Biomedical Imaging & Analysis

Lecture 6, Part 2. Fall 2014

Basic Image Processing / Filtering (II)

*[**Text**: Ch. 1 and Ch. 2 (until 2.4, Linear Filtering) of Insight into Images edited by Terry Yoo, et al.]*

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- Review: Lecture 5
- Basic concept: spatial filtering
- Basic concept: image gradient calculation
- **The Gaussian filter**
- Overview of image feature detection
- Point feature detection

The Gaussian Filter (III)

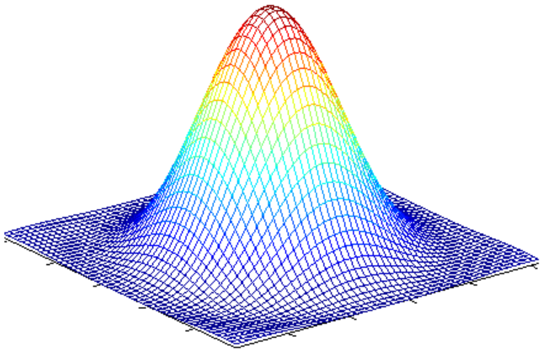
- Gaussian kernel in 1D & 2D

$$G(x;\sigma)=\frac{1}{\sqrt{2\pi}\sigma}e^{-\frac{x^2}{2\sigma^2}}$$

$$G(x,y;\sigma_x,\sigma_y)=\frac{1}{2\pi\sigma_x\sigma_y}e^{-\left(\frac{x^2}{2\sigma_x{}^2}+\frac{y^2}{2\sigma_y{}^2}\right)}$$

- First order derivative

$$G'(x;\sigma)=\frac{-x}{\sqrt{2\pi}\sigma^3}e^{-\frac{x^2}{2\sigma^2}}$$

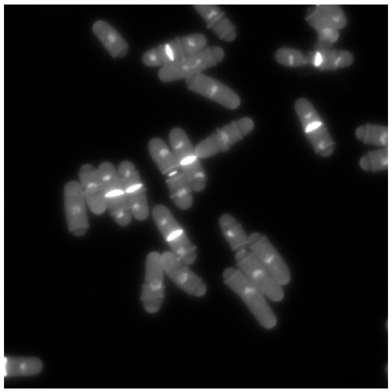


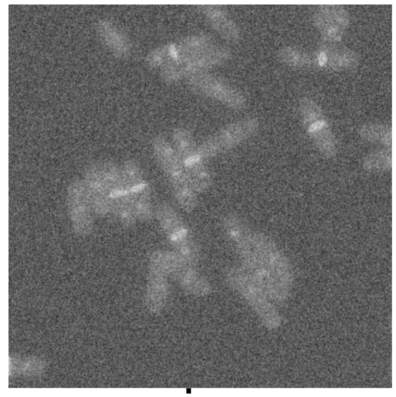
- Second order derivative

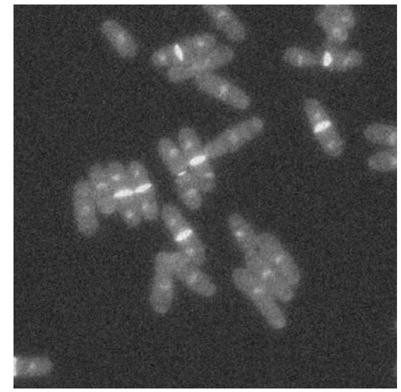
$$G''(x;\sigma)=\frac{-x}{\sqrt{2\pi}\sigma^3}e^{-\frac{x^2}{2\sigma^2}}\left[1-\frac{x^2}{\sigma^2}\right]$$

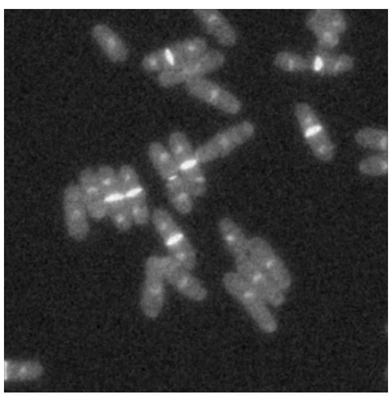
Gaussian Filter & Feature Scale

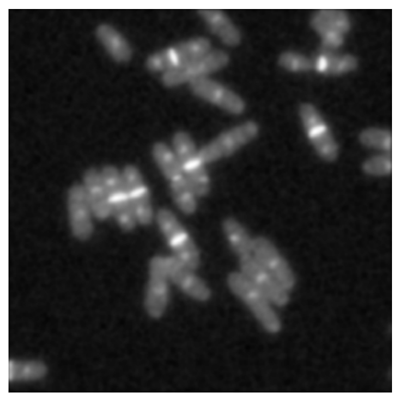
- Application I: noise suppression

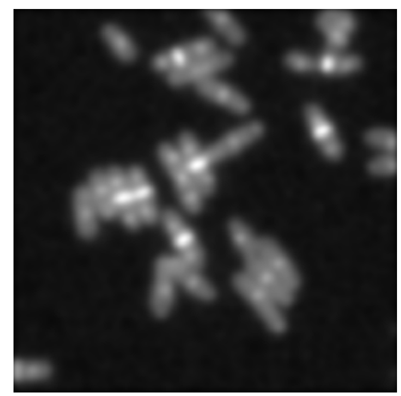

original


noise added


σ=1


σ=2


σ=5


σ=10

The Gaussian Filter (III)

- Some basic properties of a Gaussian filter
  - It is a low pass filter

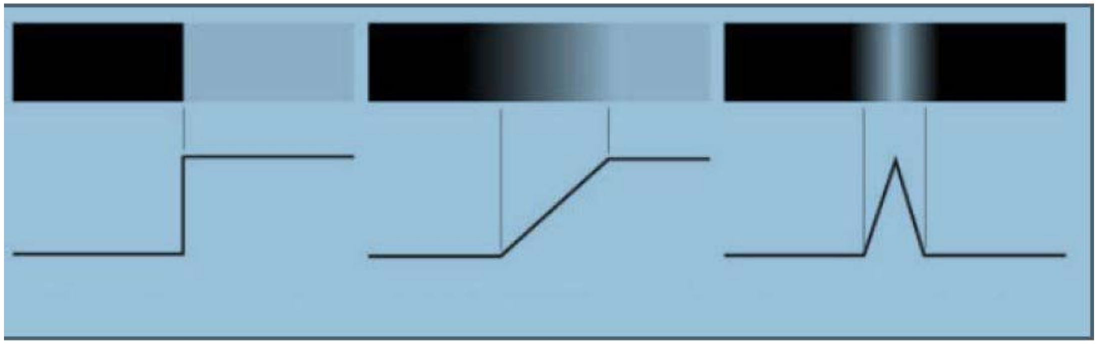
$$\frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} \frac{e^{-\frac{\sigma^2\omega^2}{2}}}{\sqrt{2\pi}}$$

  - It is separable

$$G\left(x,y;\sigma_x,\sigma_y\right) = \frac{1}{2\pi\sigma_x\sigma_y} e^{-\left(\frac{x^2}{2\sigma_x{}^2}+\frac{y^2}{2\sigma_y{}^2}\right)} = \frac{1}{\sqrt{2\pi}\sigma_x} e^{-\frac{x^2}{2\sigma_x{}^2}} \cdot \frac{1}{\sqrt{2\pi}\sigma_y} e^{-\frac{y^2}{2\sigma_y{}^2}}$$

Edge Detection

- Edge Models: can be modeled according to their intensity profiles:



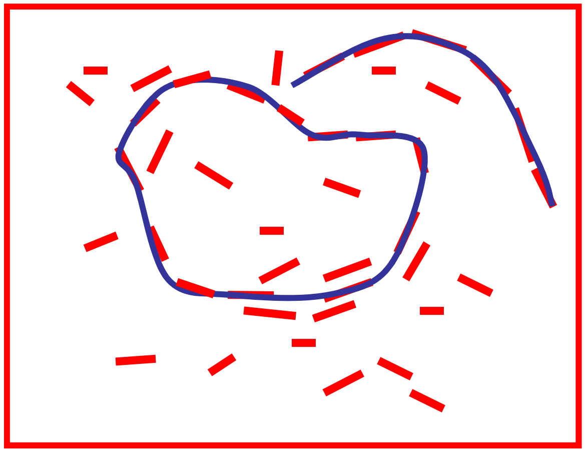
a)Step Edge   b) Ramp Edge   c) Roof Edge

Four steps for edge detection:

1. **Smoothing**: Remove noise as much as possible.
2. **Enhancement**: Apply a filter to enhance the quality of edges in the original image (ex: sharpening,contrast)
3. **Detection**: Determine which edge pixels should be thrown as a noise or retained for edge detection.
4. **Localization**: Identify the location of an edge.

