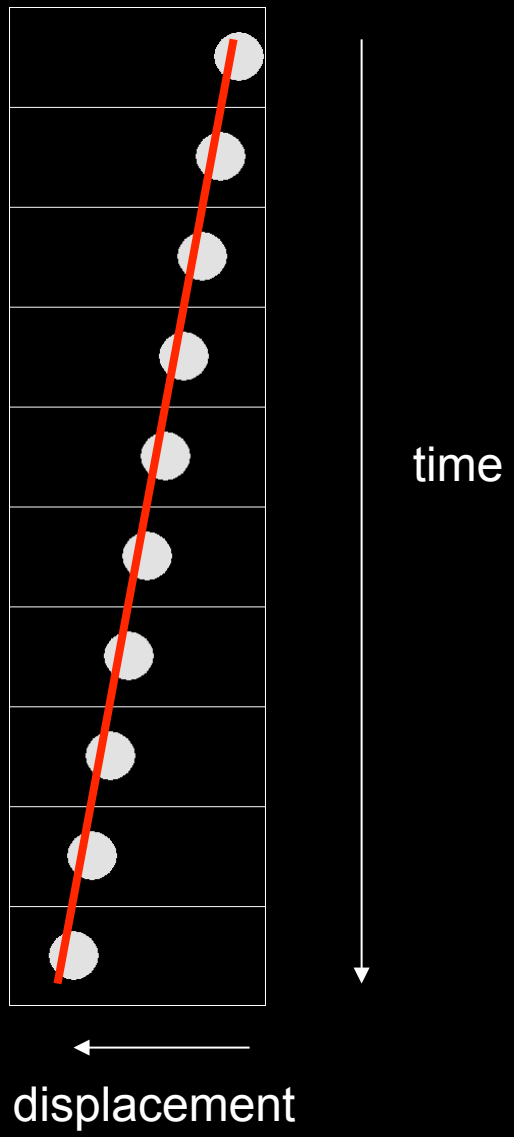
Image sequences

- Intensity dynamics
- Shape dynamics
- Position dynamics :
  - kymograph
  - tracking
- Distribution dynamics
- Optical Flow Estimation

Measurement of Movement: kymograph

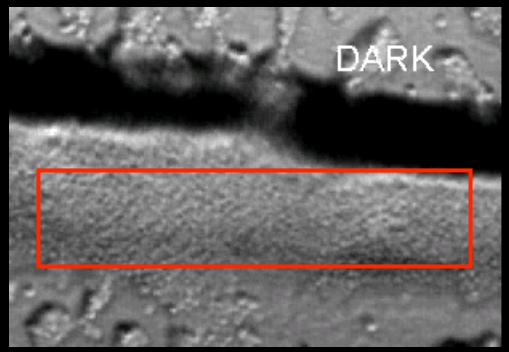


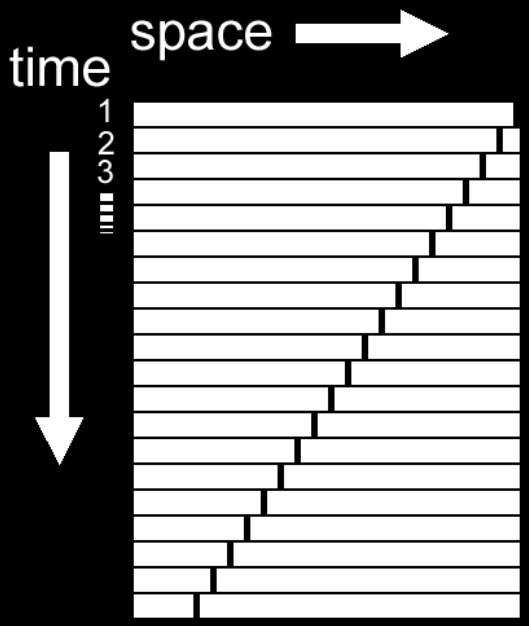
Measurement of Movement: kymograph



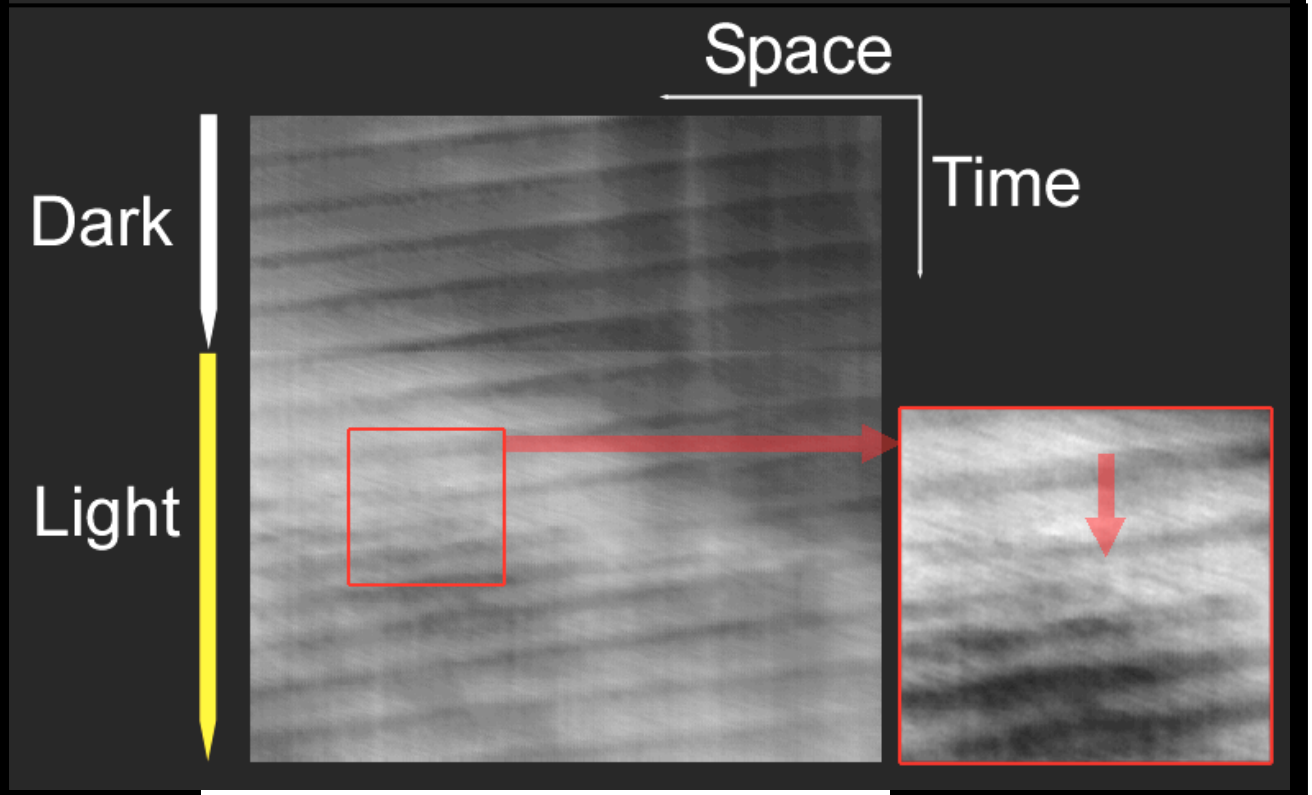
Speed = Displacement / Time

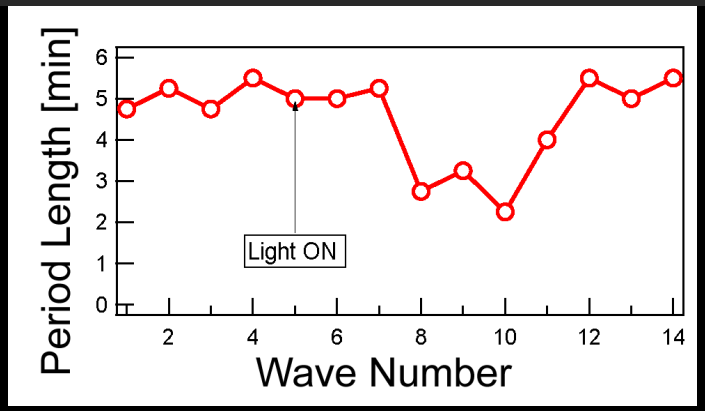
kymograph





Light Induces New Wave





Measurement of Movement

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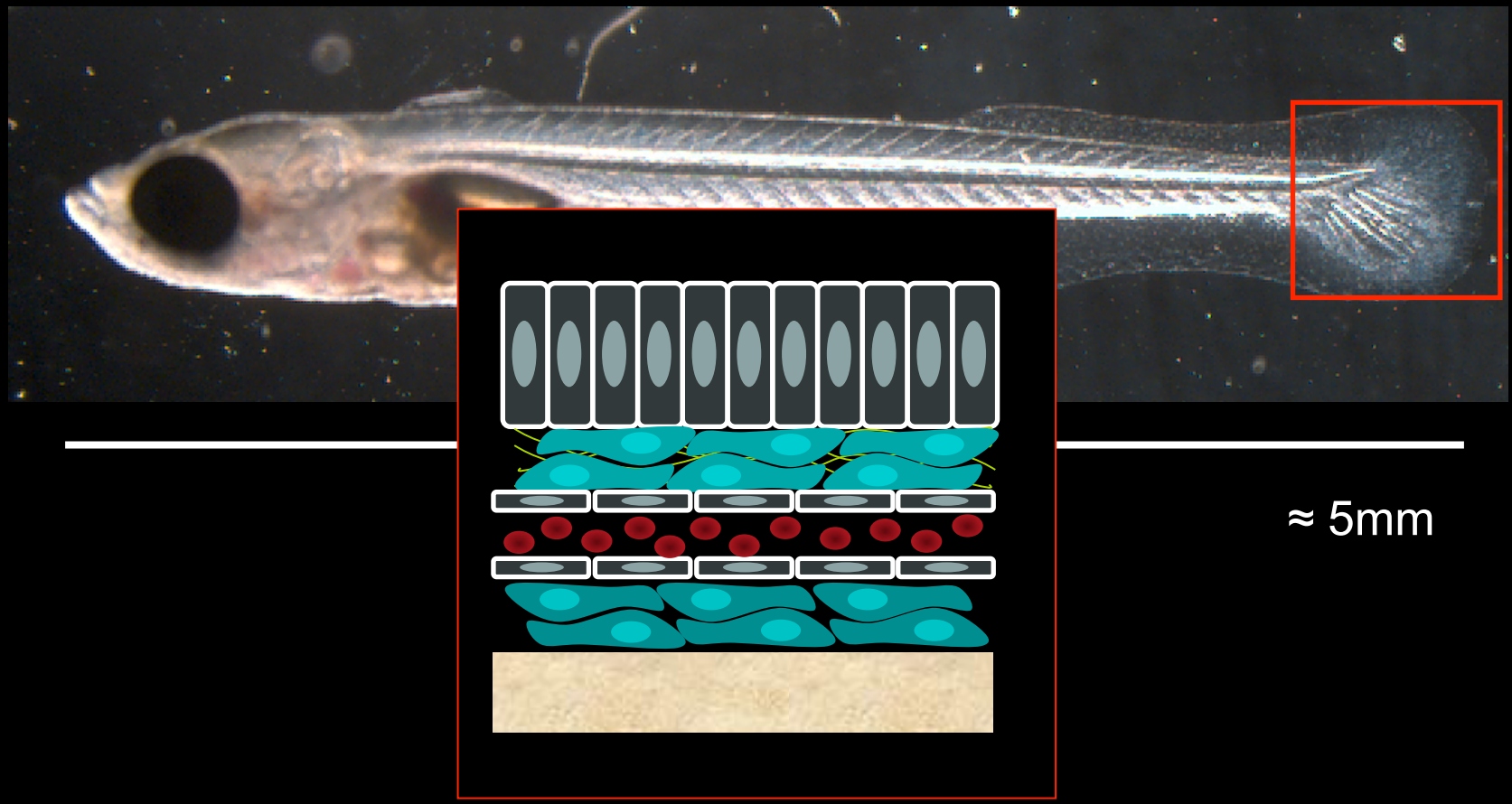
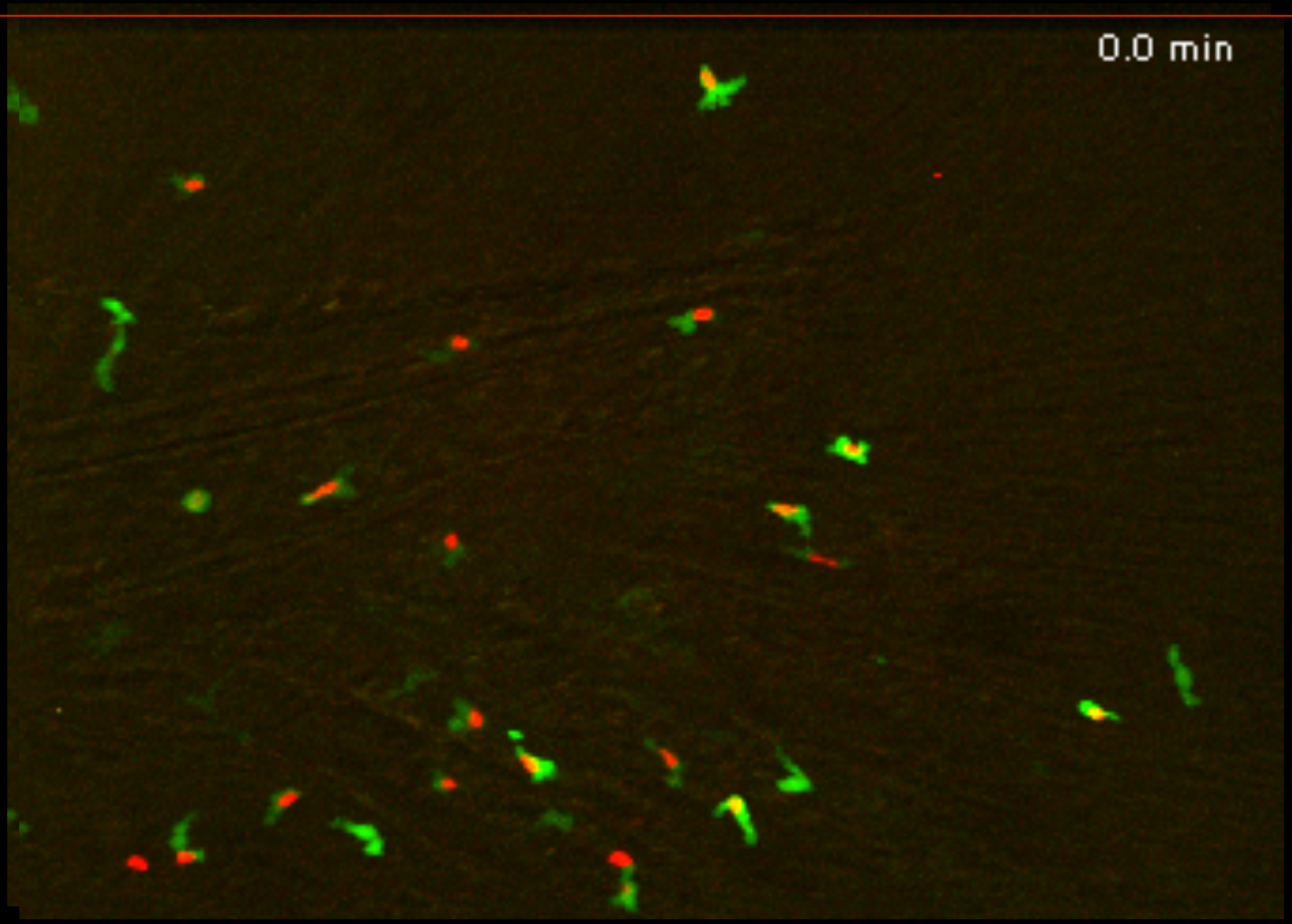
Measurement of Movement: cell tracking



Cell migration within *Medaka* tail-fin

Measurement of Movement: cell tracking

Random Migration



1. TBL (unknown protein, random insertion & screening) Green GFP
2. FMPO (myeloperoxidase promoter, HIston-mRFP, macrophage-marker) Red GFP

Grabher et al. (2007) J Leukoc Biol. 81(1):263-71

Measurement of Movement: cell tracking

Pattern Matching Method

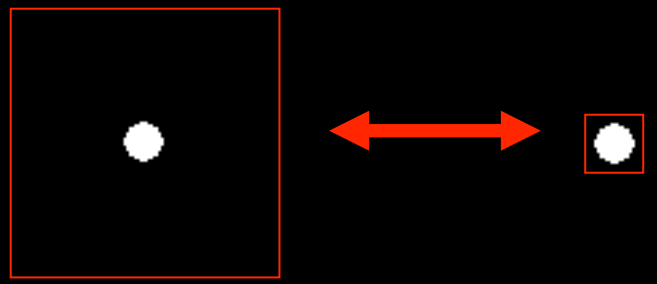


Image Frame

Kernel
(Template)

- Cross-Correlation

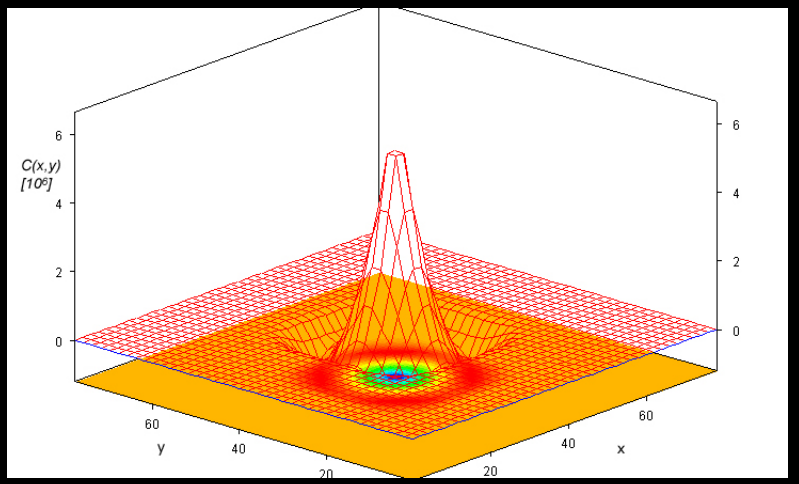
$$C(x,y) = \sum_{i=0}^{n-1}\sum_{j=0}^{m-1} I(x+i, y+j)\{K(i,j) - \overline{K}\}$$

- Sum of Absolute Difference

$$SAD(x,y) = \sum_{i=0}^{n-1}\sum_{j=0}^{m-1} \left| I(x+i, y+j) - K(i,j) \right|$$