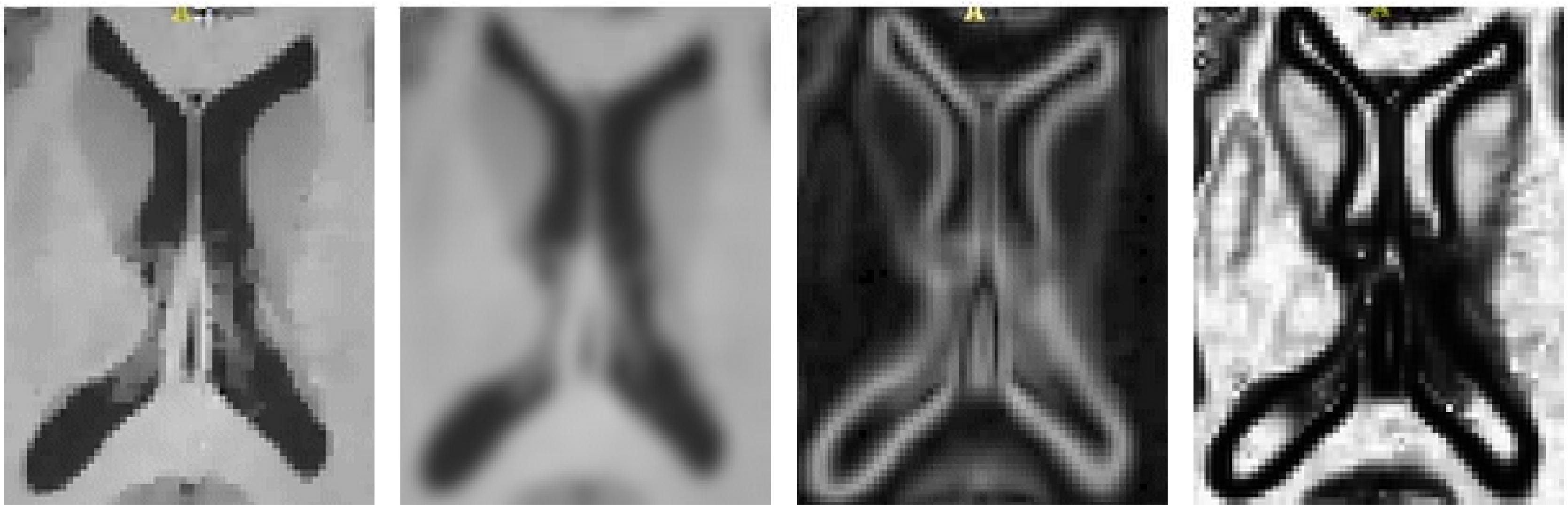
Edge Detection

- Edge Detection:
  - The process of labeling the locations in the image where the gray level's "rate of change" is high.
    - OUTPUT: "edgels" locations, direction, strength



- Edge Integration:
  - The process of combining "local" and perhaps sparse and non-contiguous "edgel"-data into meaningful, long edge curves (or closed contours) for segmentation
    - OUTPUT: edges/curves consistent with the local data

Leveraging **Gradient** Images to get Edges

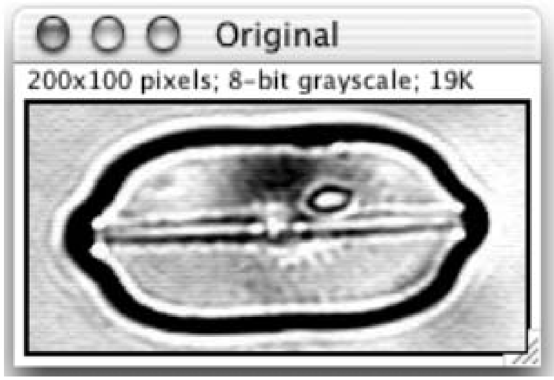


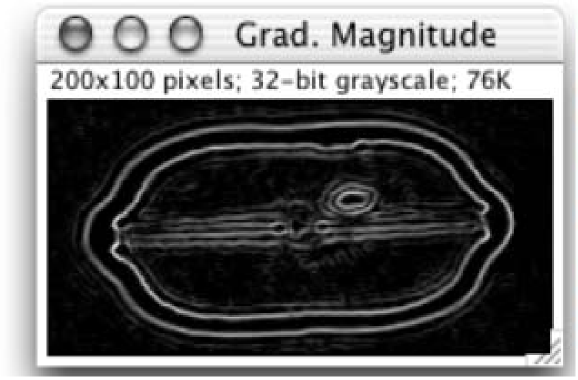
greylevel image blurred image grad. magnitude edge feature image

<u>Edge feature image</u>: The gradient magnitude is remapped to the range 0 to 1, with large values of gradient magnitude mapping to 0 and small values mapping to 1.

http://www.itksnap.org/pmwiki/pmwiki.php?n=Documentation.TutorialSectionIntroductionToAutomatic

Edge Detection





Diatom image (left) and its gradient magnitude (right).
(http://bigwww.epfl.ch/thevenaz/differentials/)

$$\nabla f = \left[\frac{\partial f}{\partial x} \; \frac{\partial f}{\partial y} \right]^{\mathrm{T}} \equiv \left[G_x \; G_y \right]^{\mathrm{T}}$$

$$|\nabla f| = \sqrt{G_x^2 + G_y^2} = \text{Edge Strength}$$

$$\angle \nabla f = \operatorname{atan}\left(\frac{G_x}{G_y} \right)$$

Then **threshold** the gradient magnitude image

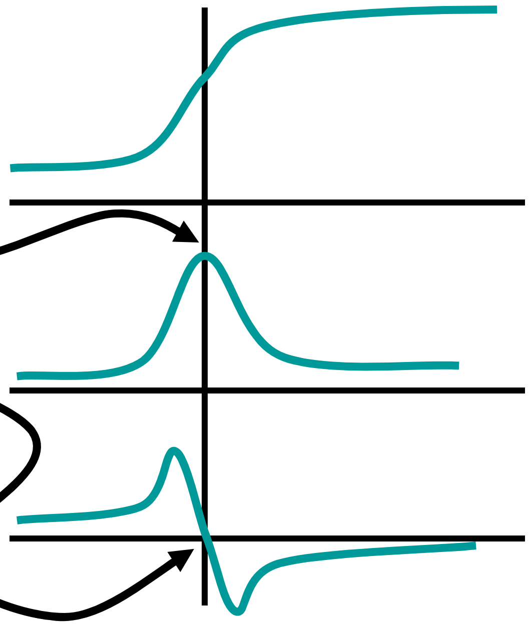
Detected edges are:
- Too thick in places
- Missing in places
- Extraneous in places

The Classics

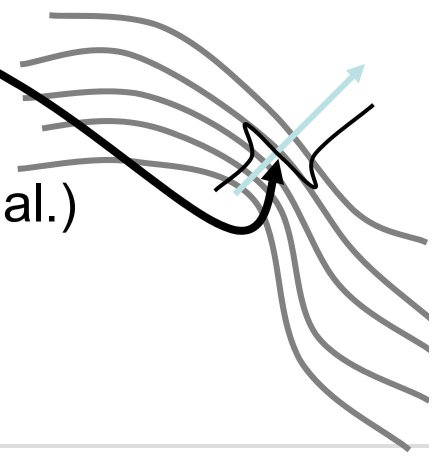
- Edge detection:
  - Sobel, Prewitt, Other gradient estimators
  - Marr Hildreth

    zero crossings of $\Delta G * I$
  - Haralick/Canny/Deriche et al.

    "optimal" directional local max of derivative



- Edge Integration:
  - tensor voting (Rom, Medioni, Williams, …)
  - dynamic programming (Shashua & Ullman)
  - generalized "grouping" processes (Lindenbaum et al.)



Detection Methods - Prewitt vs. Sobel

- Two popular edge detection filters, the only difference being the coefficients in the convolution kernel.
- Sobel has better noise suppression.
- Both fail when exposed to high level of noise (laplacian operator for a better solution)

−1	−1	−1	−1	0	1
0	0	0	−1	0	1
1	1	1	−1	0	1

Prewitt

−1	−2	−1	−1	0	1
0	0	0	−2	0	2
1	2	1	−1	0	1

Sobel

Original

Prewitt

Sobel

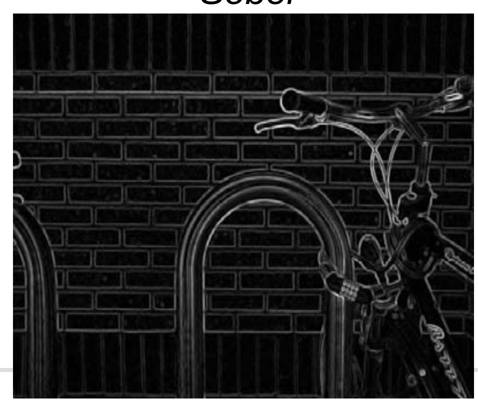


Image Gradient Calculation Under Gaussian Filtering

- A basic property of convolution

$$\frac{\partial\left(G\otimes J\right)}{\partial x}=\frac{\partial I}{\partial x}=I_x=\frac{\partial G}{\partial x}\otimes J \qquad \frac{\partial\left(G\otimes J\right)}{\partial y}=\frac{\partial I}{\partial y}=I_y=\frac{\partial G}{\partial y}\otimes J$$

- This is the basis of “Canny Edge Detection”.

Algorithm **Canny Edge Detection**

1. *Smooth the image with a Gaussian filter.*
2. *Compute the gradient magnitude and orientation using finite-difference approximations for the partial derivatives.*
3. *Apply nonmaxima suppression to the gradient magnitude.*
4. *Use the double thresholding algorithm to detect and link edges.*

Canny Edge Detection

Additional salient aspects of interest:

1) Line-thinning by Non-Maxima Suppression:
   For each pixel *I(x0,y0),compare* the edge strength along the direction perpendicular to the edge. An edge point must have its edge strength no less than its two neighbors.

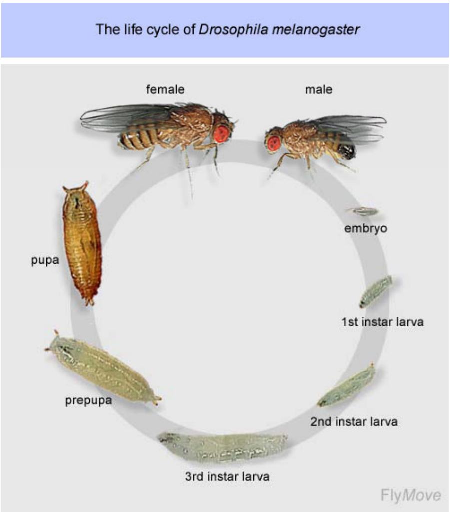
2) Thin-line 'stitching' i.e. Edge Integration:
   The process of combining "local" and perhaps sparse and non-contiguous "edgel"-data into meaningful, long edge curves (or closed contours) for segmentation

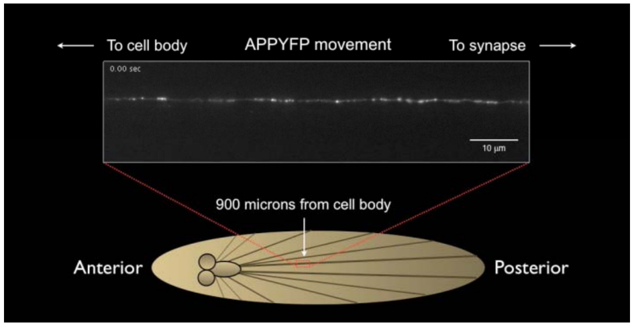
Basic idea (http://users.ecs.soton.ac.uk/msn/book/new_demo/thresholding/)

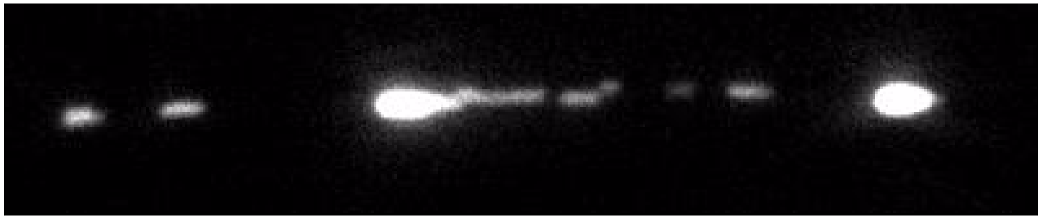
- Using two thresholds T_L *and* T_H
- Starting from a point where edge gradient magnitude higher than T_H
- Link to neighboring edge points with edge gradient magnitude higher than T_L

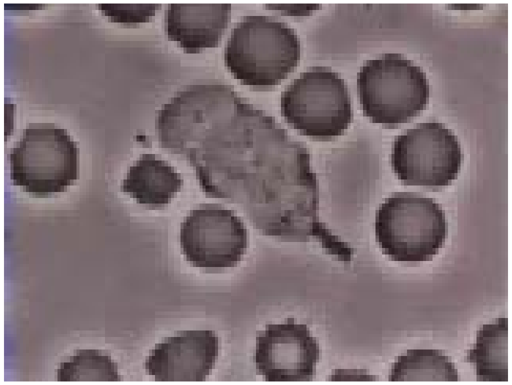
- Review: Lecture 7
- Basic concept: spatial filtering
- Basic concept: image gradient calculation
- The Gaussian filter
- Overview of image feature detection
- Point feature detection