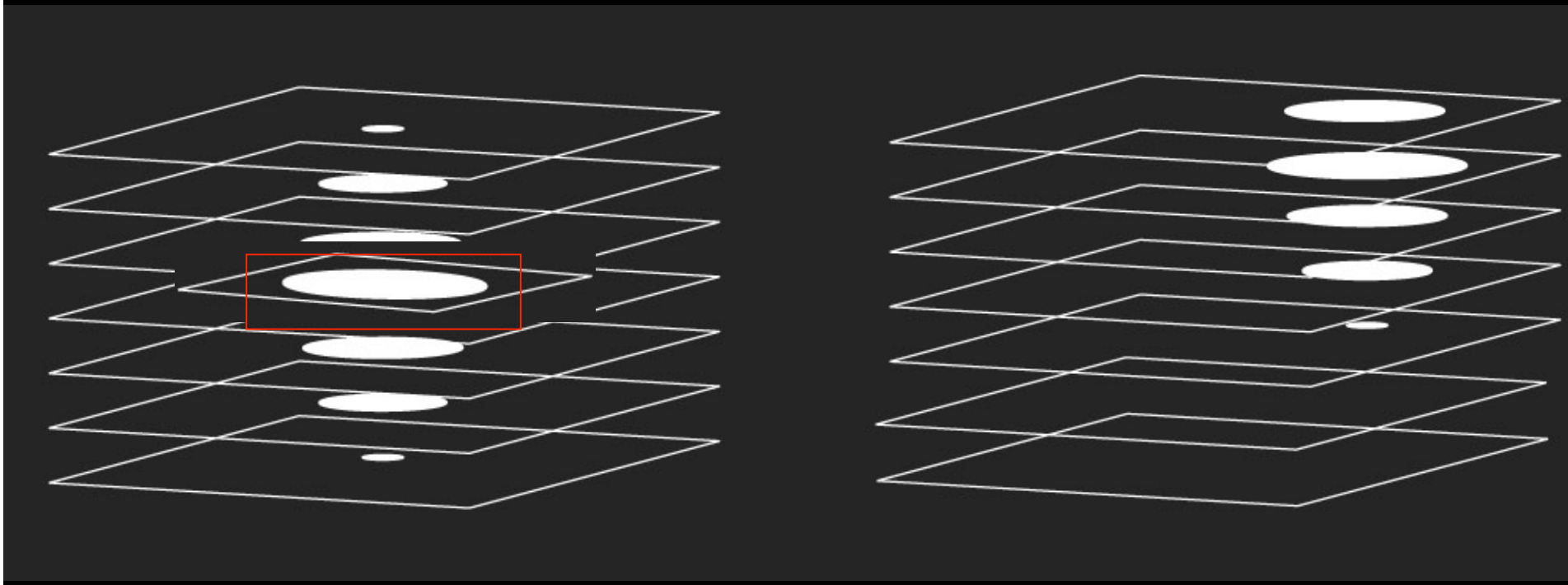
$$x_c = \frac{\sum x\{C(x,y) - T\}}{\sum \{C(x,y) - T\}}$$

$$y_c = \frac{\sum y\{C(x,y) - T\}}{\sum \{C(x,y) - T\}}$$



Measurement of Movement: cell tracking

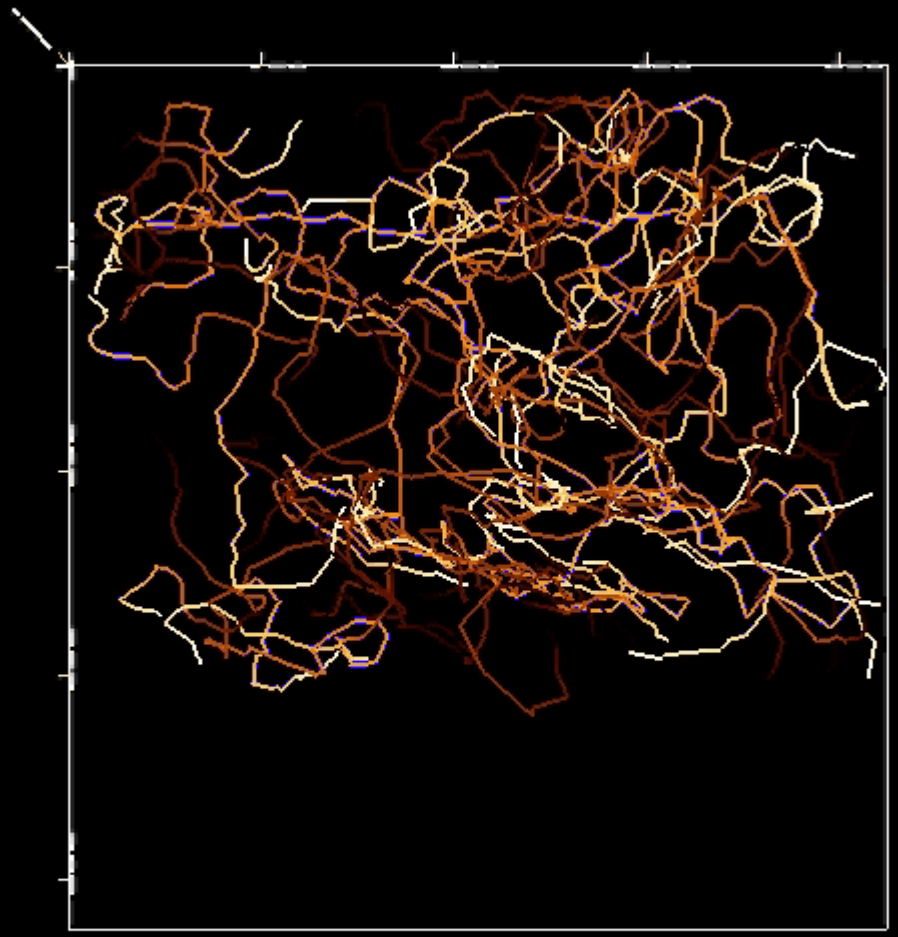
Pattern matching in 3D



t

t+1

Grabher et al. (2007) J Leukoc Biol. 81(1):263-71



3D plotting

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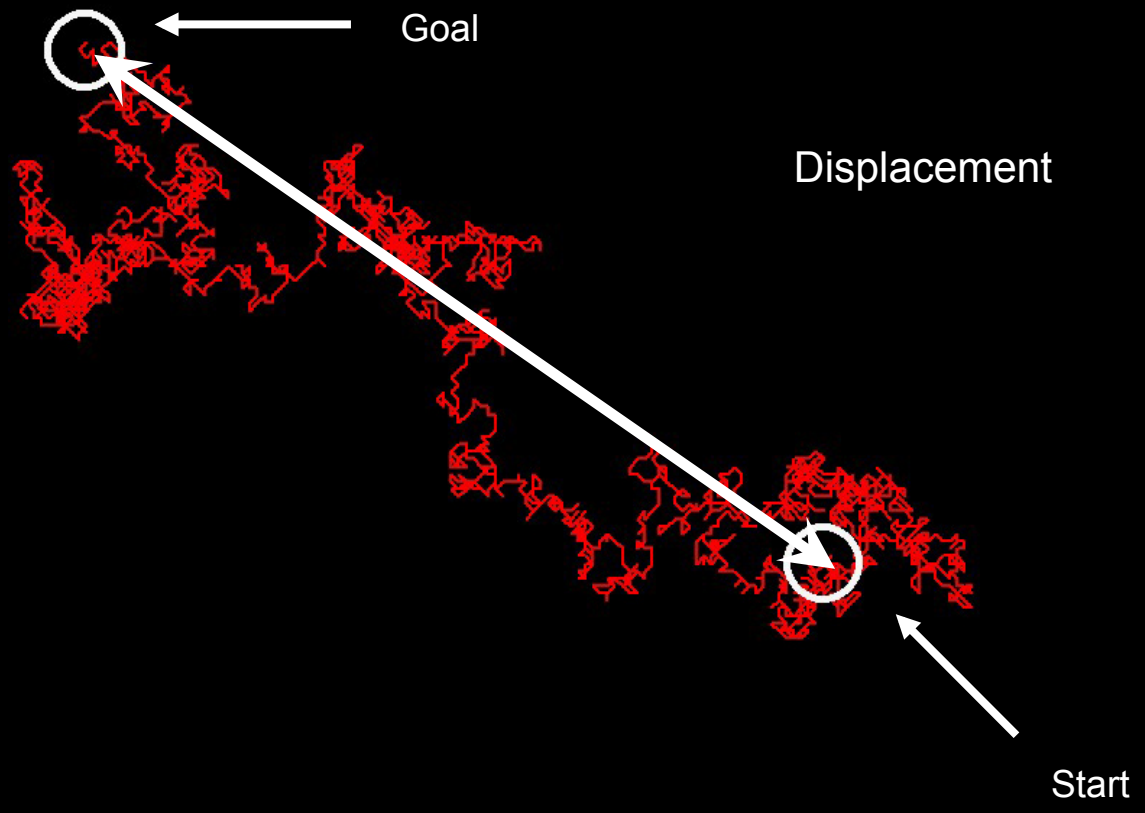
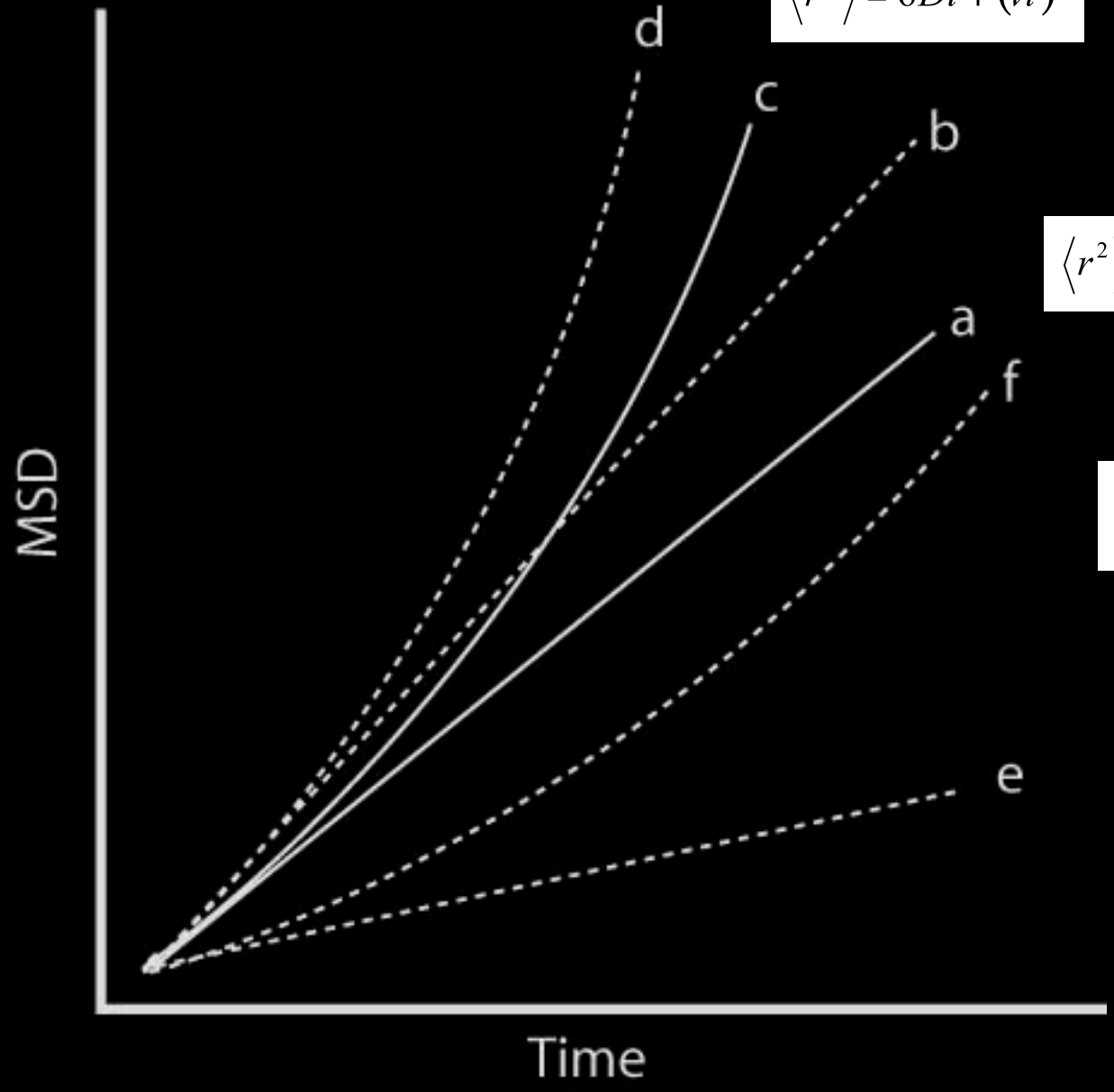
Measurement of Movement: cell tracking



Mean Square Displacement (MSD) = $\langle(\text{Displacement})^2\rangle$

Measurement of Movement: cell tracking

$$\left\langle r^2 \right\rangle = 6Dt + (vt)^2$$



$$\left\langle r^2 \right\rangle = 6Dt$$

$$\left\langle d^2(t) \right\rangle = 6\mu \left\{ t - P\left[1 - e^{-t/P}\right] \right\}$$

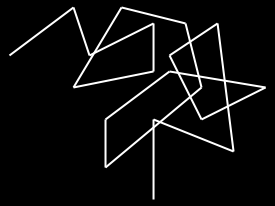
Fuerth's Formula

Measurement of Movement: cell tracking

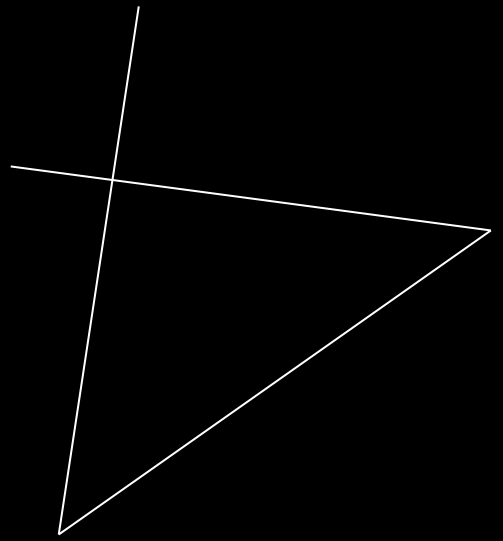
Fuerth's formula: Persistence Time

$$\left\langle d^2(t) \right\rangle = 6\mu \left\{ t - P\left[1 - e^{-t/P}\right] \right\}$$

"characteristic amount of time between directional changes in cell movement" [*1]

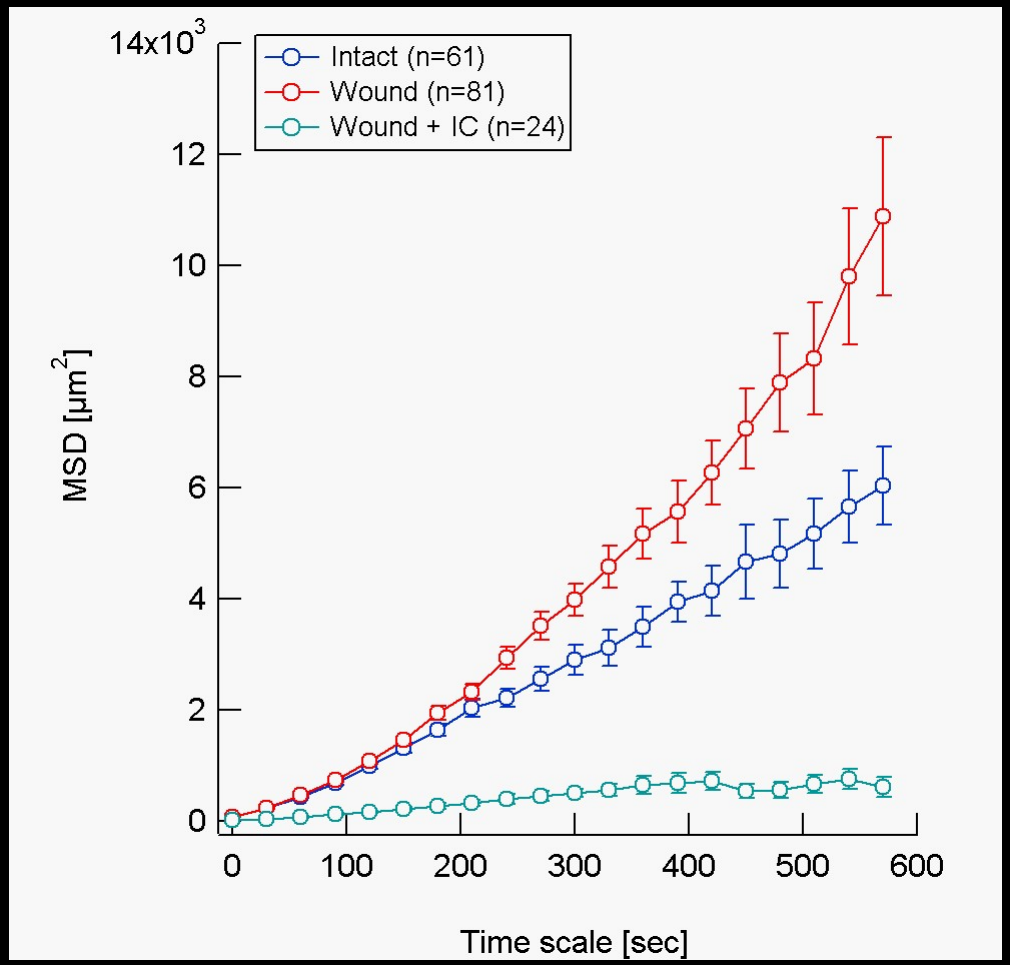


Short Persistence Time



Long Persistence Time

1. Bergman & Zygourakis (1999) Biomaterials
2. Selmeczi et. al. (2005) Biophys. J.
3. Fuerth (1920) Z. Physik. 2:244-256



μ: Random Migration Coefficient
P: Persistence Time

← Random Walk + Directed Motion

μ = 291 ± 23.4 µm^2/min
P = 282 ± 40 sec

← Random Walk

μ = 114 ± 1.8 µm^2/min
P = 76 ± 6 sec

	Average Velocity [µm/min]	Persistence
Intact (61 tracks)	14.1 ± 10.1	0.48 ± 0.21
Wounded (81 tracks)	14.3 ± 8.7	0.67 ± 0.20
Wounded +Inhibitor (24 tracks)	5.9 ± 5.4	0.40 ± 0.22

μ = 17.4 ± 1.8 µm^2/min
P = 44 ± 28 sec

Lower Cell Activity and Suppressed Chemotaxis (PI3 kinase inhibitor)