Feature Detection: Points vs Clusters / Regions



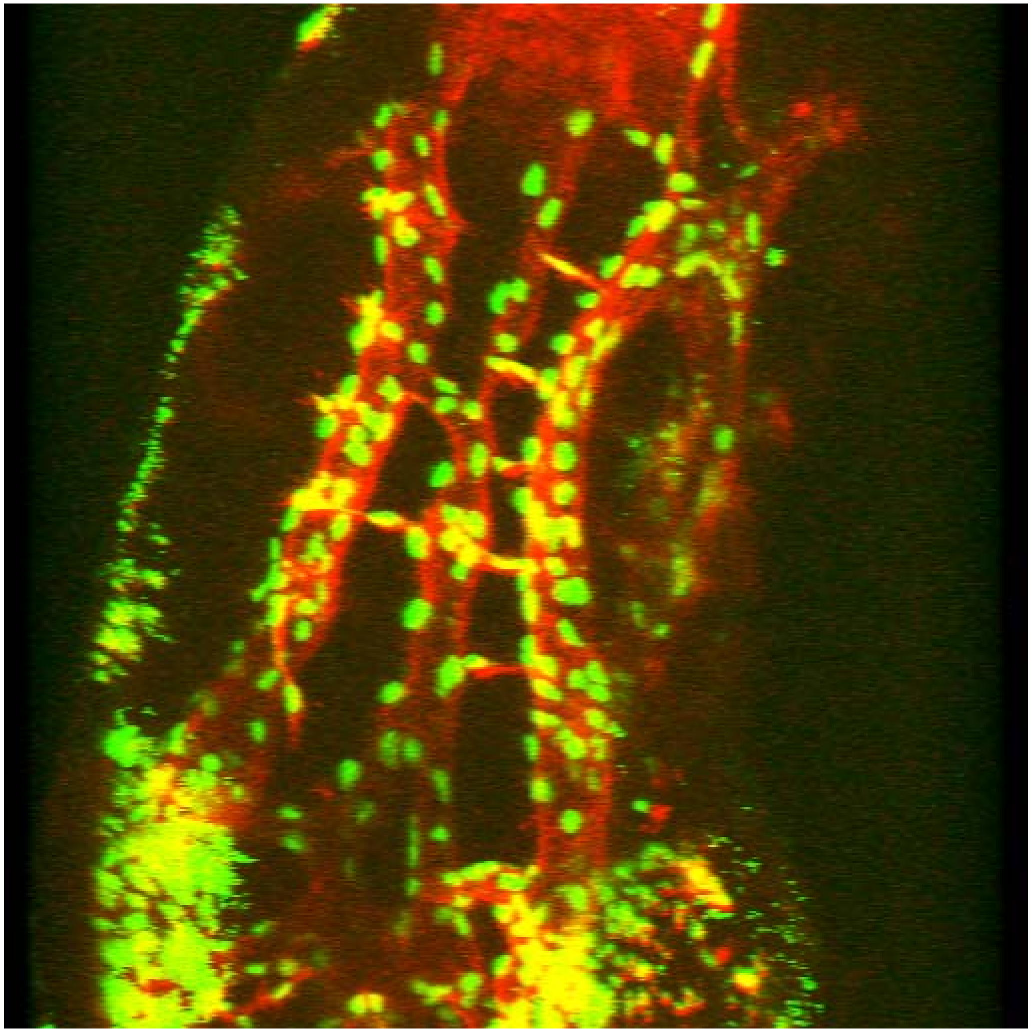




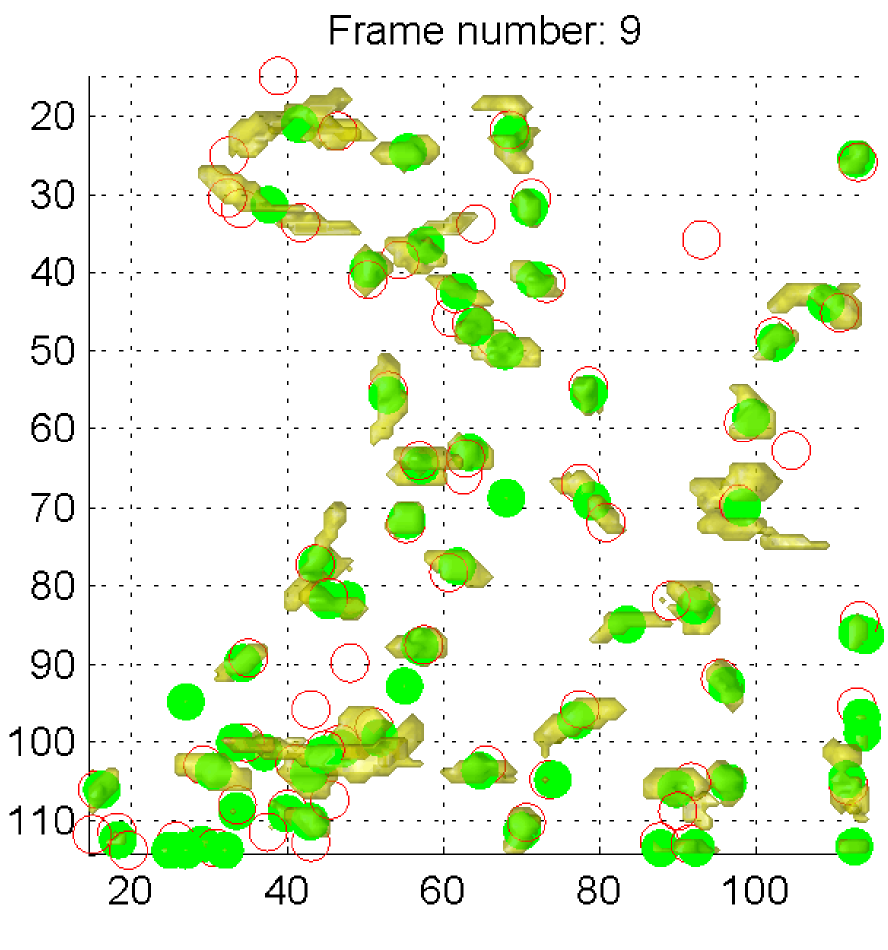


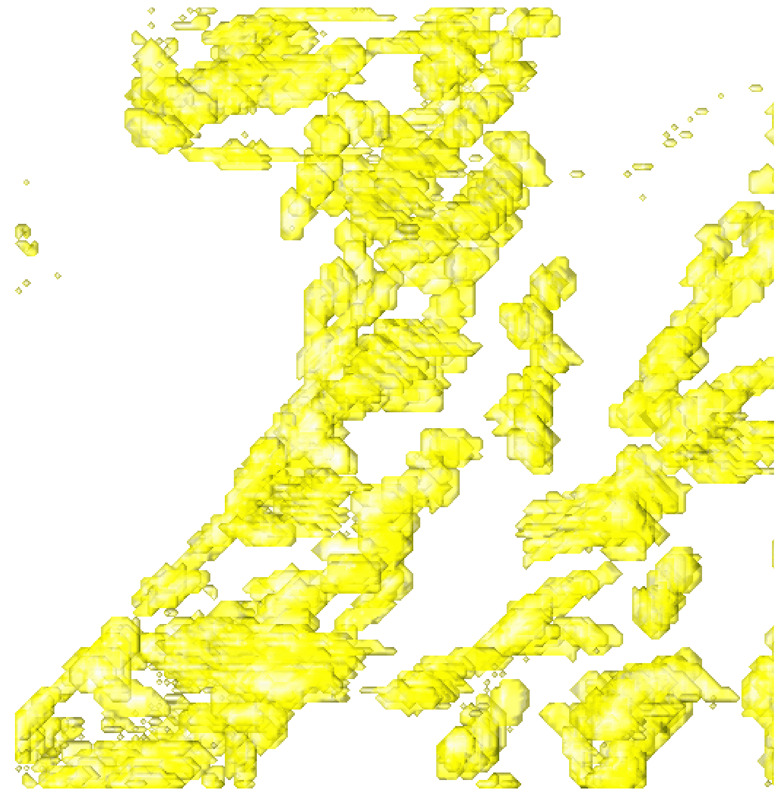
A neutrophil chasing a bacterium.
Devreotes Lab, Johns Hopkins U.

Feature Detection: Regions



Application: Tracking Endothelial Cell Nuclei during Embryonic Vascular Growth





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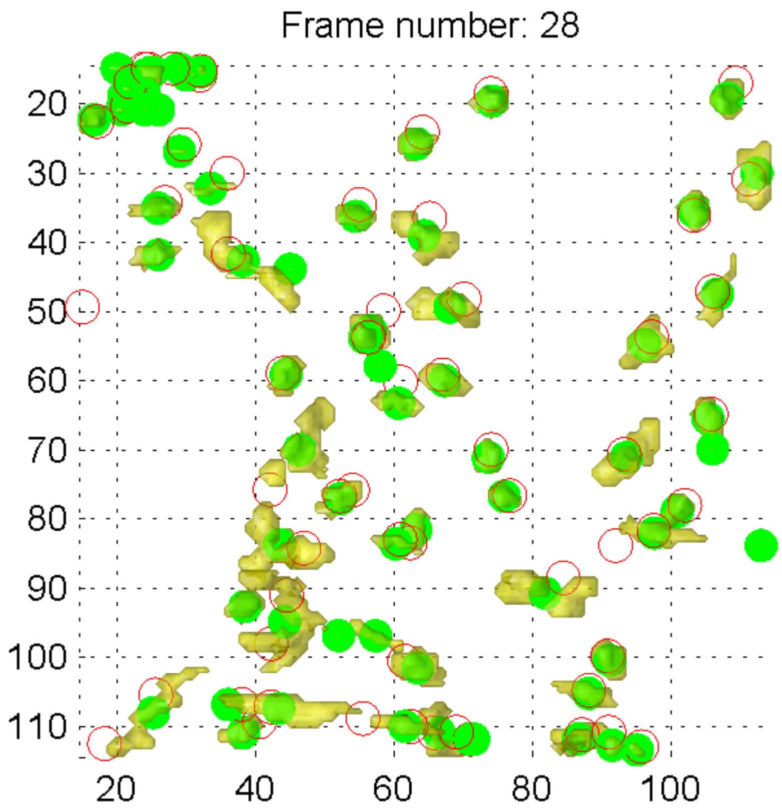
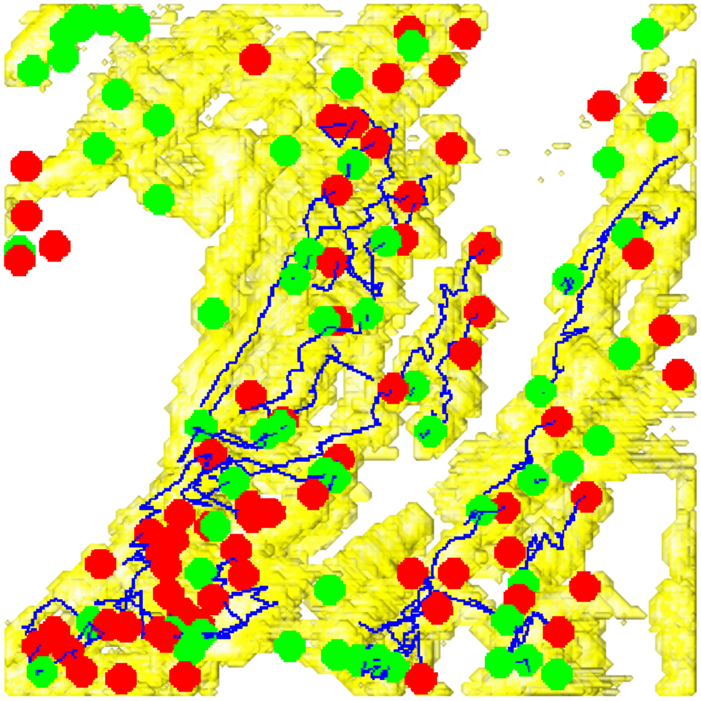
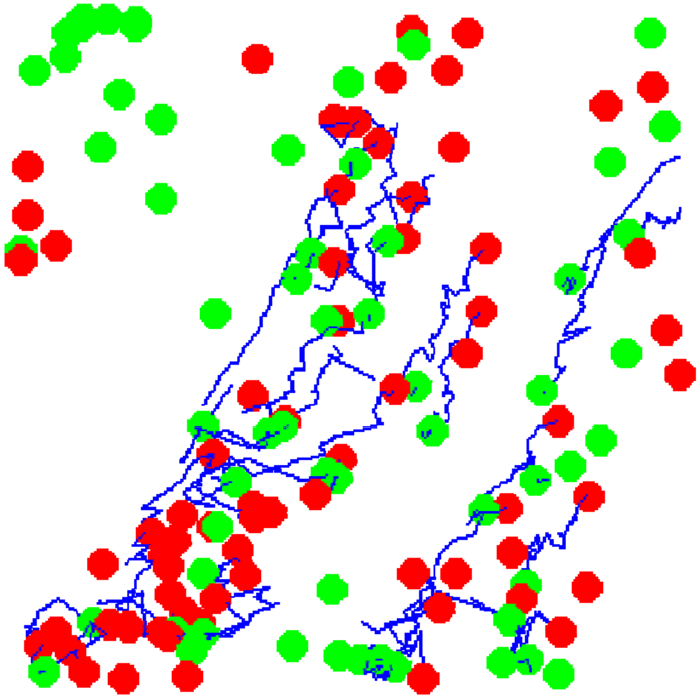




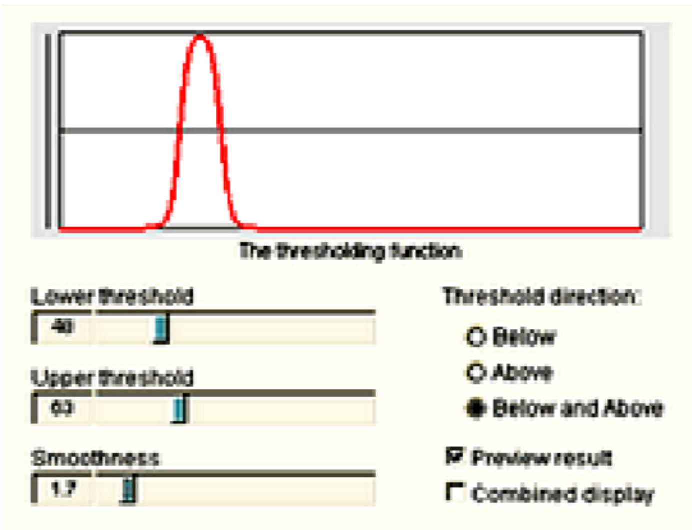
Application: Tracking Endothelial Cell Nuclei during Embryonic Vascular Growth

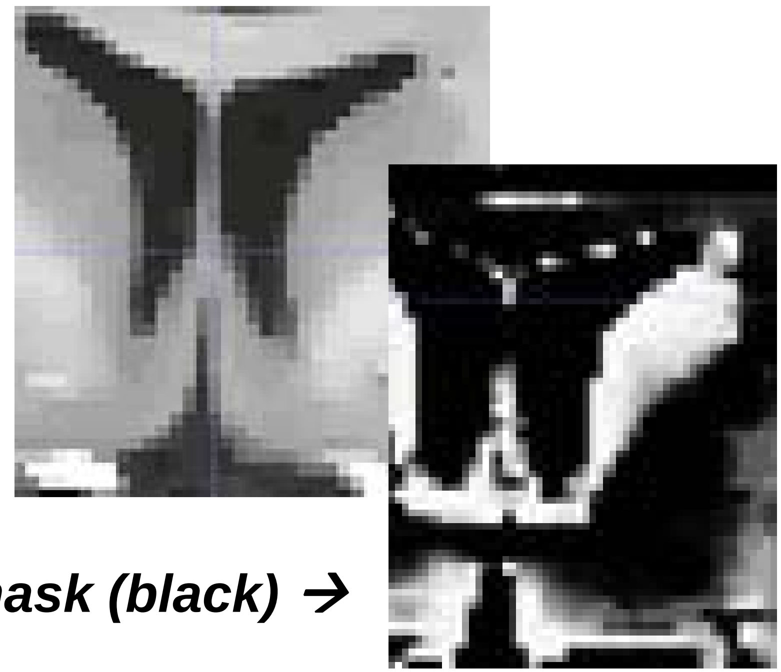


1. Extract STL surfaces of nuclei from the VTKs of the zebrafish at each time-instant / frame.
2. Load these STLs into a code that converts the mesh into a voxelized binary volume.
3. Find the number of cells from the binary volume and also identify centroids of each identified cell.
4. Save centroids in a matrix that loads to the new cell tracker code.
5. Export tracks and point locations.

100% accuracy was achieved in identifying nucleus locations in this method, which turned out to be superior to 'clustering' points representing nuclei and tracking their centroids.

Pre-processing: Thresholding





Bi-thresholded ***mask (black)*** **→**

- Provides boundaries within which more specific region-growing segmentation techniques can operate.
- Reduces the size of the problem in terms of image-space to be processed.

Beyond Thresholding…

- A simple threshold becomes a linear discriminant on the histogram, dividing the distribution of image intensity values into two classes. Eg: “bone” and “not-bone”.
- **Thresholding is particularly useful for x-ray CT data where intensity values have an intuitive mapping to physical density.**
- More complex, multivalued data, such as MRI or color cryosections, **require more sophisticated statistical techniques** as simple thresholding may fail to capture the global boundary and shape properties of the object, leading to **noisy boundaries and holes** inside the object.

- Review: Lecture 5
- Basic concept: spatial filtering
- Basic concept: image gradient calculation
- The Gaussian filter
- Overview of image feature detection
- **Point feature detection**

Point Feature Detection (I)

- In bioimaging a point is more often referred to as a particle or a single particle. Point detection is also referred to as "(single) particle detection".

- Some literature uses "particle detection" and "particle tracking" interchangeably. This may cause confusion.

- Detection of point features is particularly important for bioimage analysis because many cellular structures are diffraction limited and appear as particles.

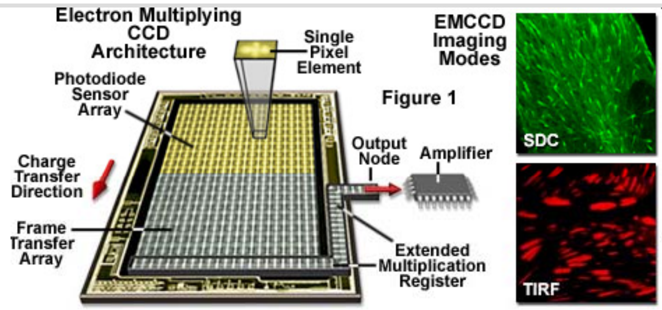
Point Feature Detection (II) – Partial Volume Effect

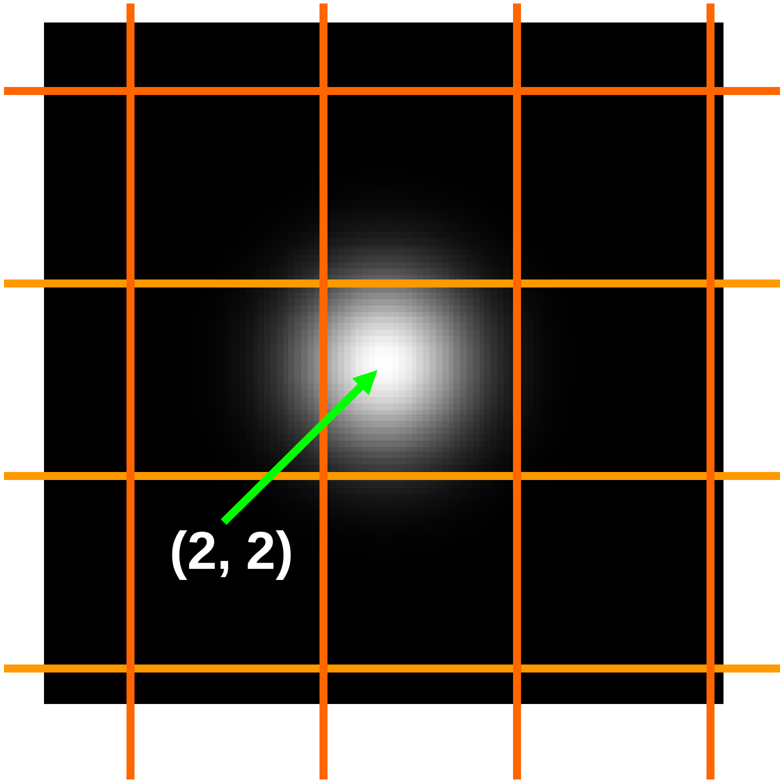
- What information is extracted from feature detection:
  - point position: sub-pixel resolutions are often required.
  - point intensity: may contain information about the number of molecules within the diffraction limit.

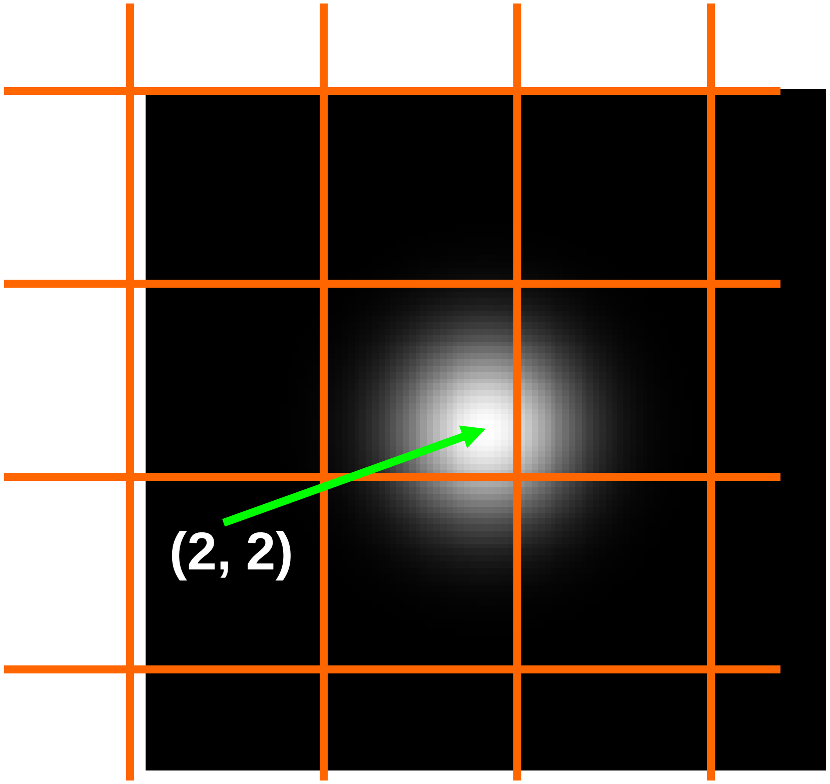
The **partial volume effect** can be defined as the loss of apparent activity in small objects or regions because of the limited resolution of the imaging system.

If the object or region to be imaged is less than twice the full width at half maximum (FWHM) resolution in x-, y- and z-dimension of the imaging system, the resultant activity in the object or region is underestimated.

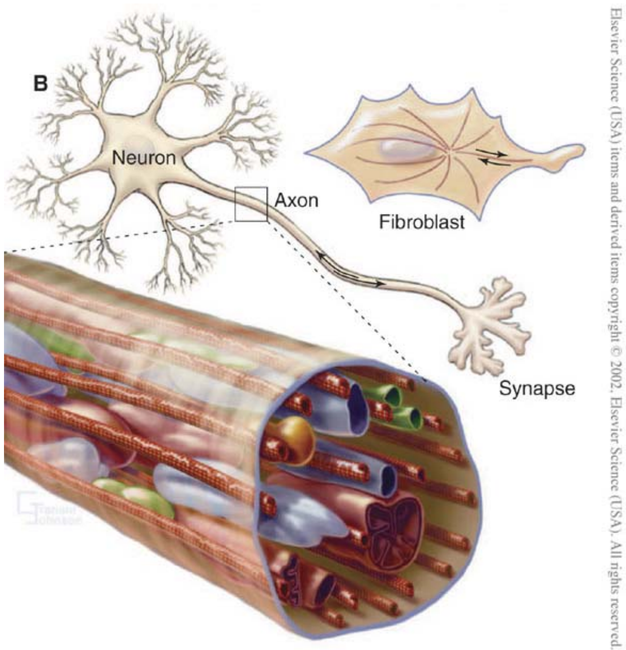
Pixel Resolution Limit in Point Detection