Grabher et al. (2007) J Leukoc Biol. 81(1):263-71

Image Sequences: Overview

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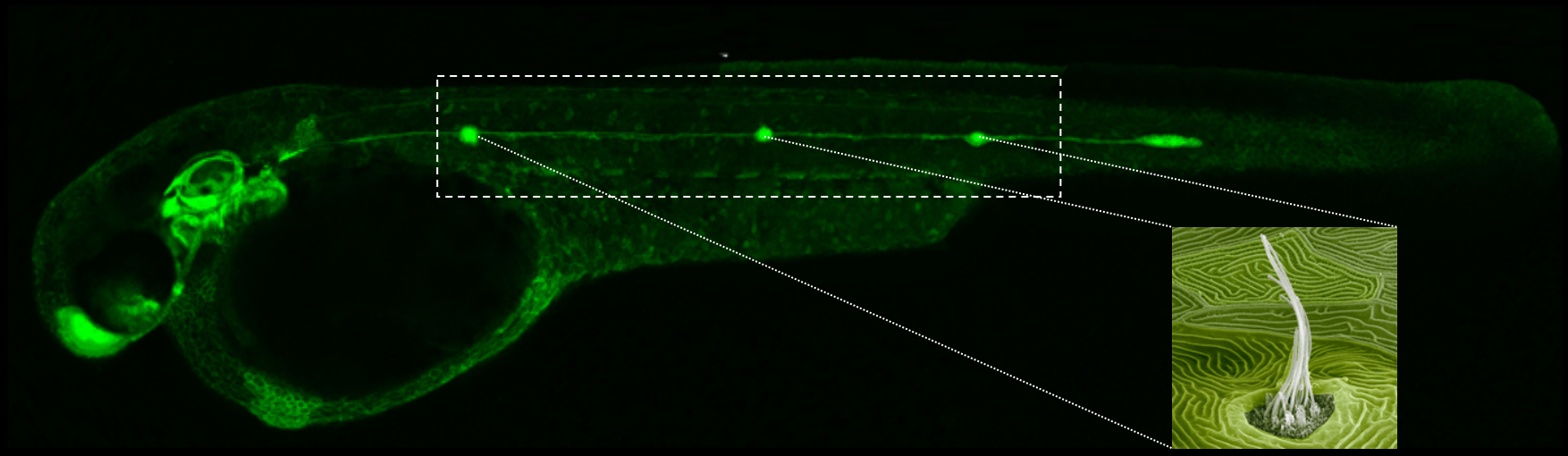
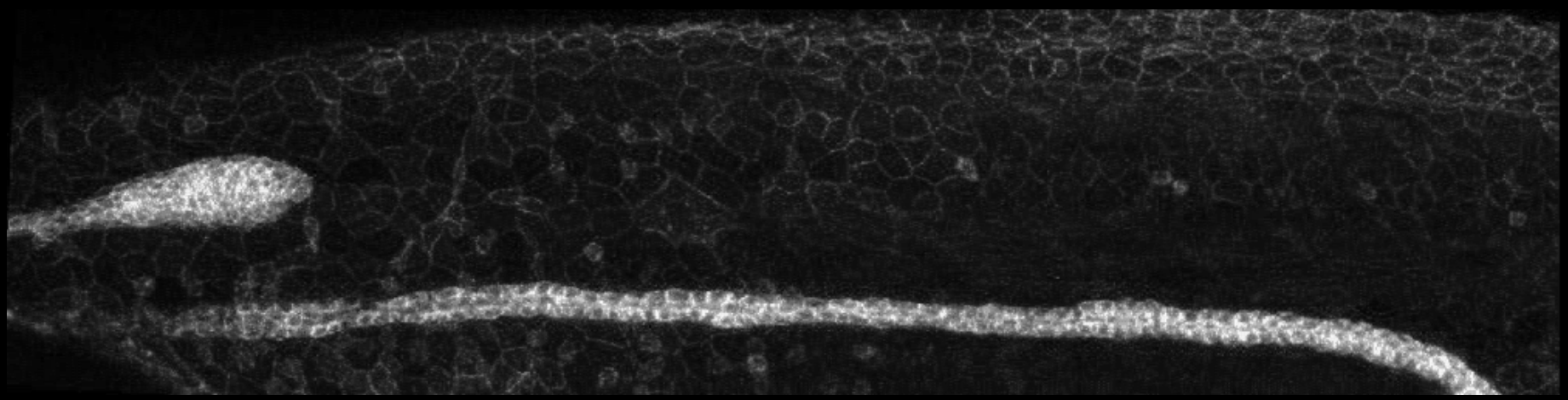
The zebrafish lateral line:
A model system for in vivo collective cell migration

Darren Gilmour lab@EMBL

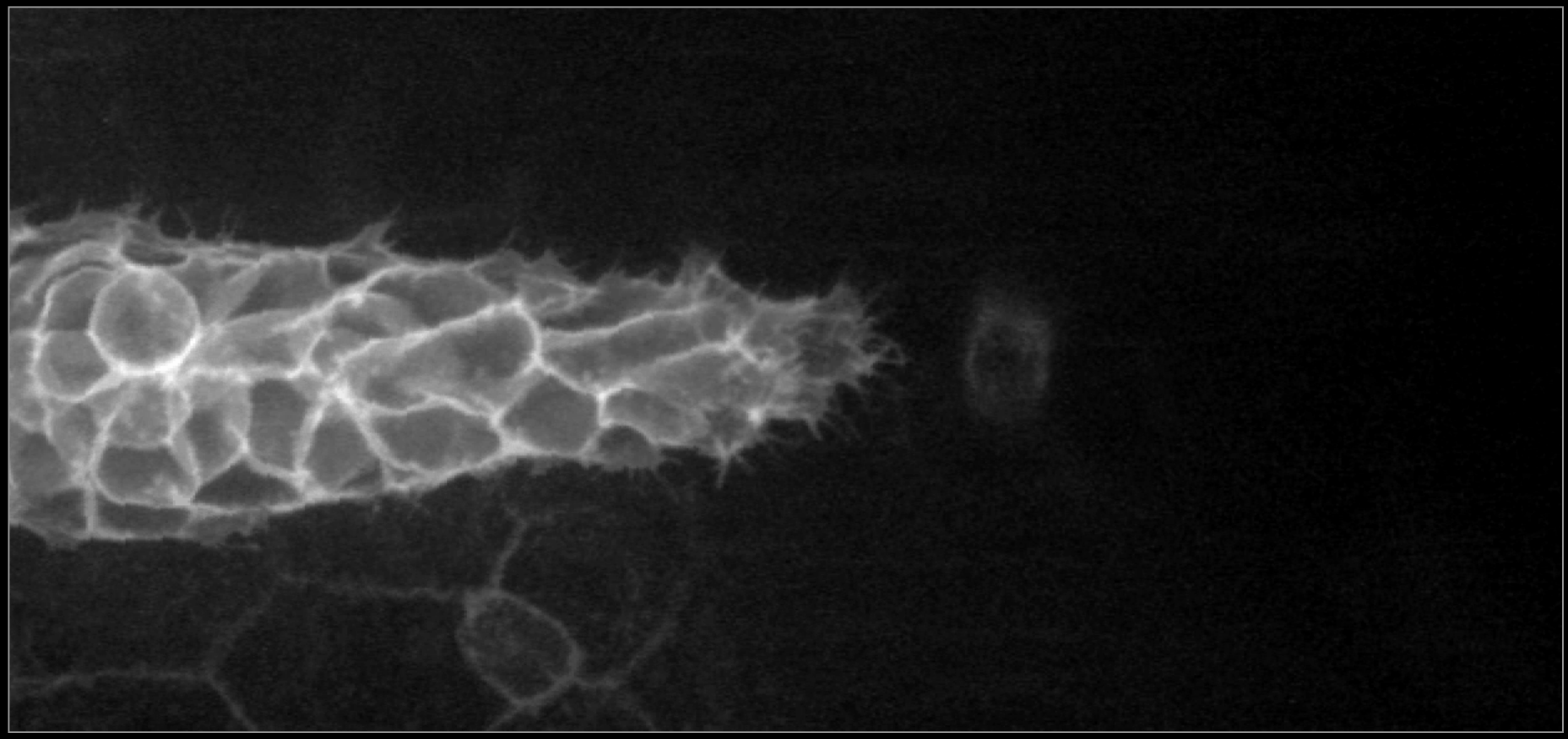




- The cells migrate efficiently as a highly coordinated unit along a predefined path.
- The forming organs repeatedly appear *de novo* within the moving tissue.
- Cells fated to become organs decelerate at the trailing edge and arrest migration.

How does this group move in a coordinated way?

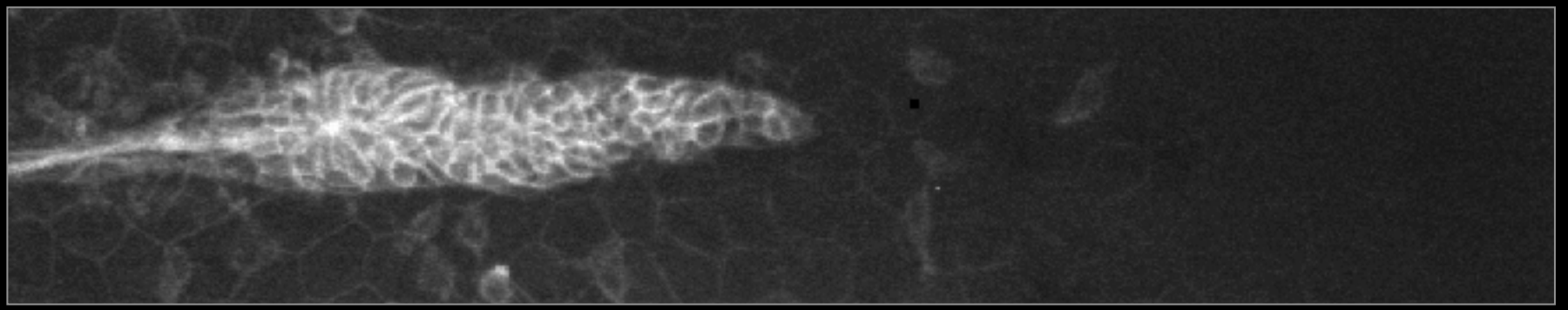
Darren Gilmour lab@EMBL

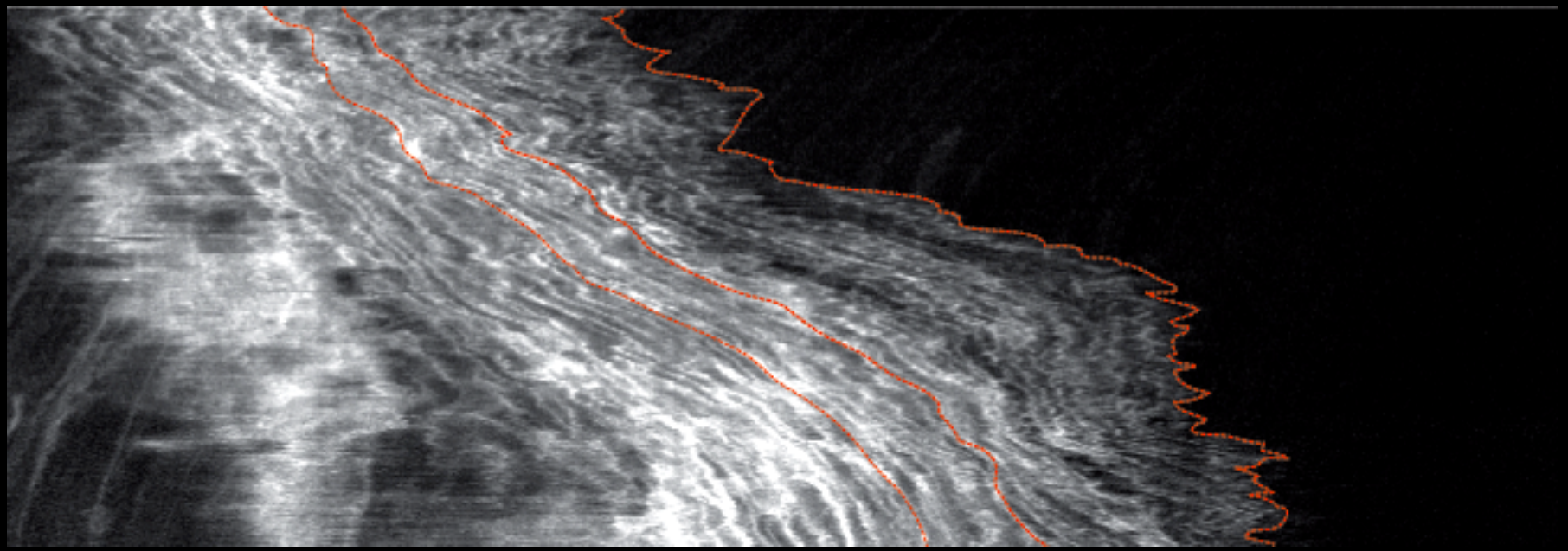


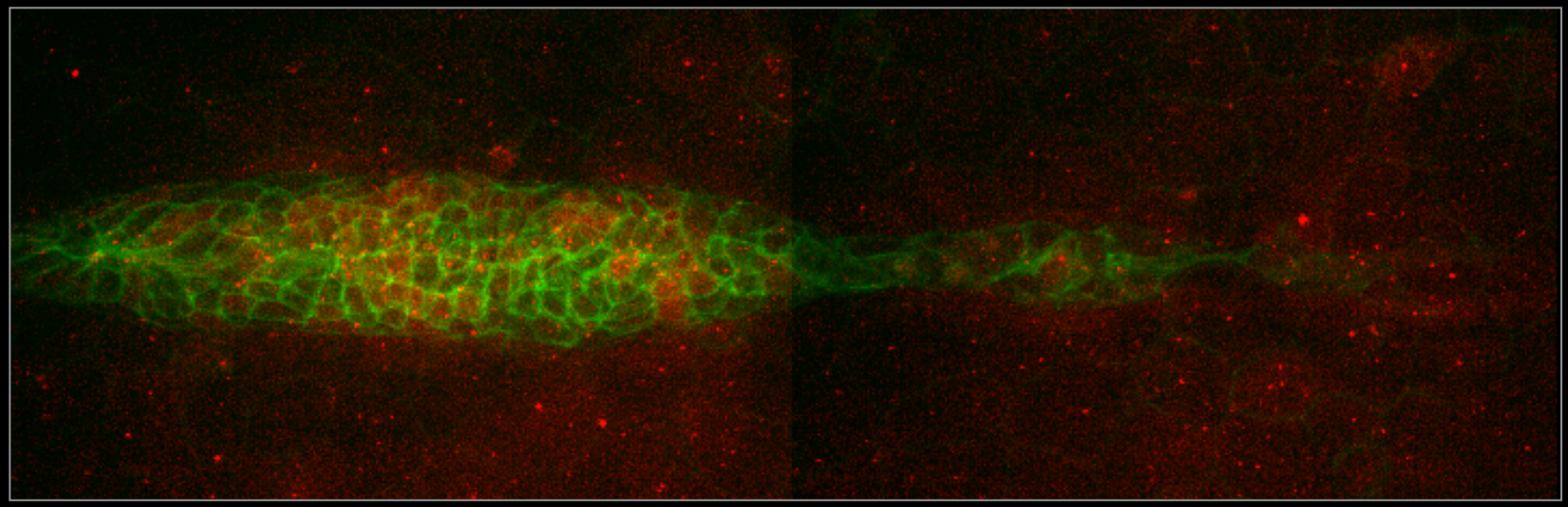
Many cells of the pLLP appear to respond directly to extrinsic guidance cues.

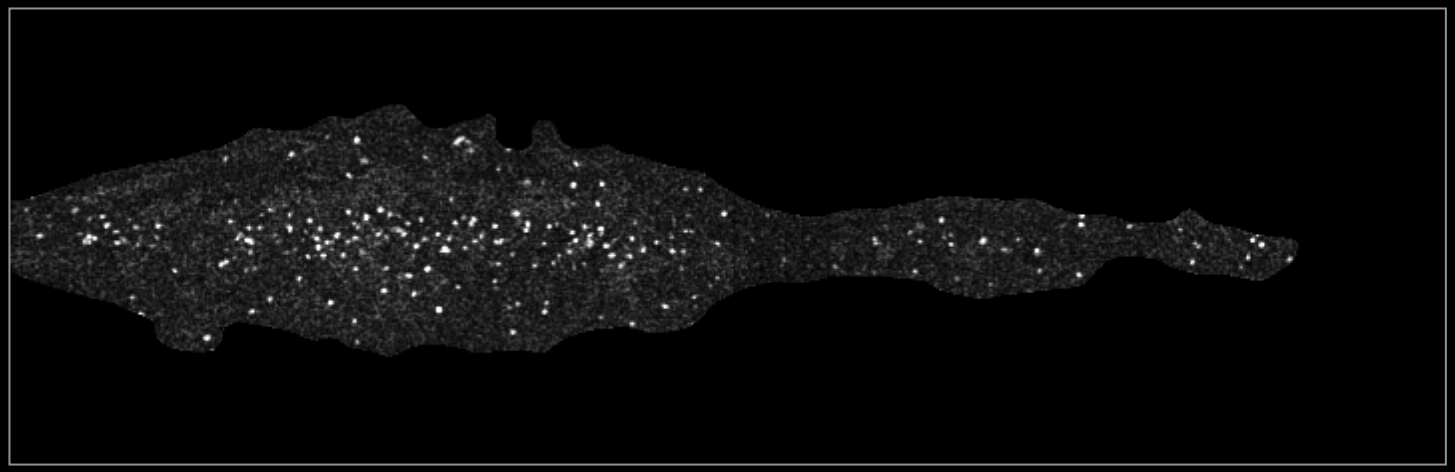
Loss of FGF signaling *uncouples* the migration of leaders and followers

Darren Gilmour lab@EMBL









Task: Gulcin's Project

quantitative analysis of the formation of hair cell "garlic" aggregates

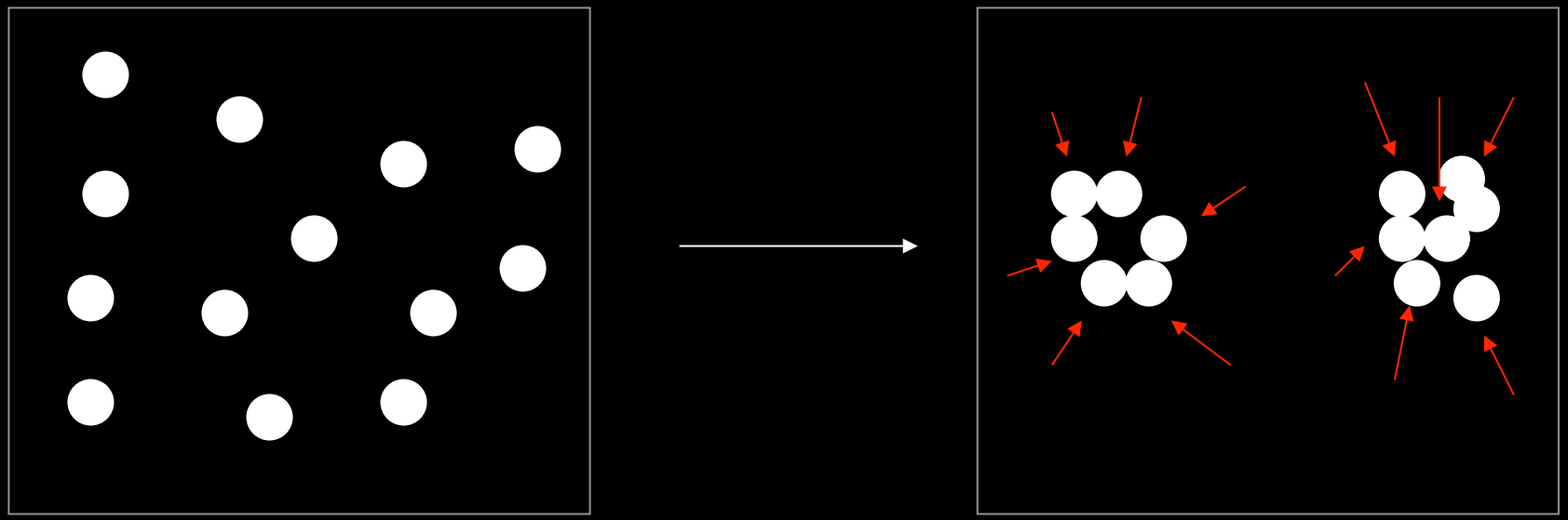
Tool: Centrin labeling and image analysis

FGF inhibitor, two hours after the wash-out FGF inhibitor

Analysis of Centrin Distribution Dynamics

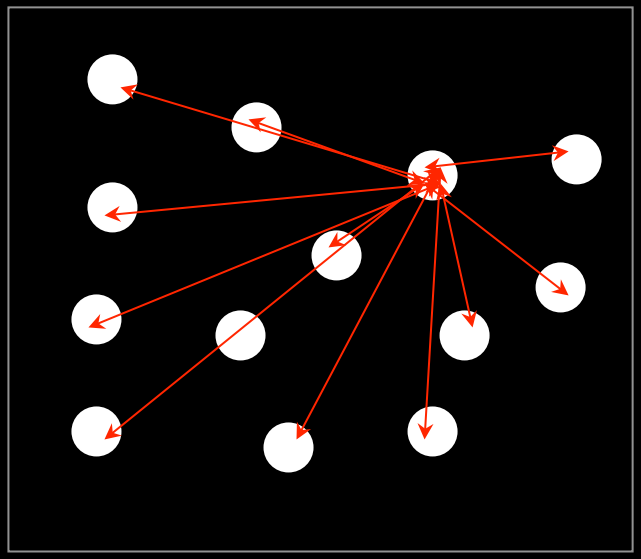
General Parameters - Velocity
- Directionality