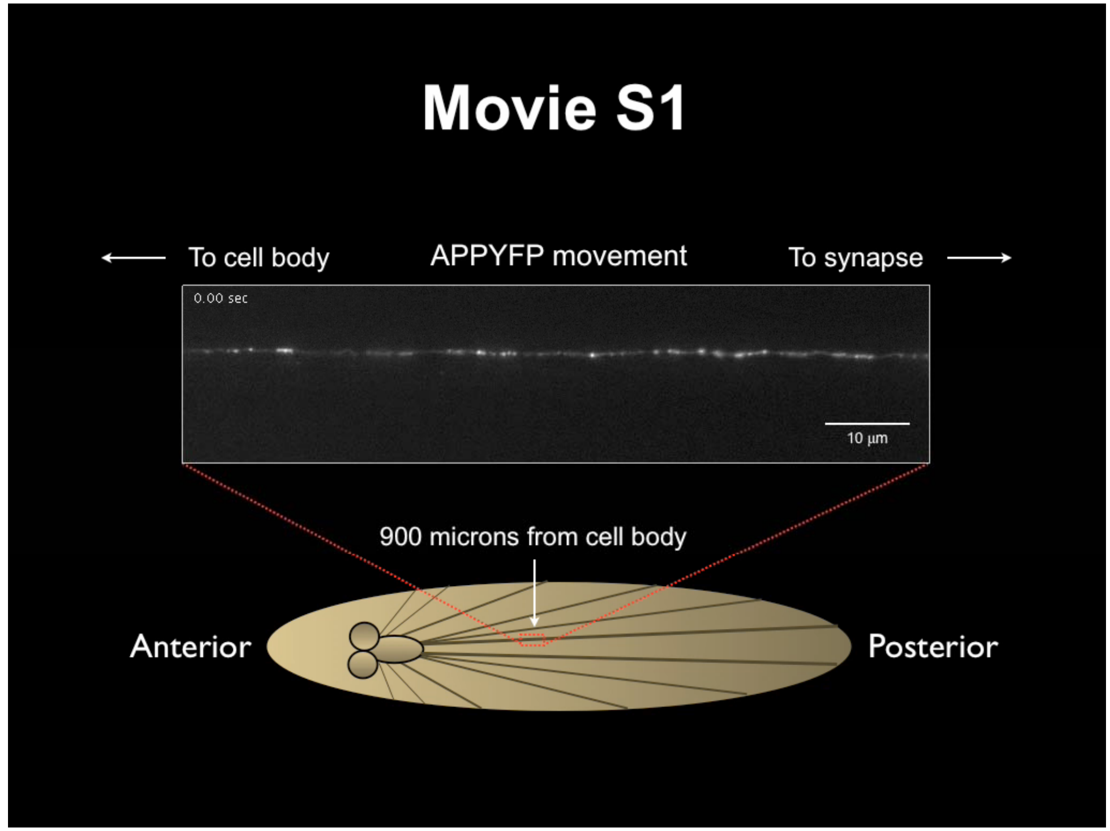




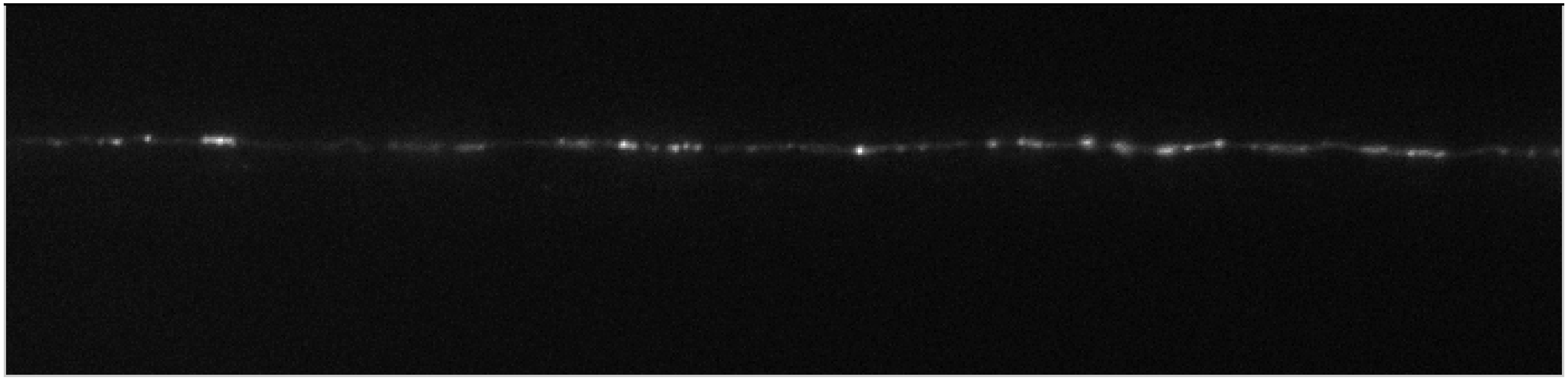


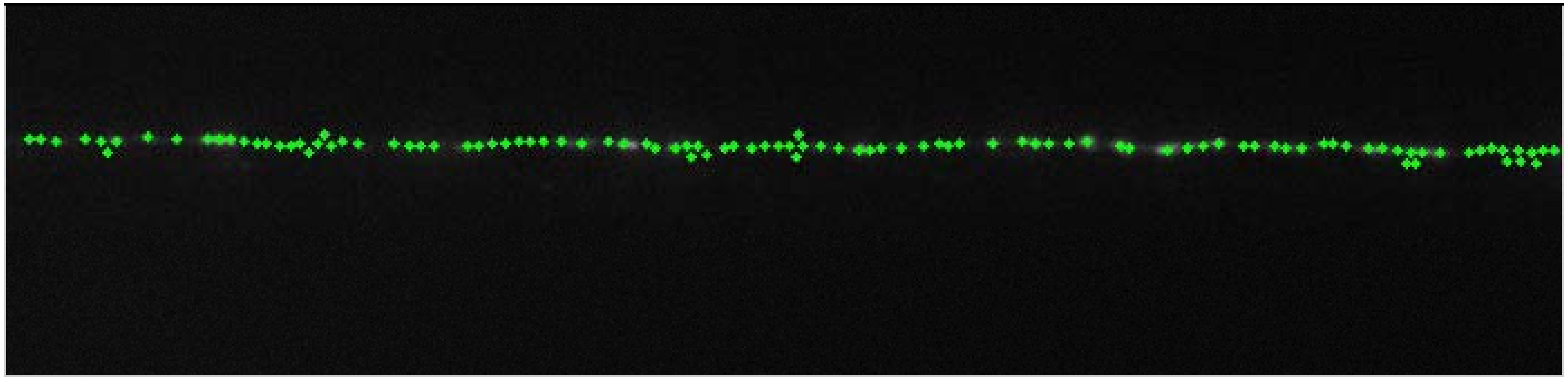
Application: Axonal Cargo Transport





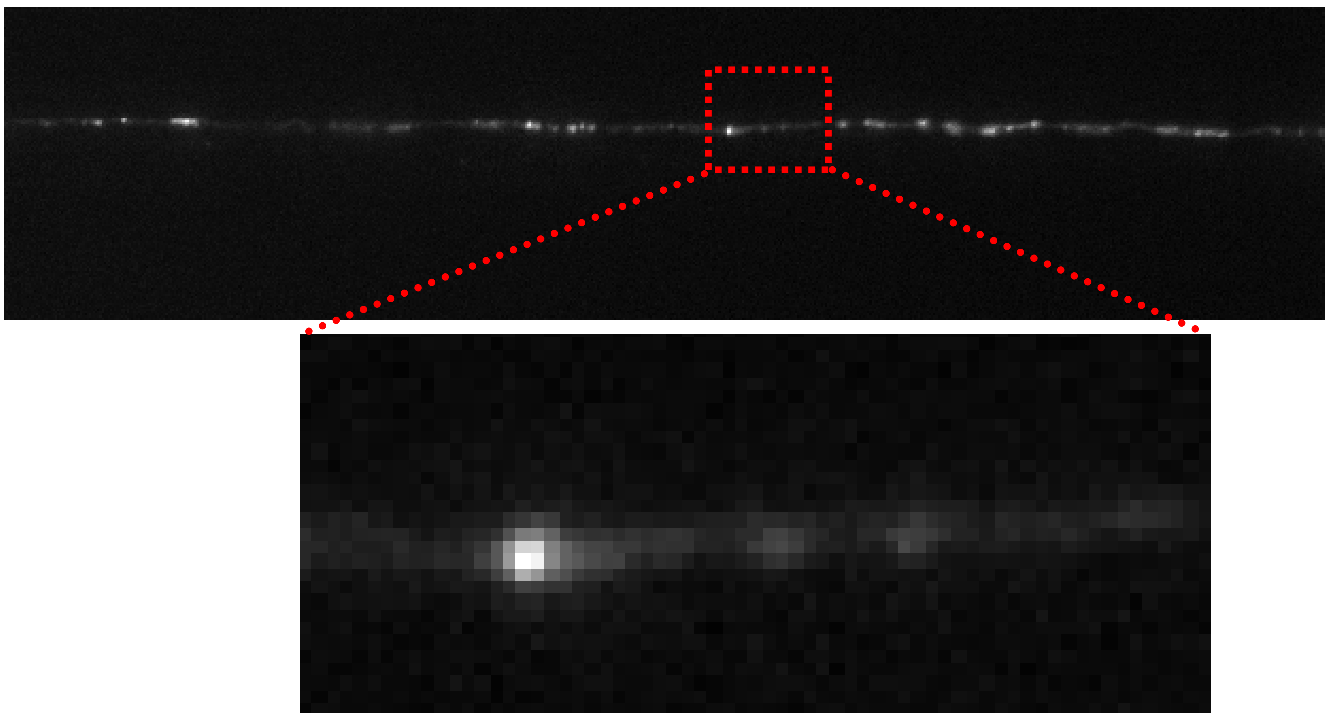
Particle Detection Demo



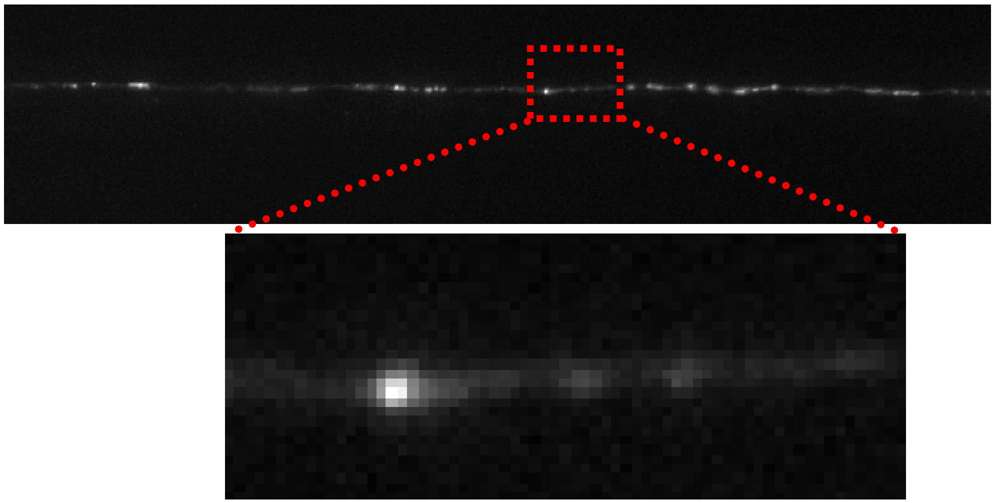


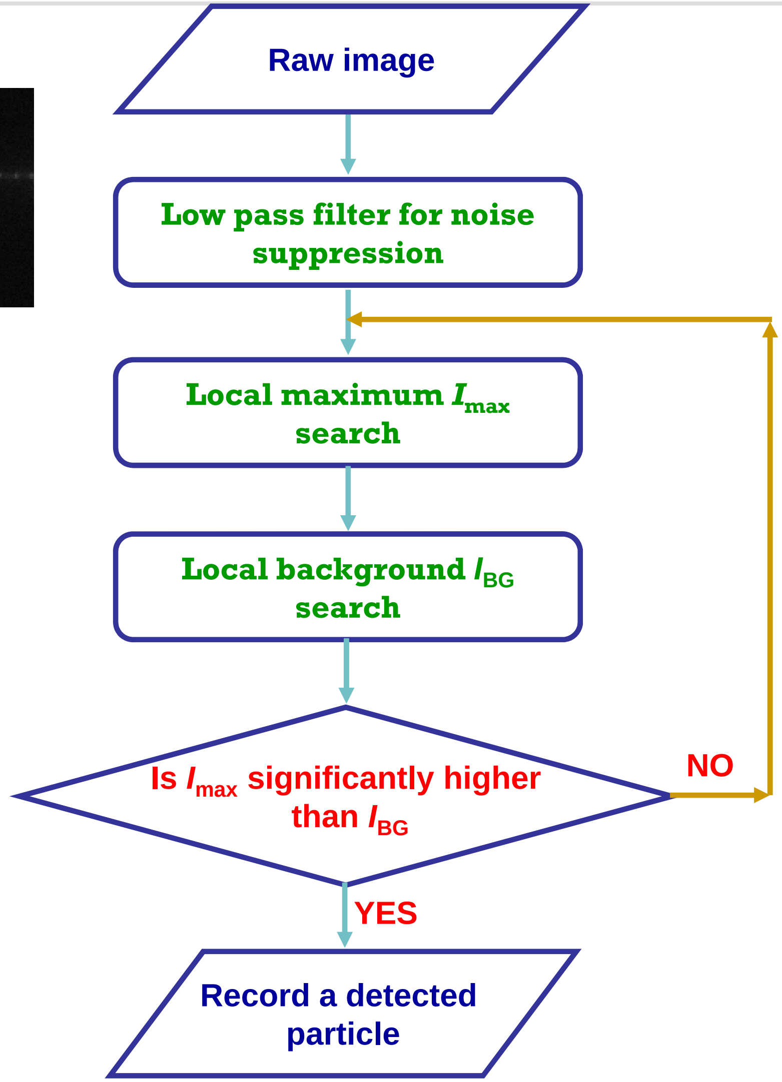
What is a Particle?

- ONE perspective: A point/particle is a local intensity maximum whose level is substantially higher than its local background neighborhood.



Basic Principle of Particle Detection





Step 1: Low Pass Filter (I)

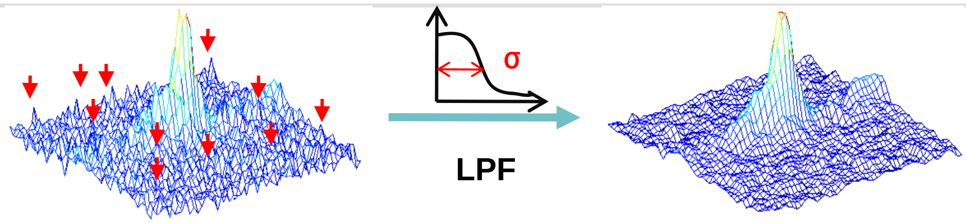
- The Fourier transform of a Gaussian kernel is Gaussian.

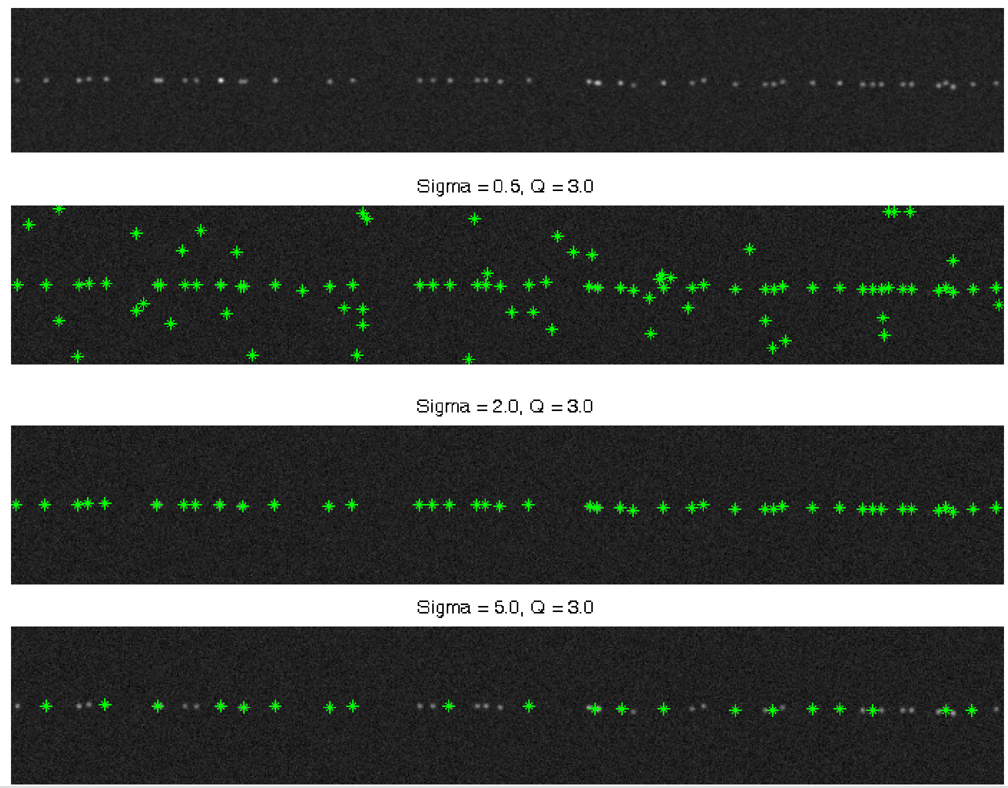
$$\frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} \frac{e^{-\frac{\sigma^2\omega^2}{2}}}{\sqrt{2\pi}}$$

- Impact of σ selection