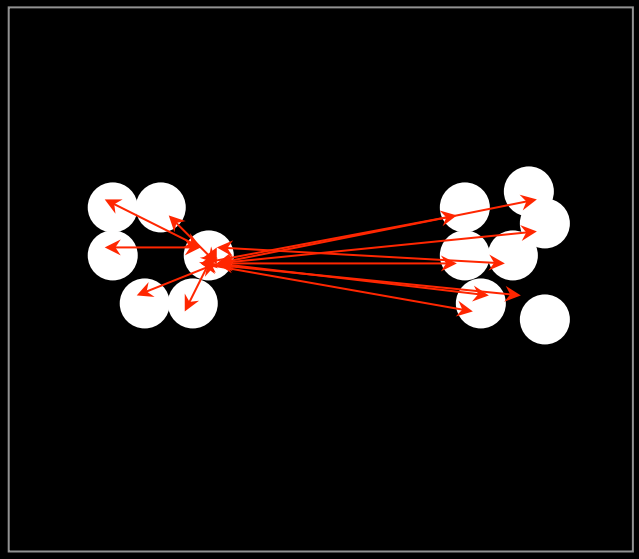
Distribution Function - how close are the centrins?
→ **Sparse to Dense**

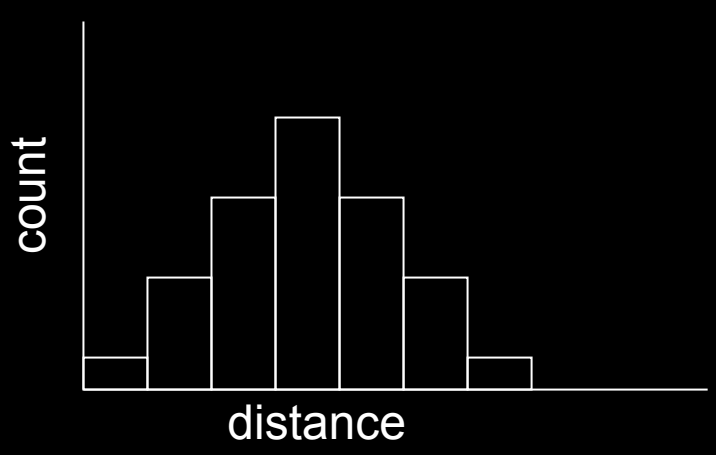


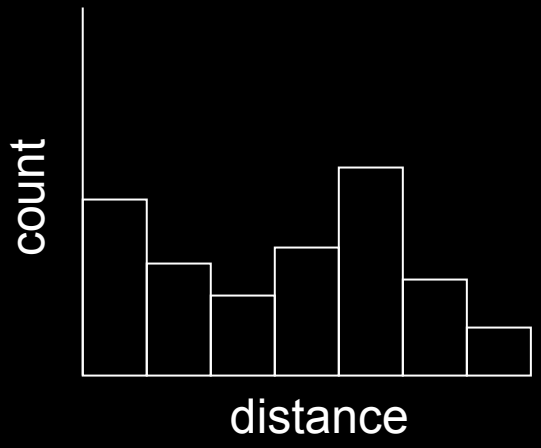
Analysis of Centrin Distribution Dynamics 2. Centrin – Centrin distance

Distribution Function



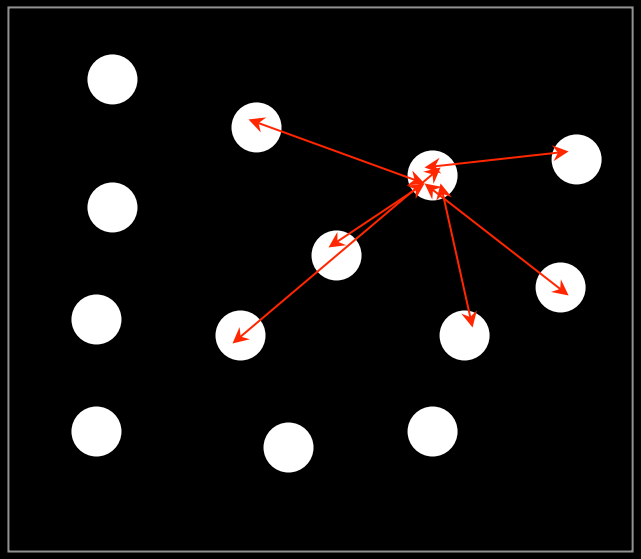


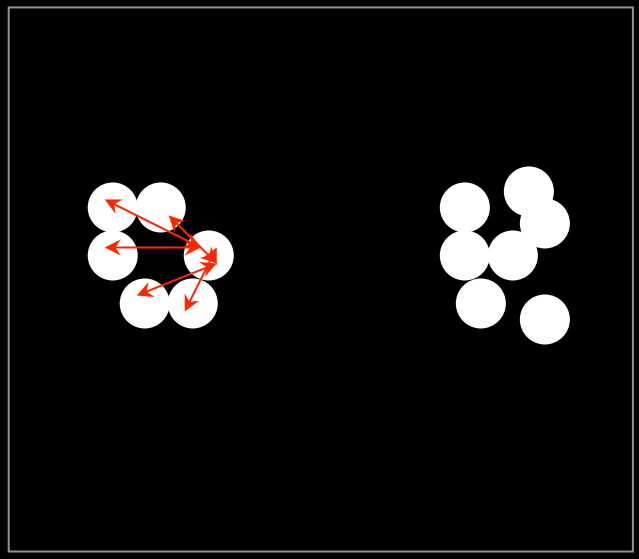




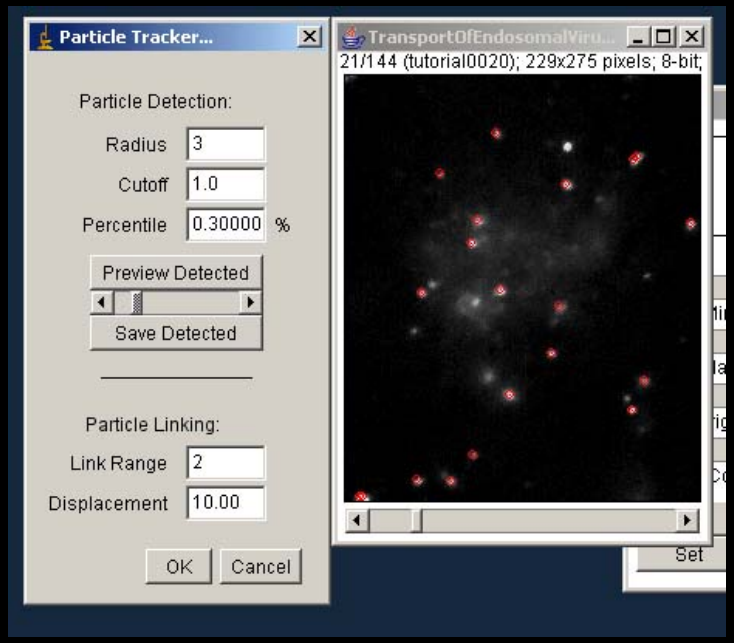
Analysis of Centrin Distribution Dynamics 2. Centrin – Centrin distance

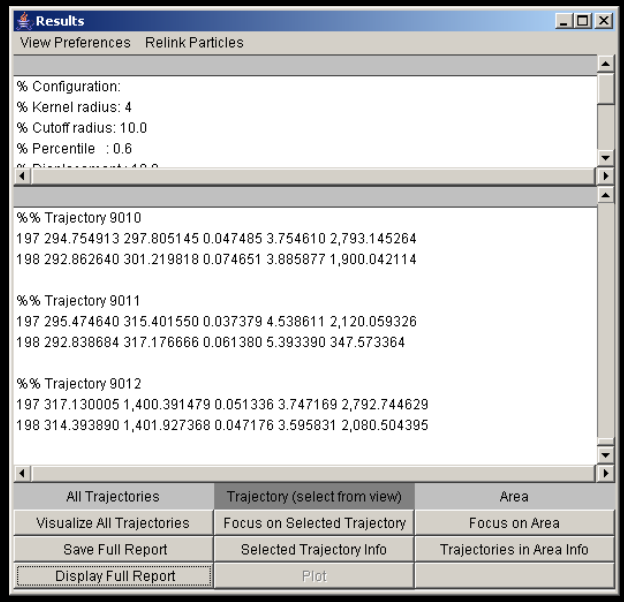
Average distance to nearest 5 centrins



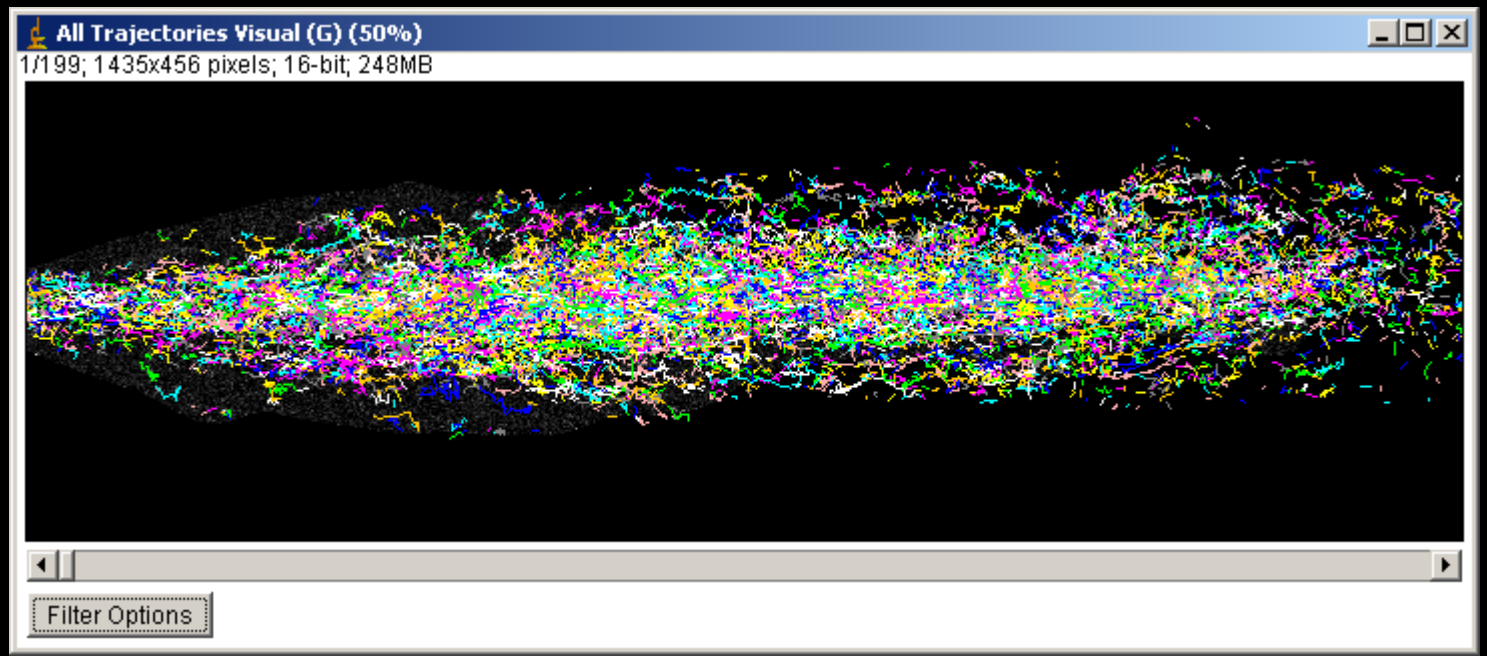


Particle Tracking: using ImageJ Particle Tracker



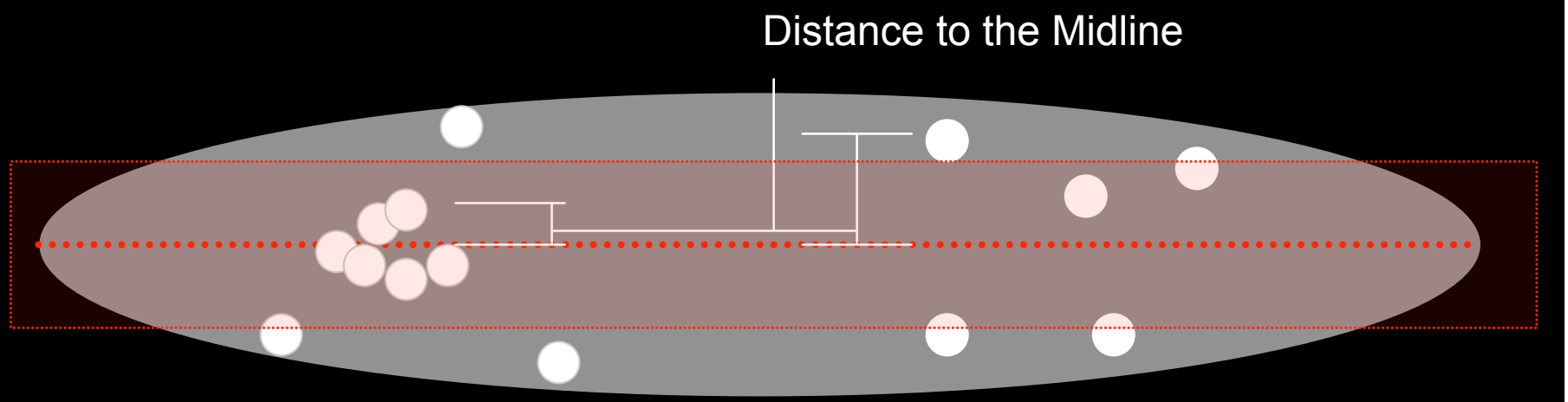


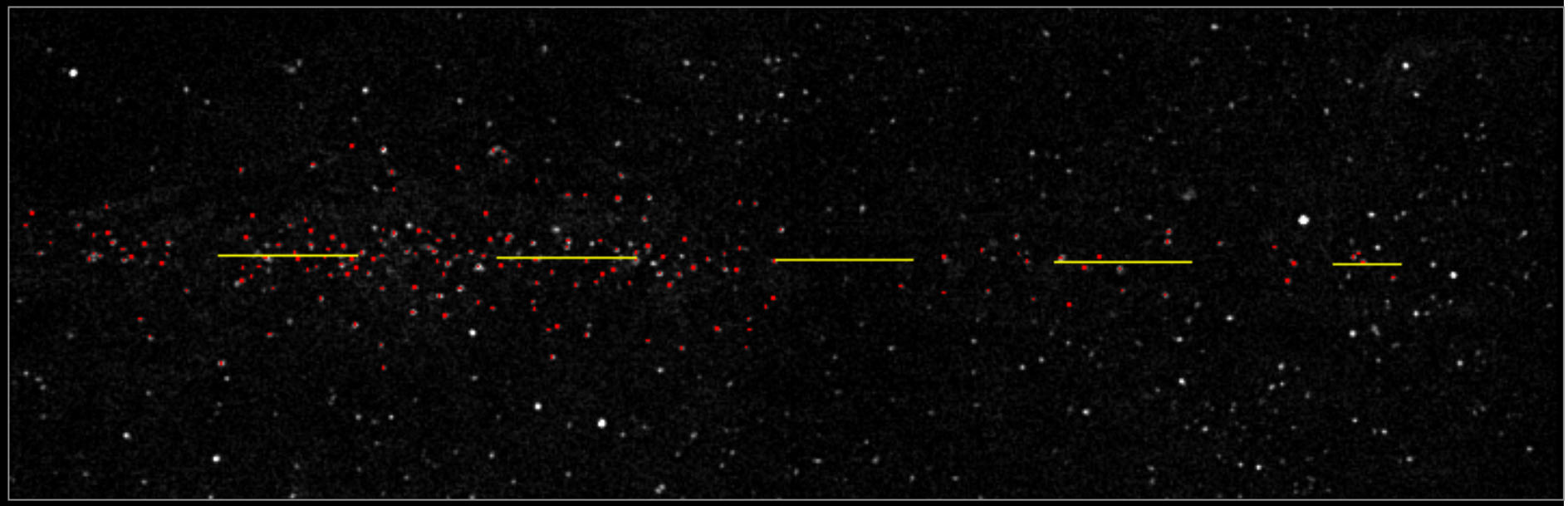
9012 trajectories



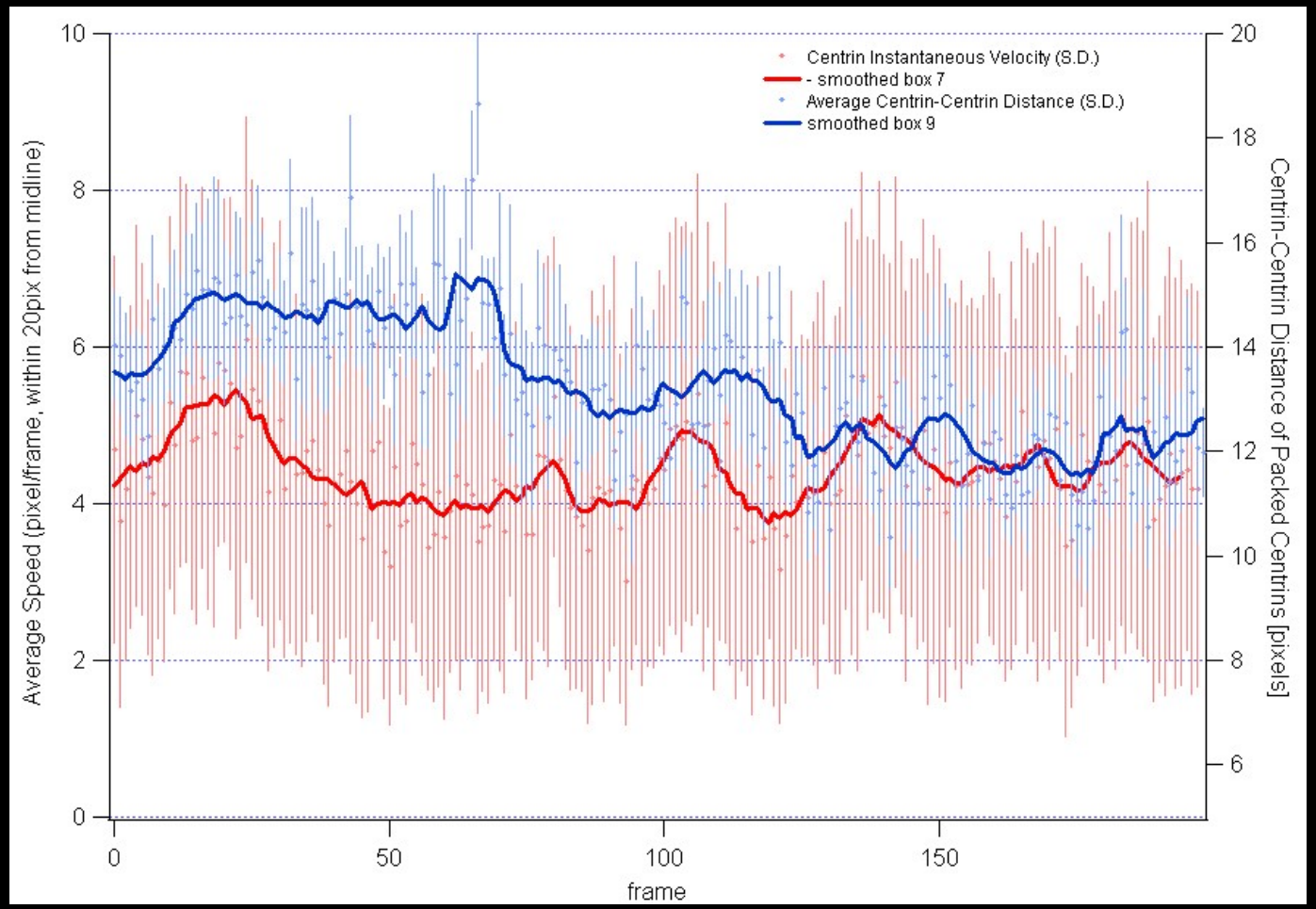
Export data to IgorPro

Analysis of Centrin Distribution Dynamics 1. distance to the “primodium midline”





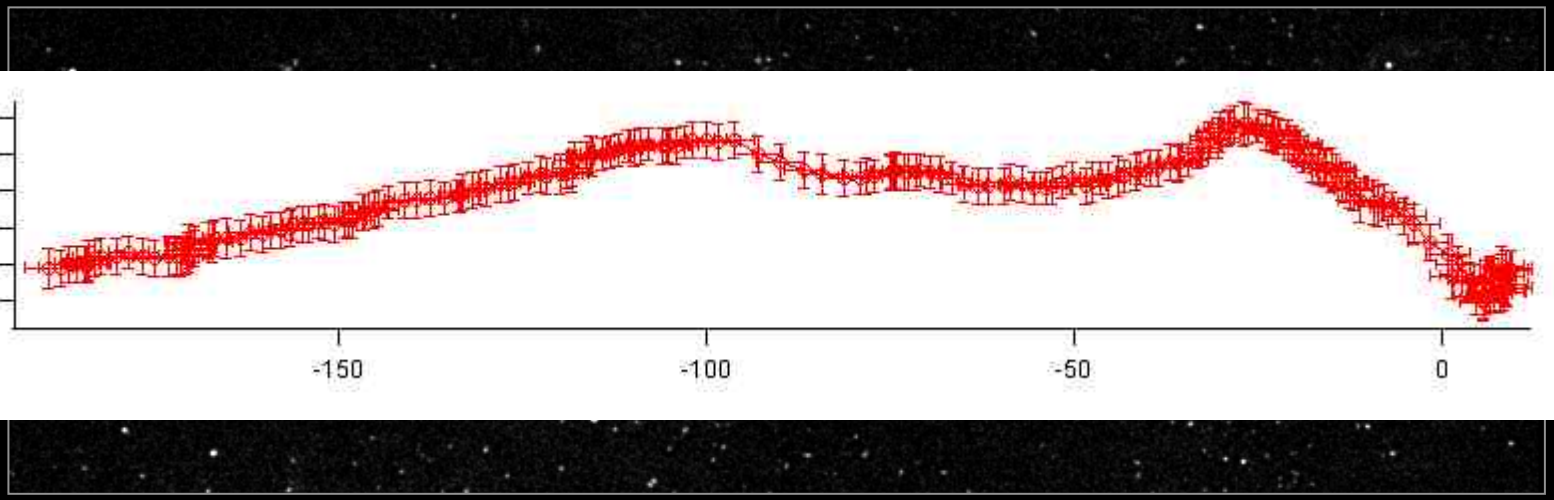
Analysis of Centrin Distribution Dynamics 4. Clustering and velocity

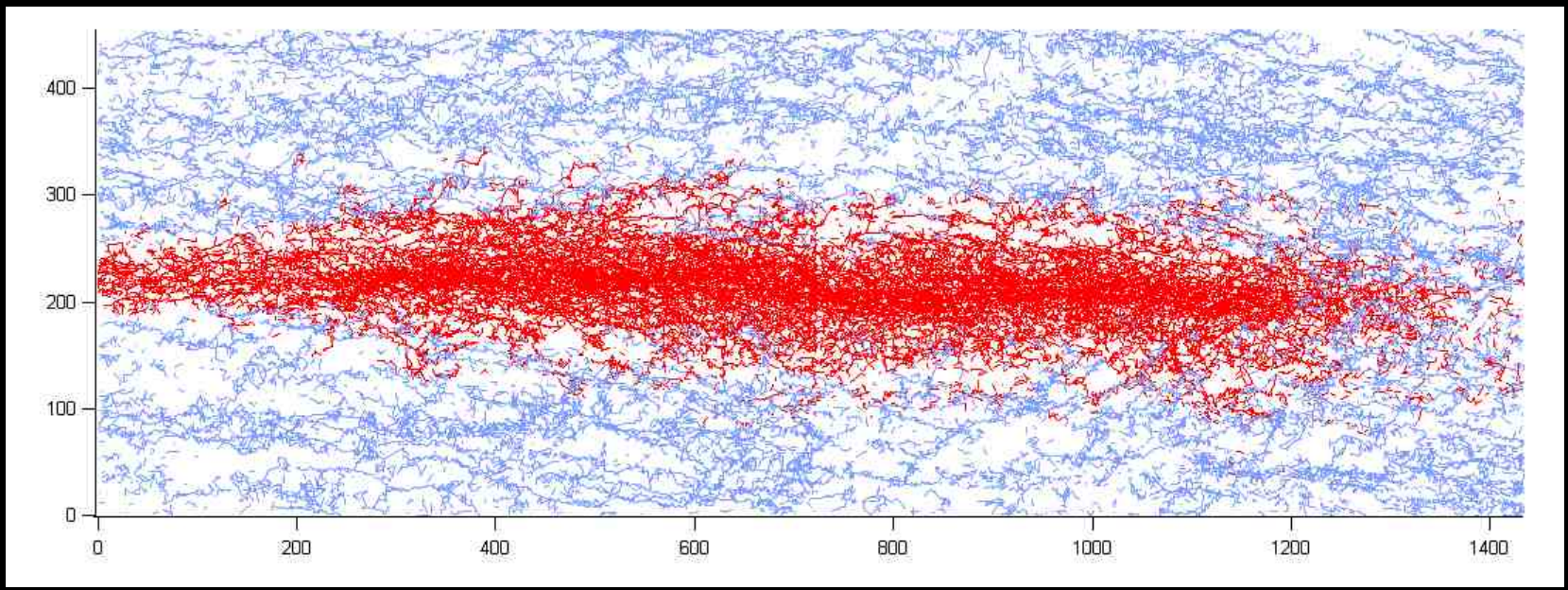


Velocity Decrease → (delay) → Clustering 1
→ cells resume movement → Clustering 2

Particle Tracking: Registration – Correction for the background movement

Background movement





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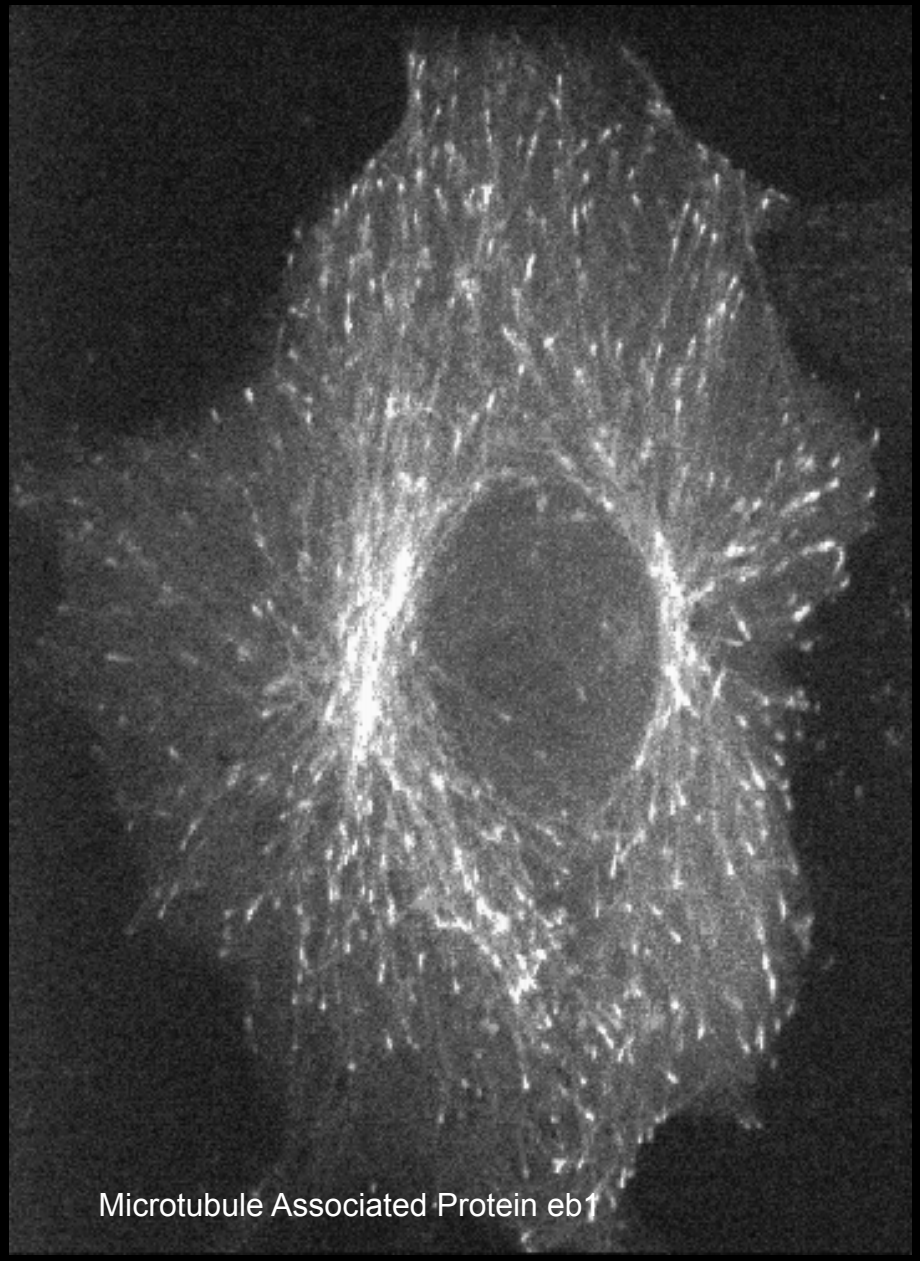
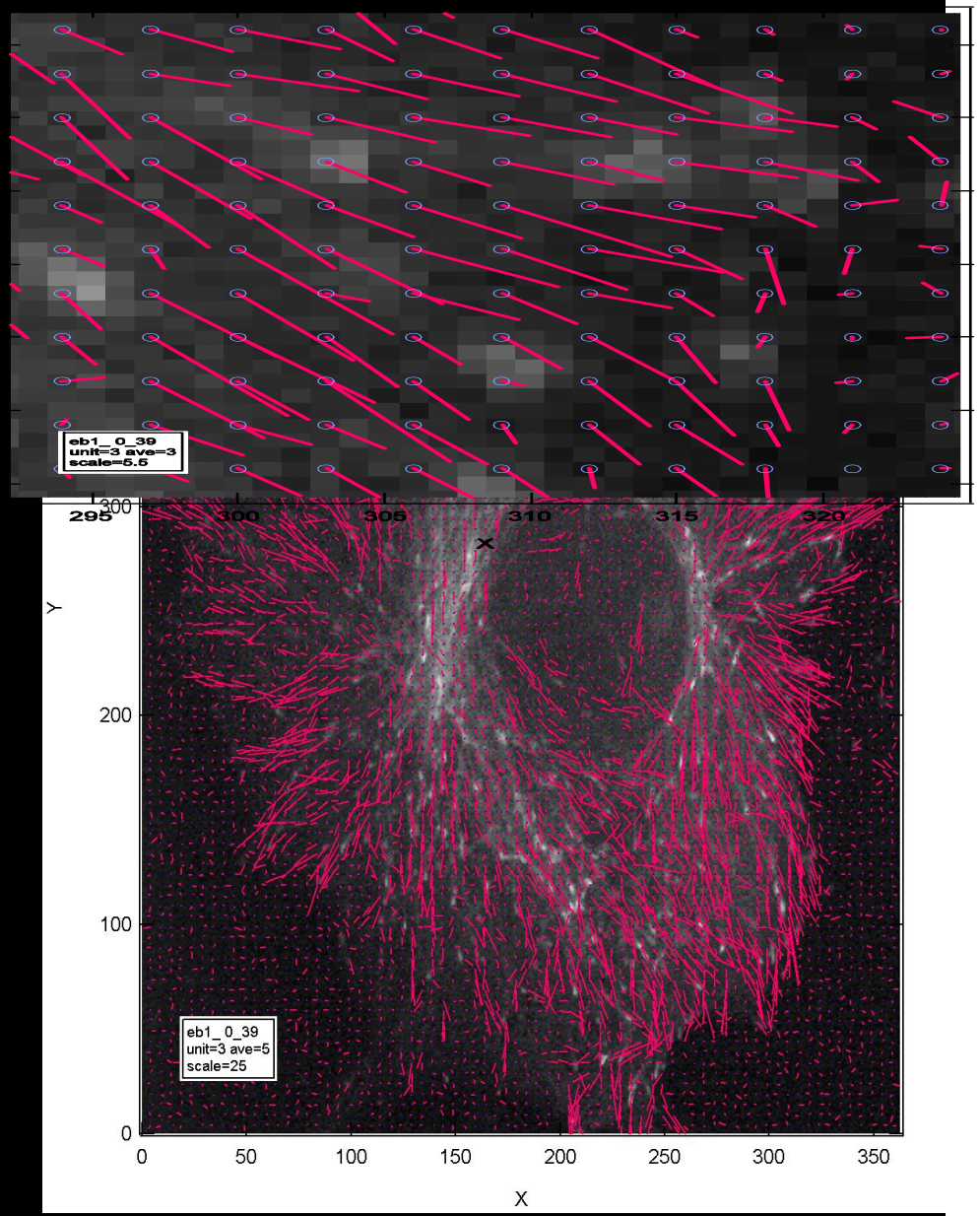
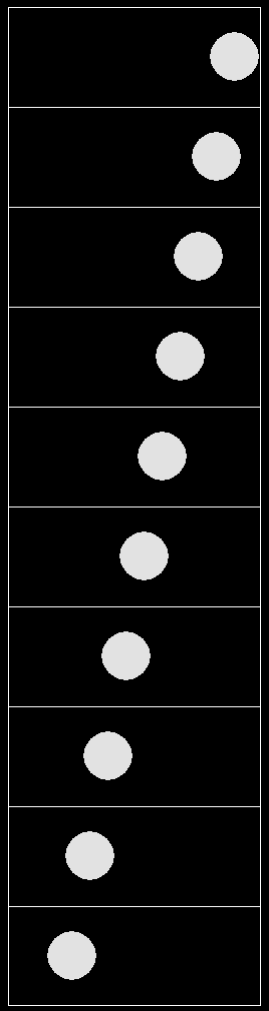
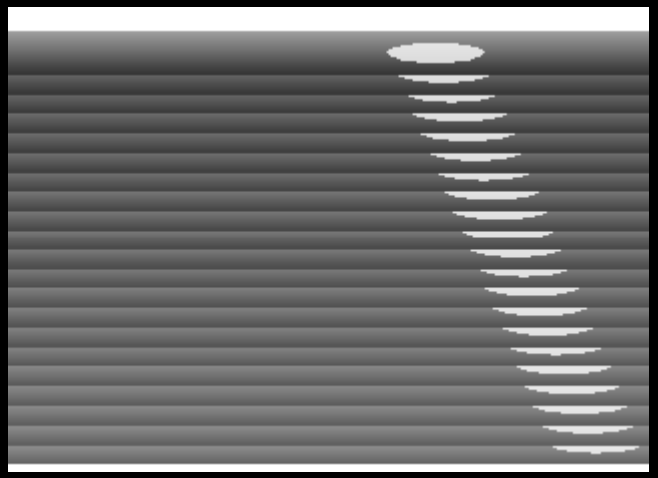
- Optical Flow Estimation

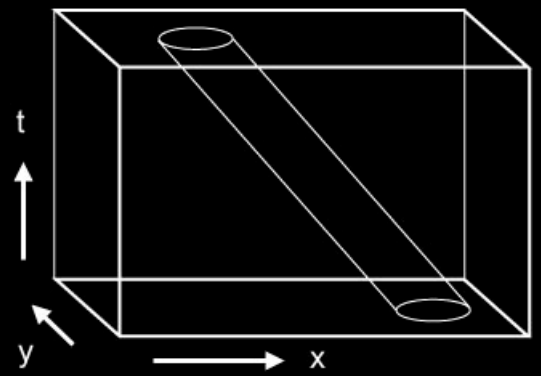
Measurement of Movement Igor Pro, Wavemetrics Optical Flow Estimation