  - A small σ allows weaker features to be picked up but at the expense of more false positives.

  - A large σ selects strong features but at the expense of more true negatives.





Step 1: Low Pass Filter (II)

- Impact of σ selection
  - Applying a σ that is too large will cause substantial shifting and merging of features.
  - Applying a σ that is too small can not effectively suppress noise.
- Using a small σ is usually preferred.
- A commonly used strategy of selecting σ is to set it to be the Rayleigh limit.

$$3\sigma = \frac{0.61 \cdot \lambda}{NA}$$

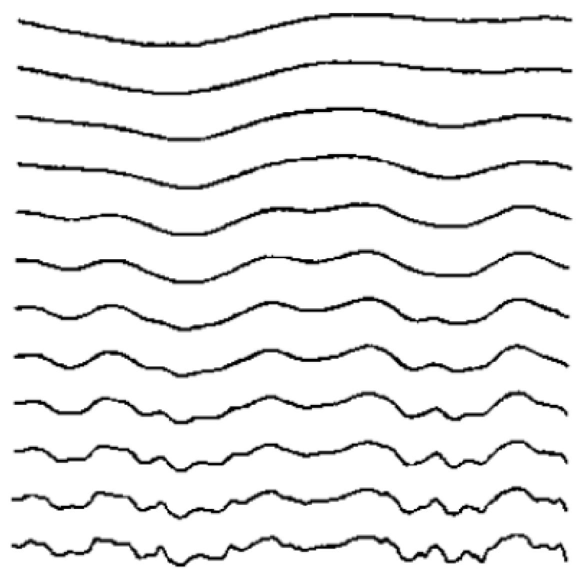
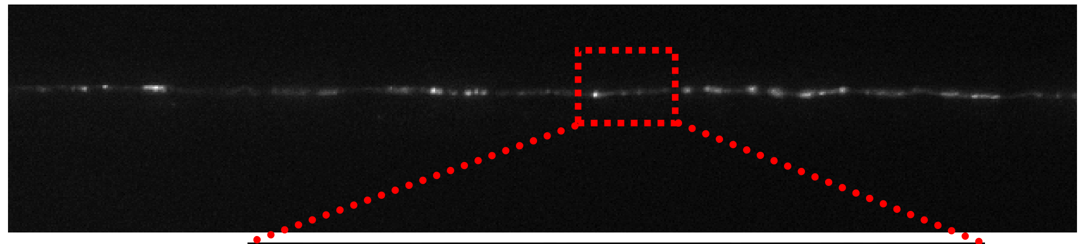


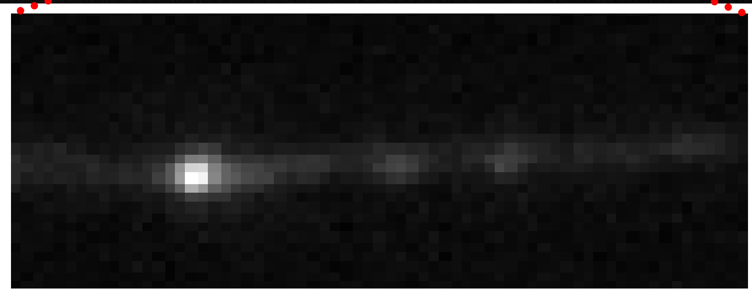
Figure 1. A sequence of gaussian smoothings of a waveform, with σ decreasing from top to bottom. Each graph is a constant-σ profile from the scale-space image.