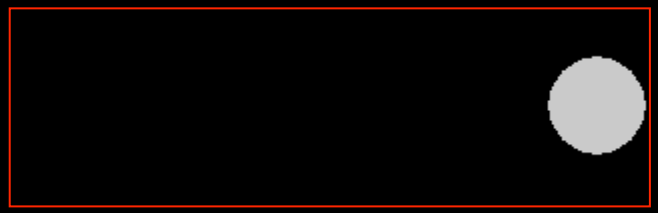


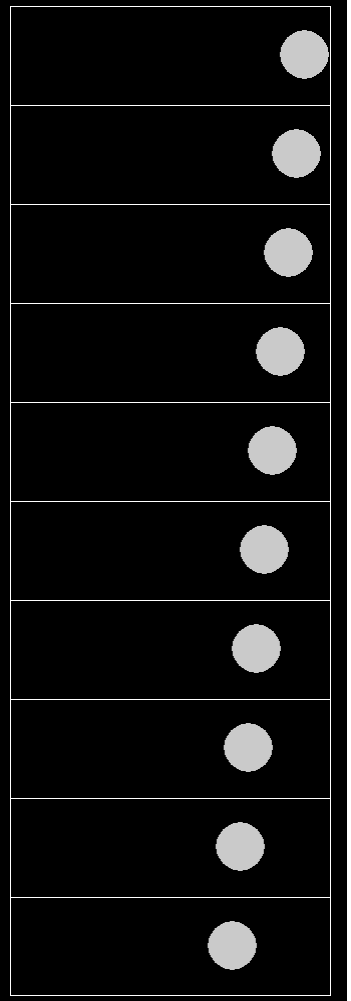


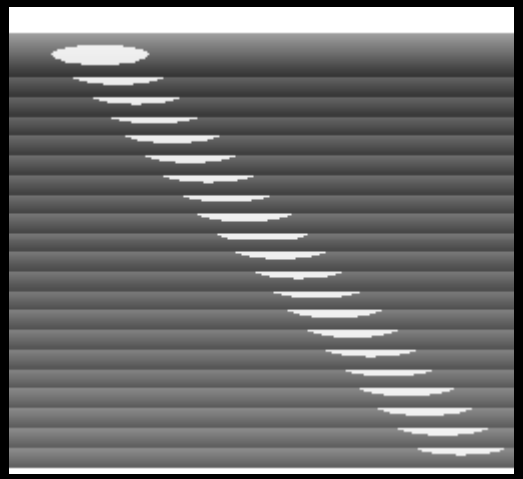


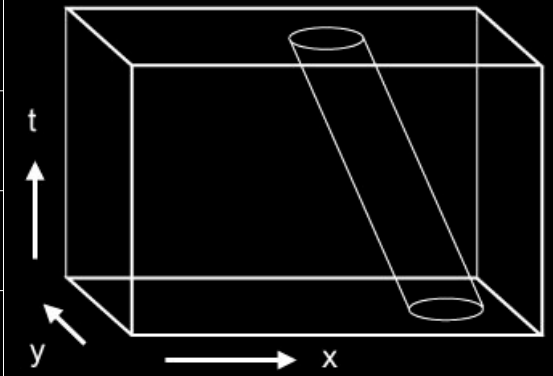



t
y
x



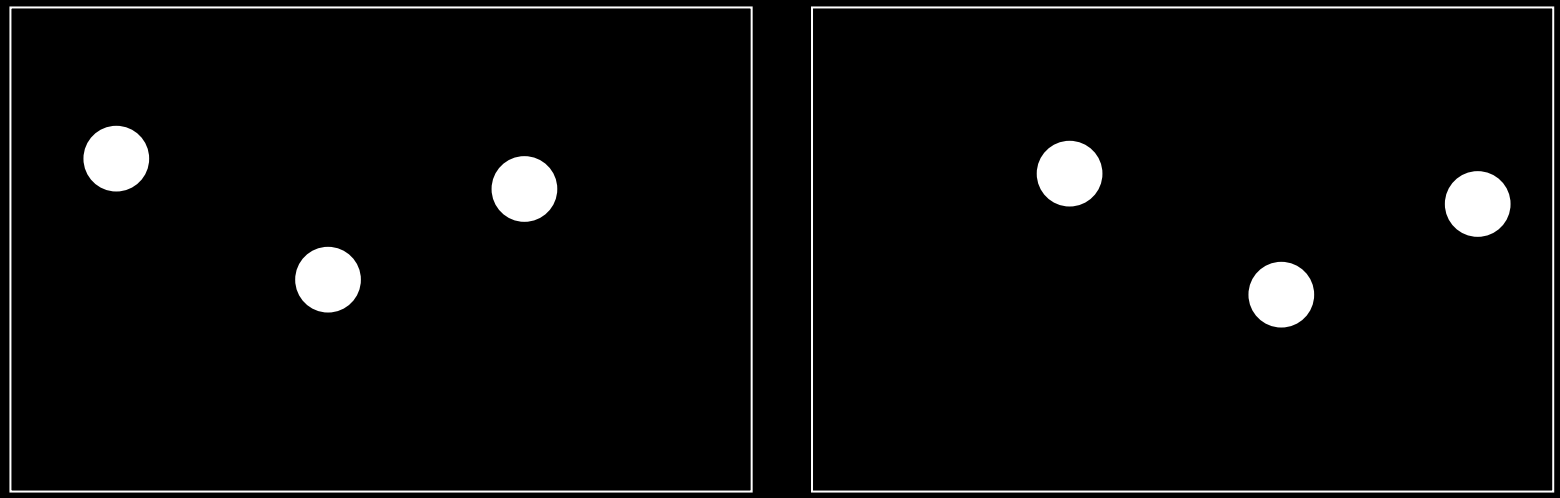





t
y
x

$$\frac{\partial f}{\partial t} + \frac{\partial f}{\partial x} v_x + \frac{\partial f}{\partial y} v_y = 0$$

Optical Flow Equation



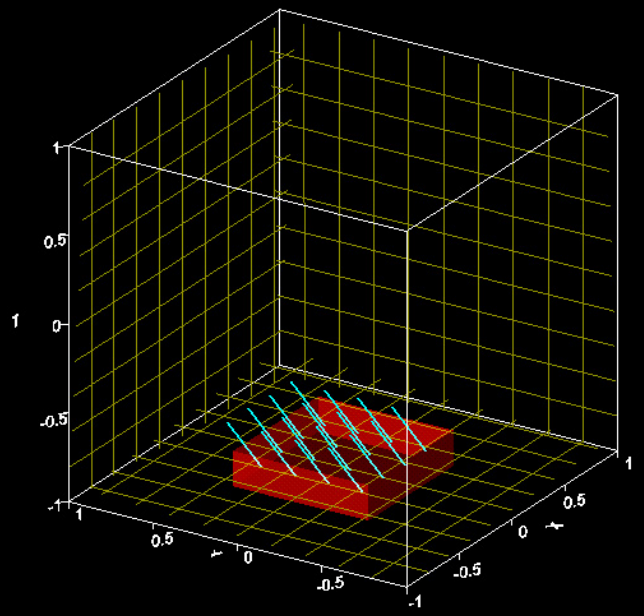
t **t+1**

Under-determined → Need another constraint…

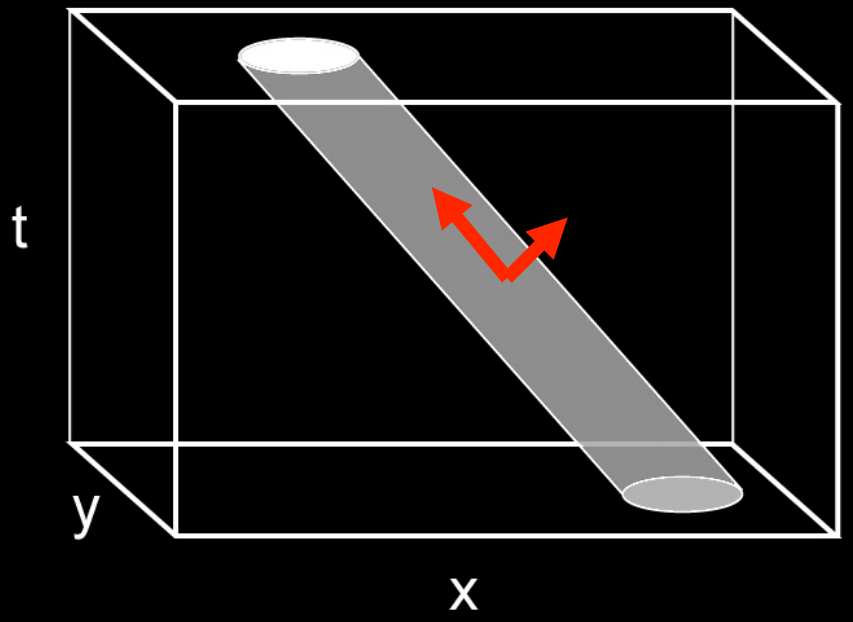
Second Constraint - I

Spatial Local Smoothness

$$\frac{\partial \mathbf{v}}{\partial x} = \frac{\partial \mathbf{v}}{\partial y} = 0$$



Structure Tensor Approach



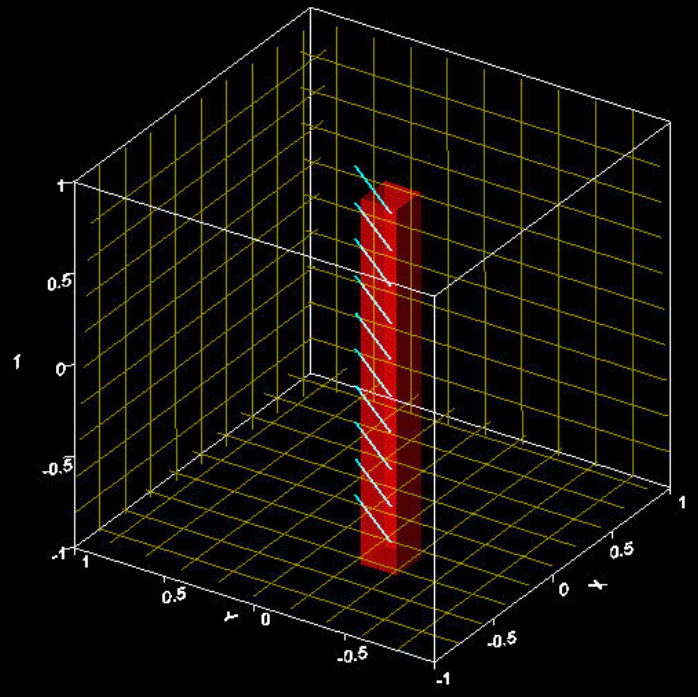
$$J = \begin{bmatrix} \frac{\partial I}{\partial x}\cdot\frac{\partial I}{\partial x} & \frac{\partial I}{\partial x}\cdot\frac{\partial I}{\partial y} & \frac{\partial I}{\partial x}\cdot\frac{\partial I}{\partial t} \\ \frac{\partial I}{\partial x}\cdot\frac{\partial I}{\partial y} & \frac{\partial I}{\partial y}\cdot\frac{\partial I}{\partial y} & \frac{\partial I}{\partial y}\cdot\frac{\partial I}{\partial t} \\ \frac{\partial I}{\partial x}\cdot\frac{\partial I}{\partial t} & \frac{\partial I}{\partial y}\cdot\frac{\partial I}{\partial t} & \frac{\partial I}{\partial t}\cdot\frac{\partial I}{\partial t} \end{bmatrix}$$

eigenvector

Second Constraint - II

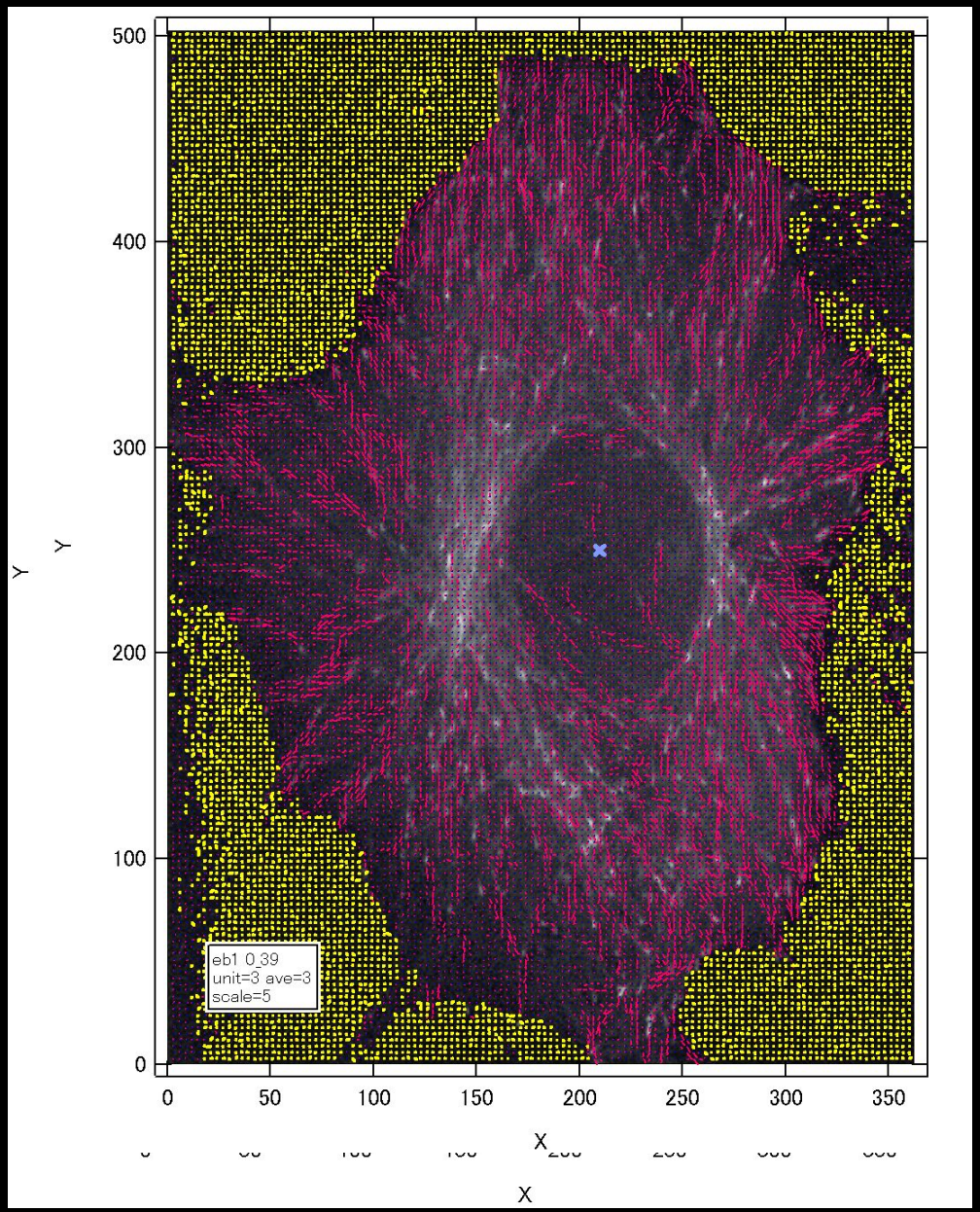
Temporal Local Smoothness

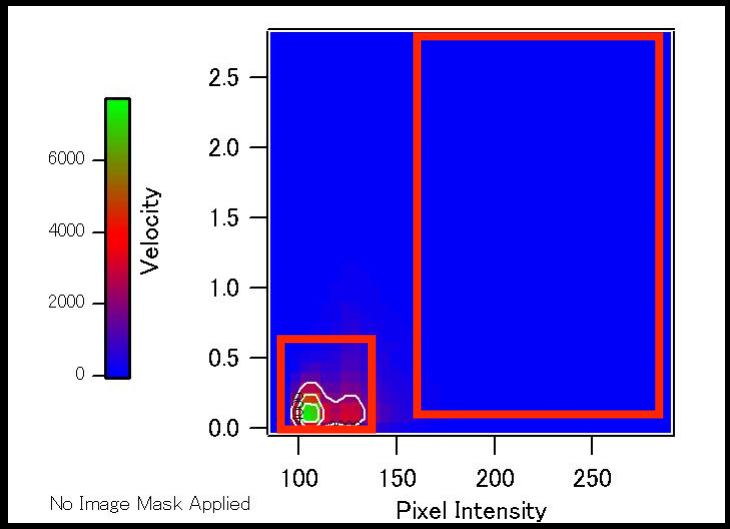
$$\frac{\partial v}{\partial t} = 0$$

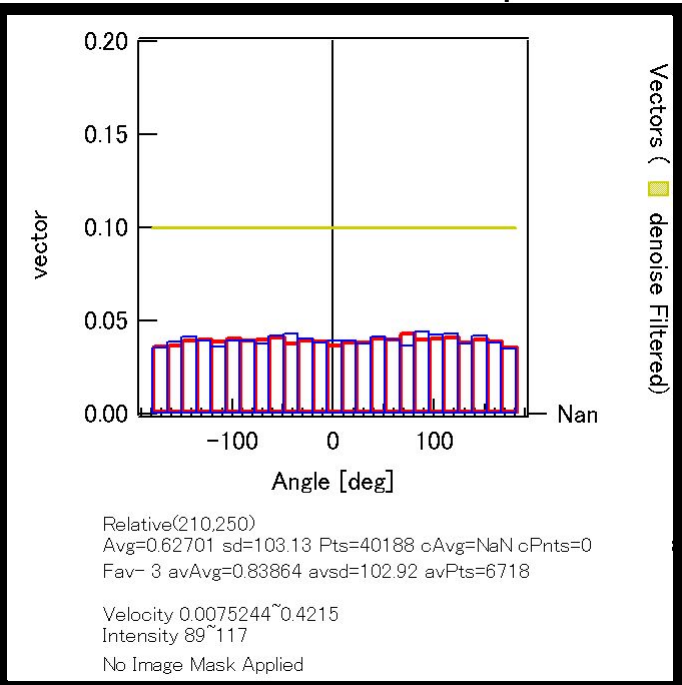


Measurement of Movement

Filtering of Vector Field by Speed & Pixel Intensity

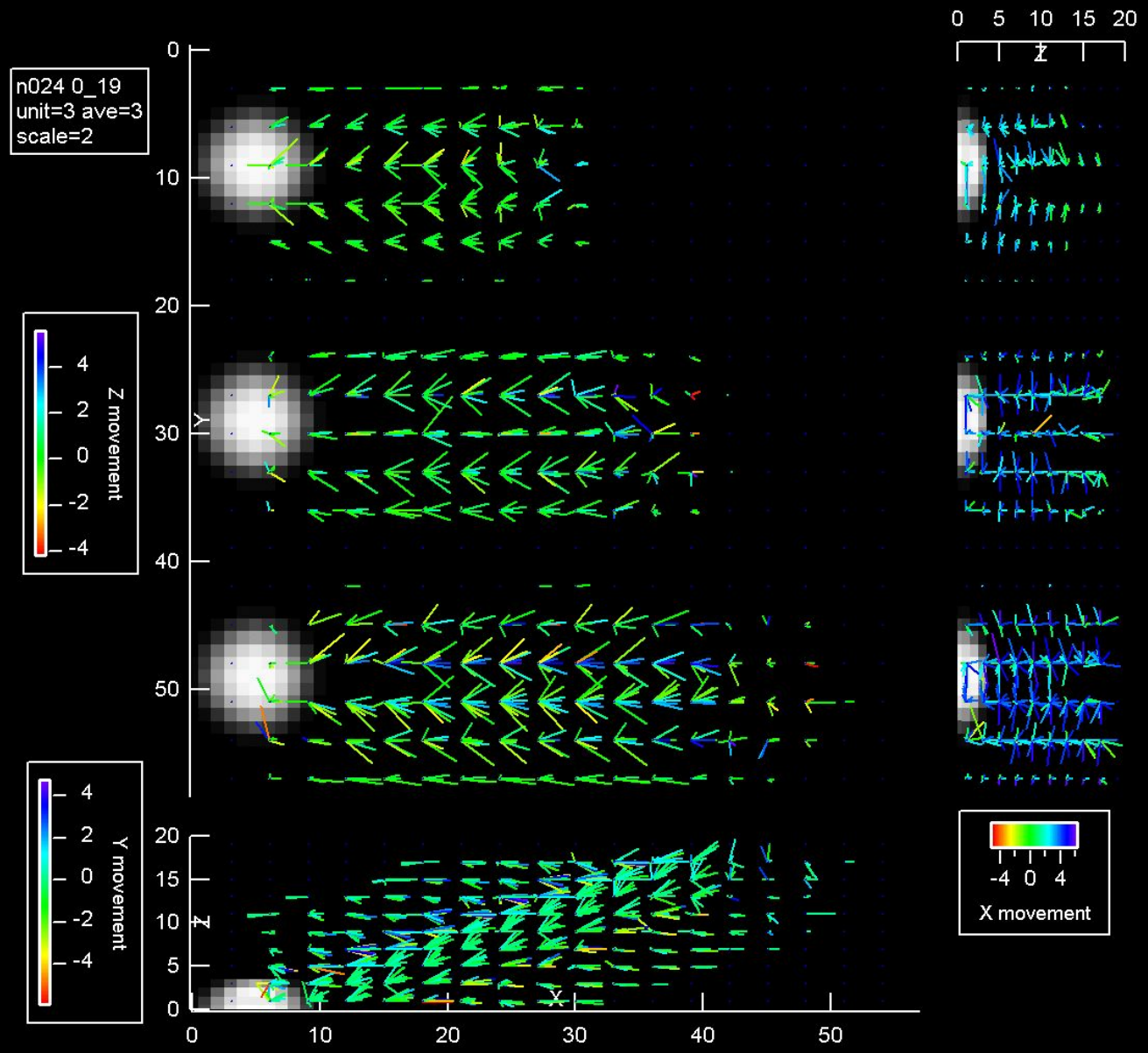






Measurement of Movement: Optical Flow Estimation

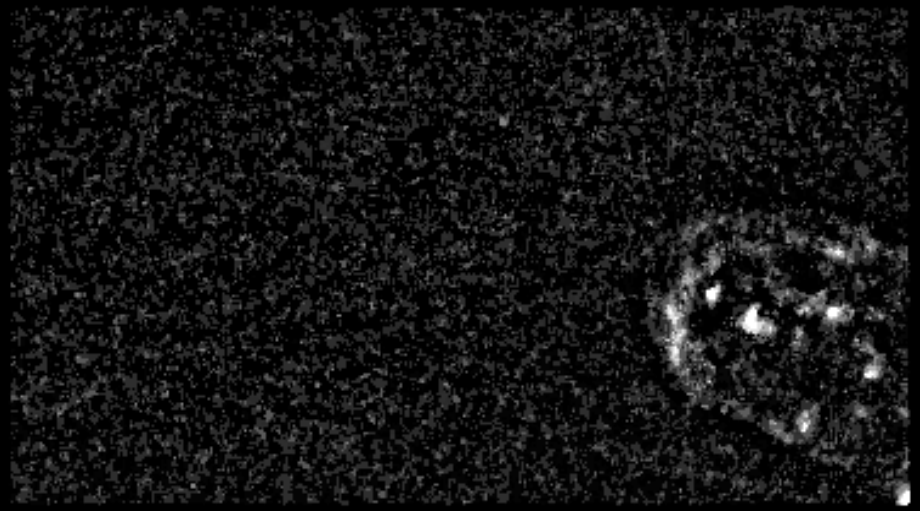
virtual 3D beads



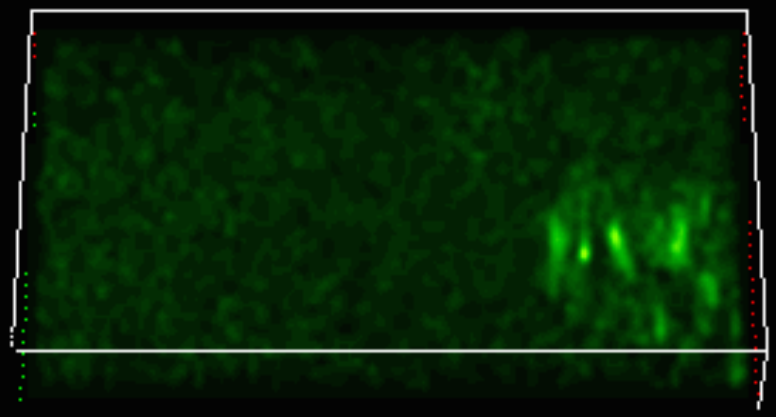
Measurement of Movement: Optical Flow Estimation 3D

3-D *Dictyostelium* Slug Cell Movement

Z-projection

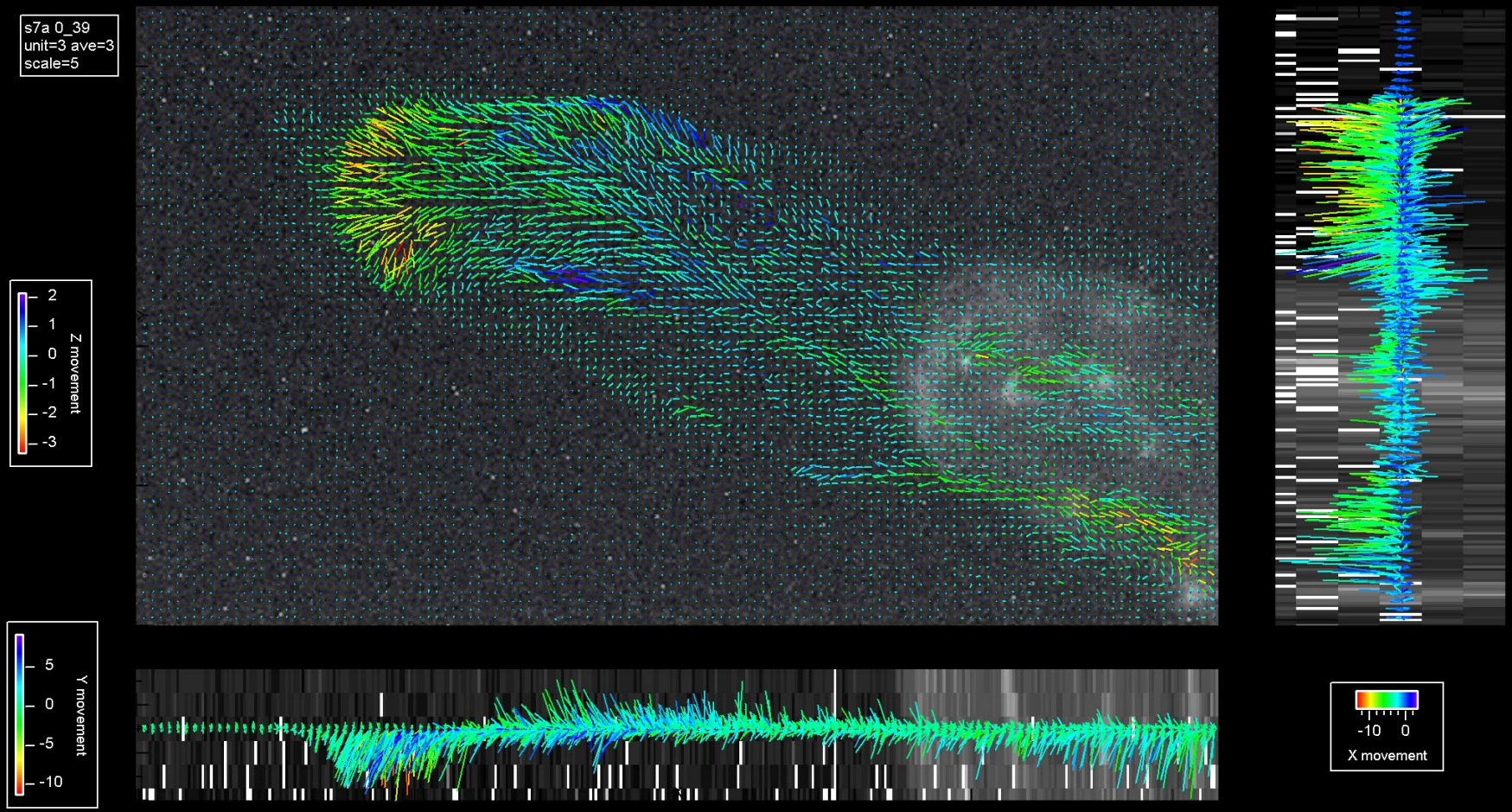


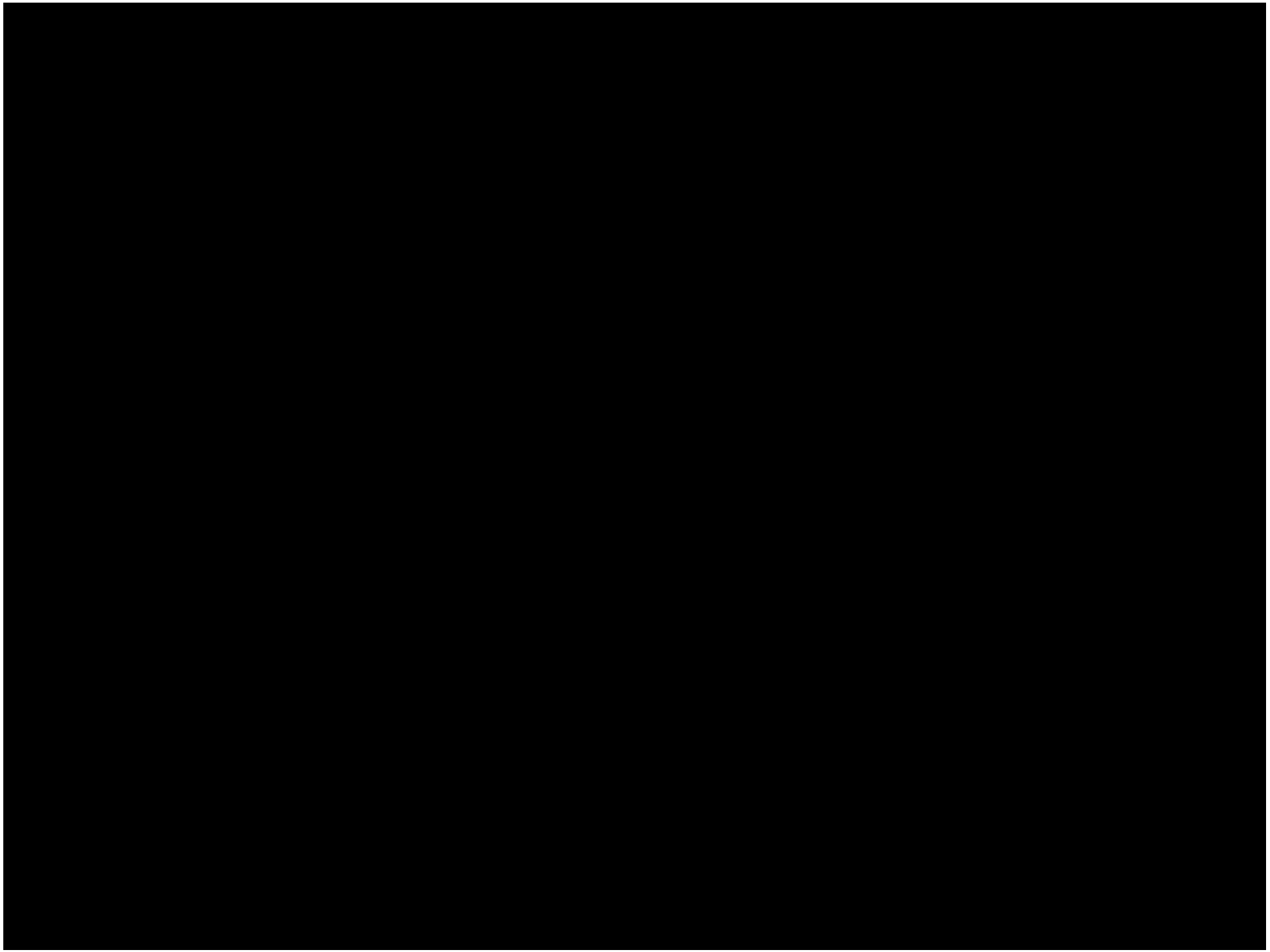
Max.point-projection
3D rendering



Measurement of Movement: Optical Flow Estimation 3D

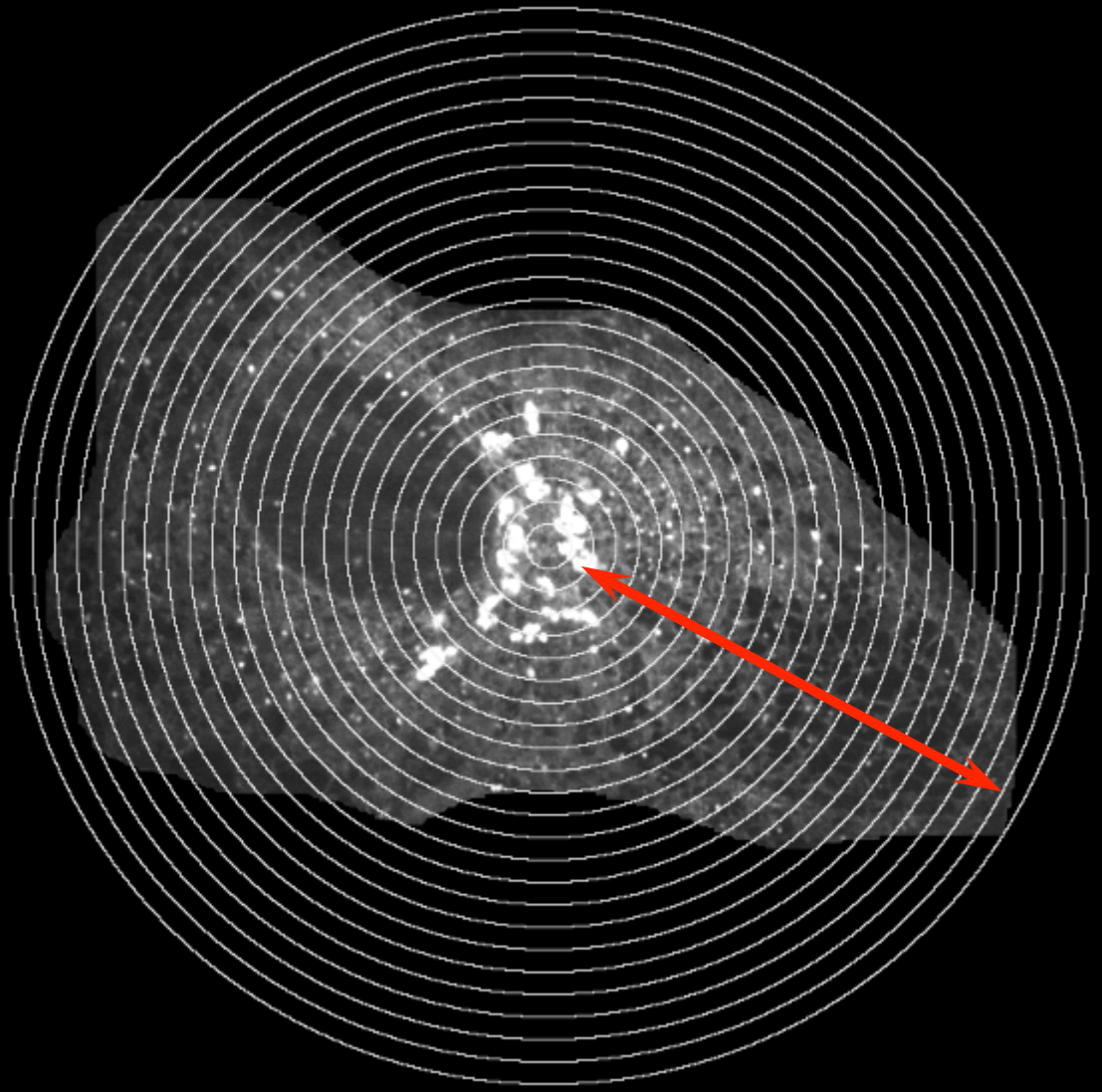
3-D *Dictyostelium* Slug Cell Movement



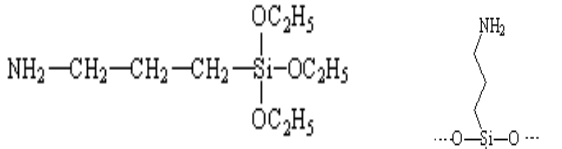


Vesicle Transport

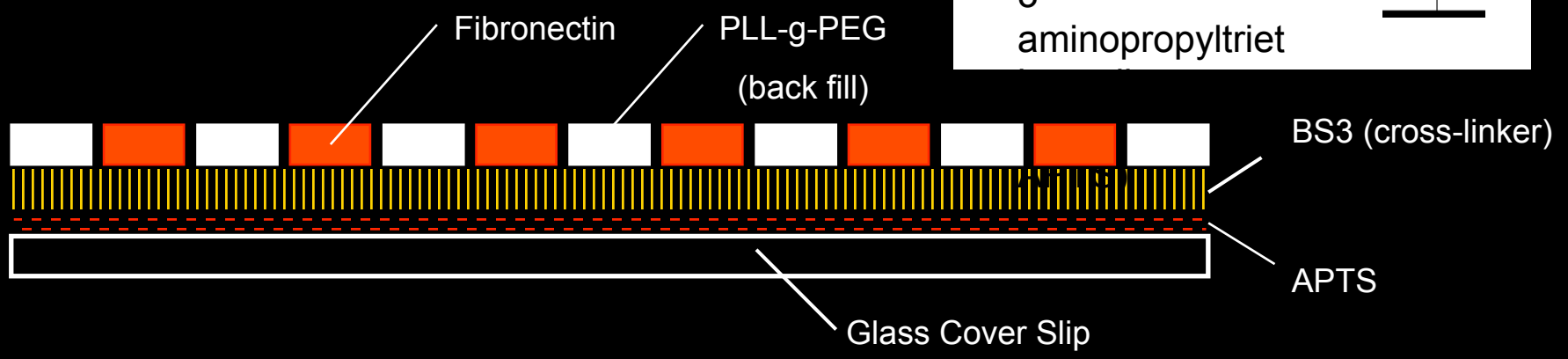
- Simulation: Initial Protein Distribution -

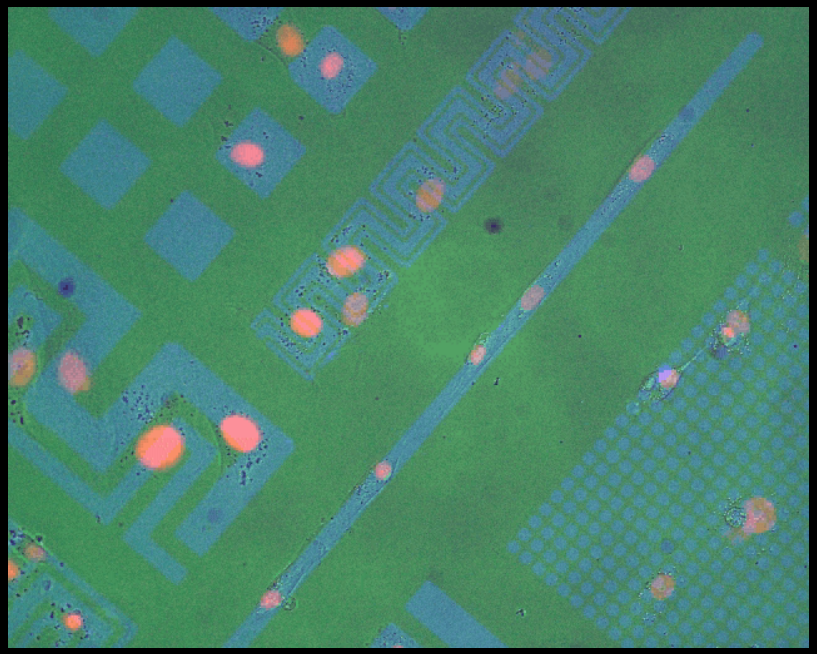


Patterned Substrate
→ constant boundary condition

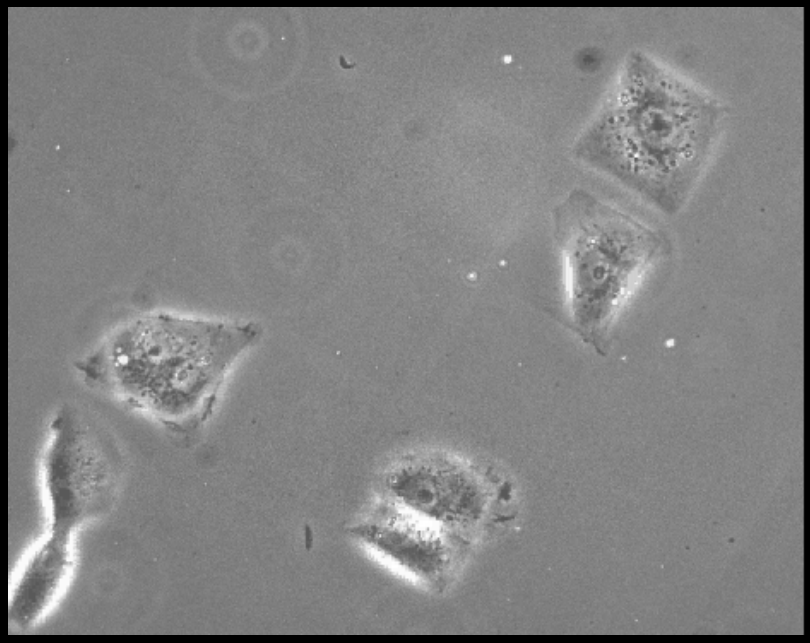


3-
aminopropyltriet

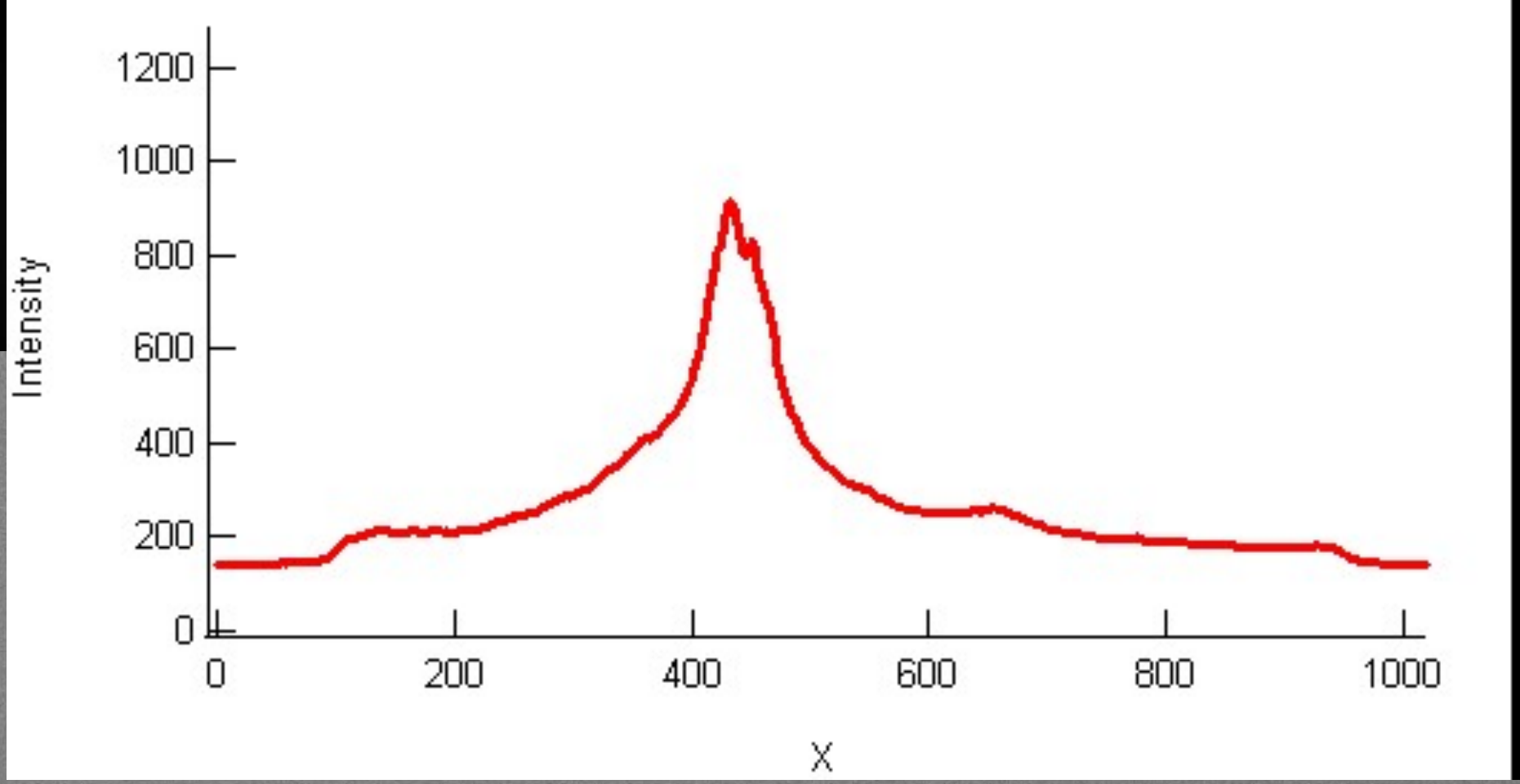


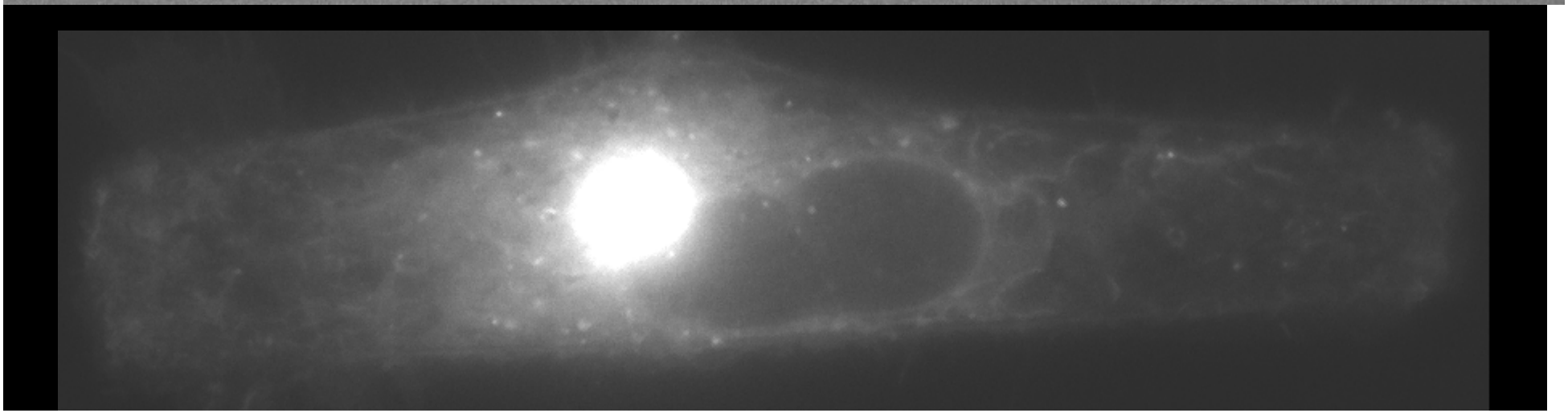


Line width: 10 μm

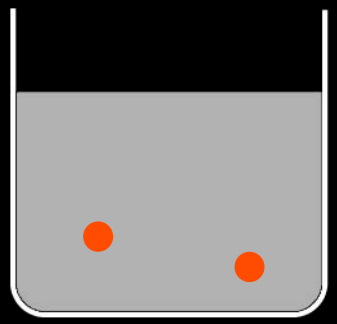


Squares: 50 x 50 μm

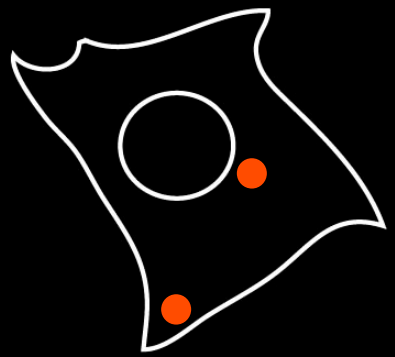

Intensity
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
X



Biochemistry
（solution）



Topo-biochemistry
(in vivo)



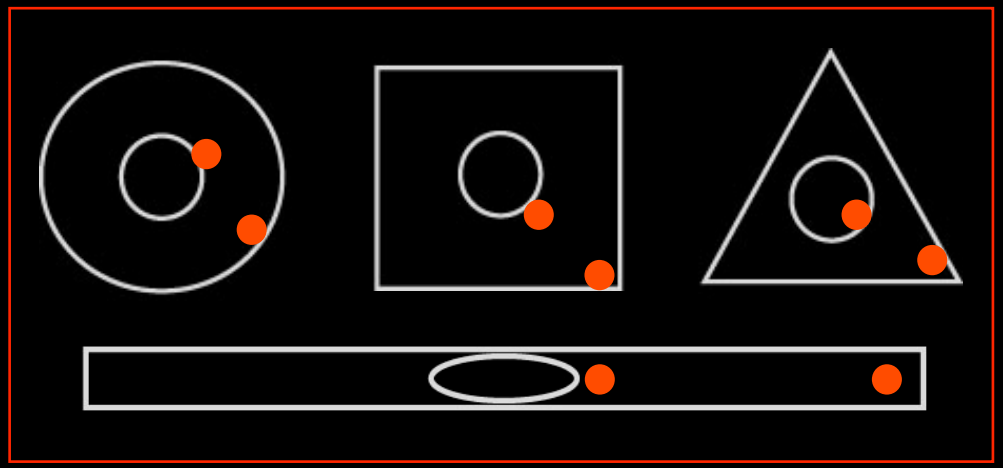
$$[E]+[S] \underset{k2}{\overset{k1}{\rightleftarrows}} [ES] \xrightarrow{k3} [P]$$

B([E], [S], [ES], [P], t)

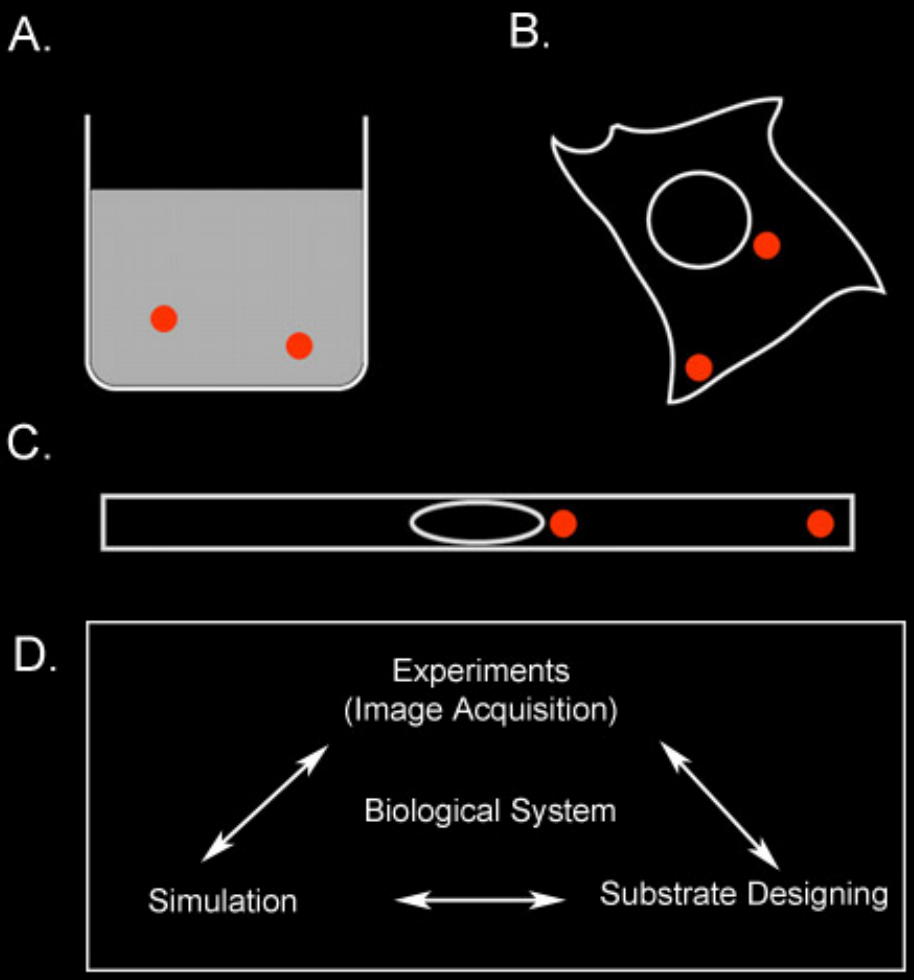
B([E], [S], [ES], [P], t, x)

B([E], [S], [ES], [P], t, x, y)

B([E], [S], [ES], [P], t, x, y, z)



Topobiochemistry



Endoplasmic Reticulum

The Golgi apparatus

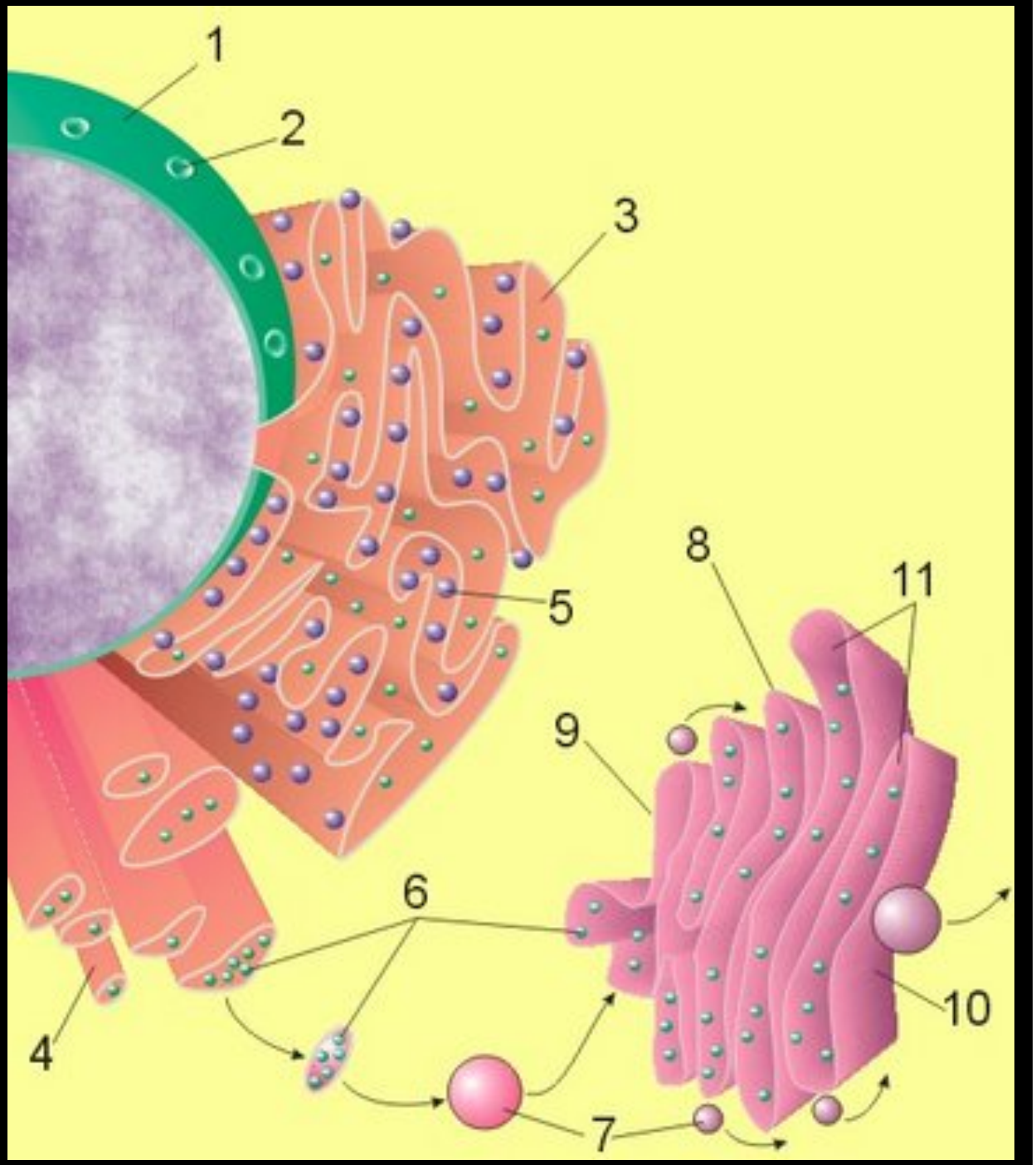


Image taken from Wikipedia

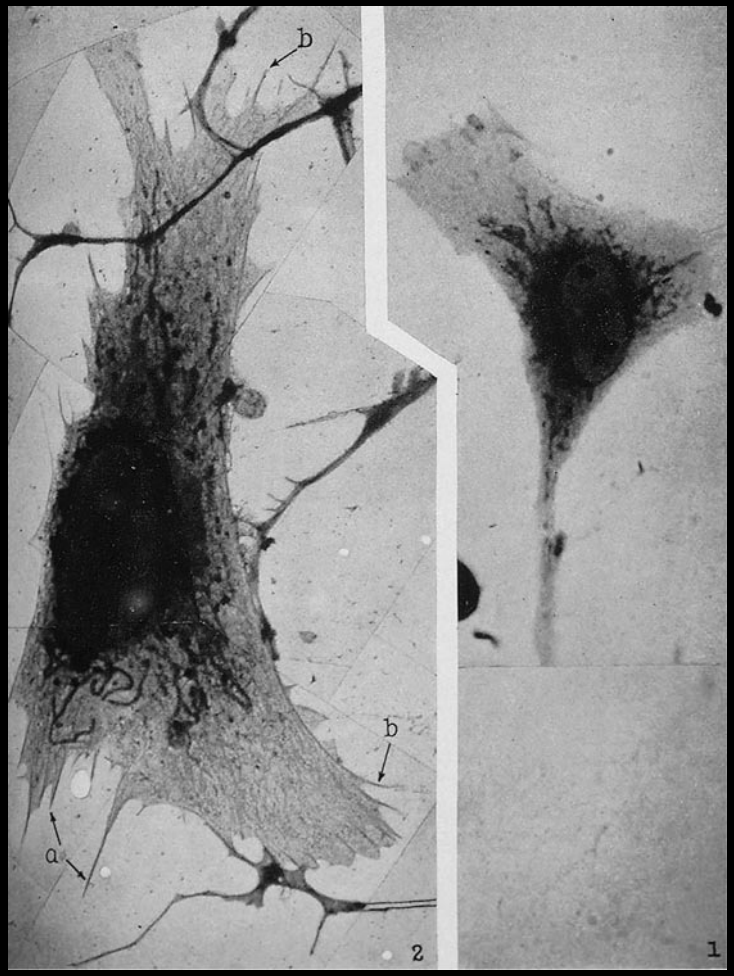
The Golgi Apparatus

In 1897, studying the nervous system with his black reaction, Golgi noticed in neurons an intracellular structure, whose existence he officially reported in April 1898. This structure was designated by Golgi "internal reticular apparatus" and was soon named after him as Golgi apparatus (or much later as the Golgi complex and is frequently referred to nowadays only as "the Golgi").

Golgi's drawings of the "internal reticular apparatus" that he observed in spinal ganglia (the different drawings illustrate the variety of features Golgi observed with his metal impregnation, from *Opera Omnia*). This intracellular structure is universally known nowadays as "Golgi apparatus".

http://nobelprize.org/medicine/articles/golgi/

Endoplasmic Reticulum



This famous first electron micrograph of an intact cell was published in *The Journal of Experimental Medicine* in March 1945, in "A Study of Tissue Culture Cells by Electron Microscopy," by Keith R. Porter, Albert Claude, and Ernest F. Fullam. The cell is a cultured fibroblast originating from a chick embryo, which was grown by Porter on polyvinyl film, then peeled off and transferred to a wire specimen grid. The cell was fixed with osmium tetroxide, washed and then dried in order to prevent evaporation in the electron microscopeUs vacuum chamber. Magnified 1600 times, this first electron micrograph of a cell reveals mitochondria, the Golgi apparatus and a "lace-like reticulum" which Porter later named the "endoplasmic reticulum". The electron microsope used for this historic image was an RCA EMB model, operated by Fullam at the Interchemical Corporation in New York City.

http://www.rockefeller.edu/rucal/journey/journey.html

http://www.nature.com/ncb/journal/v1/n4/fig_tab/ncb0899_E99_F1.html

Perspectives -Re-Defining Organelle-

Intracellular Organelle → Protein Dynamics

(Static View)

Protein Dynamics → Intracellular Organelle

(Dynamic View)