A. Witkin, *Scale-space filtering*, ICASSP 1984.

Step 2: Local Maximum Detection

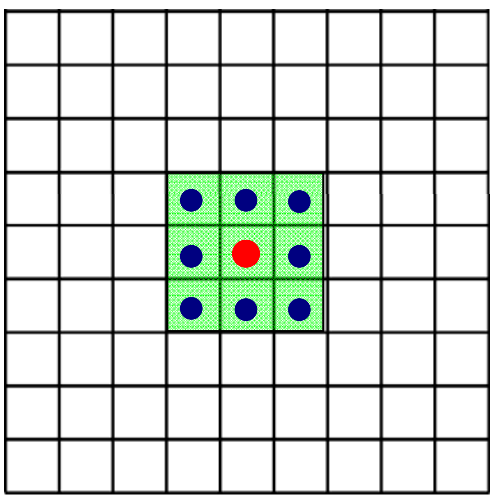
- A local maxima has an intensity that is no smaller than those of its neighbors.



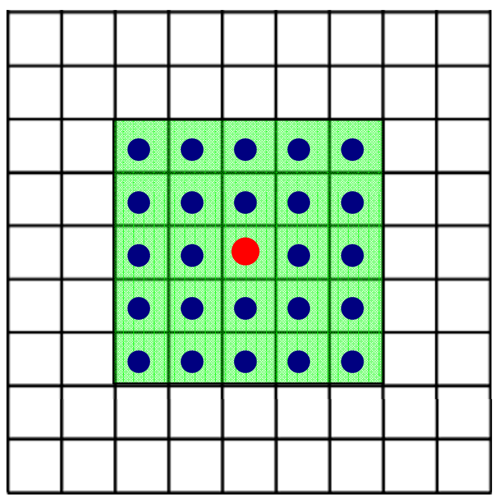


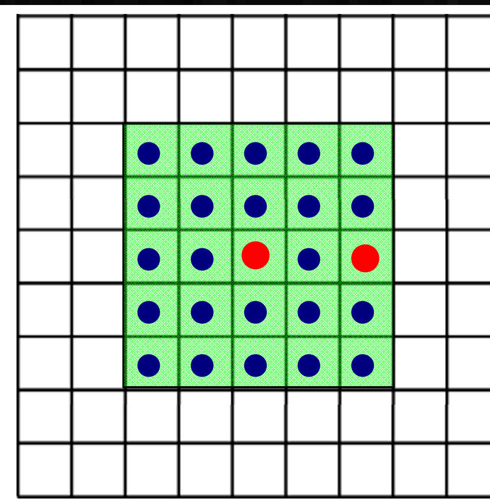
- Large masks give more stable results but lower detection resolution.

3X3 mask



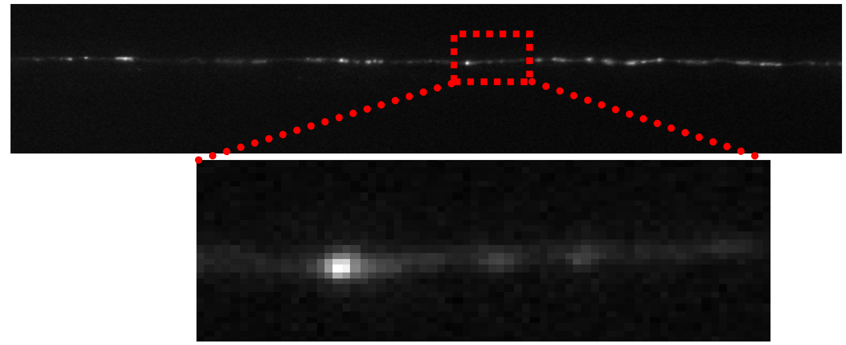
5X5 mask



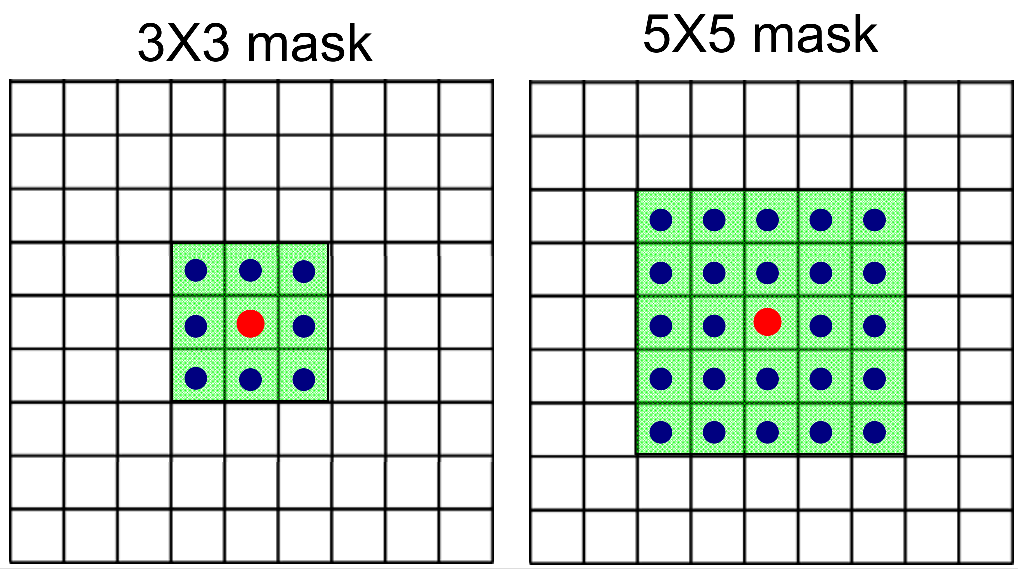


Step 3: Local Background Detection

- A local minima has an intensity level that is no higher than those of its neighbors.



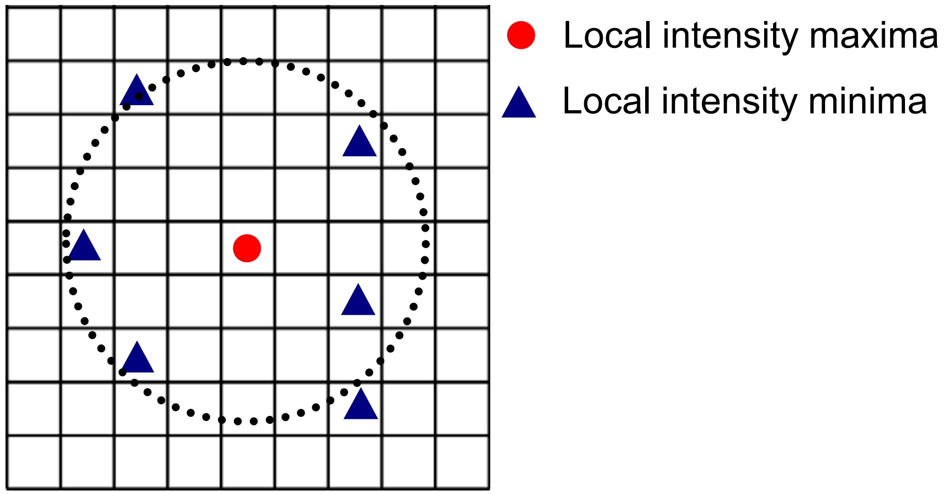
- Local background is detected through detection of local intensity minima.

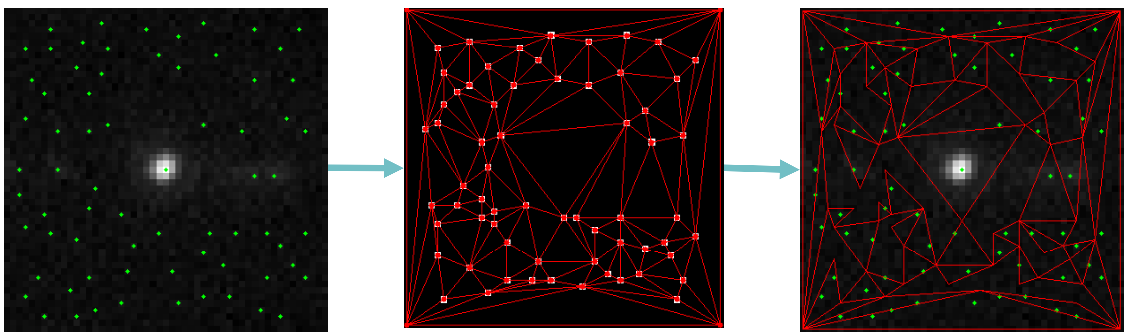


Step 3: Establishing Correspondence Between Local Maxima and Local Minima

- Different approaches can be used to establish correspondence between local maxima and local minima.

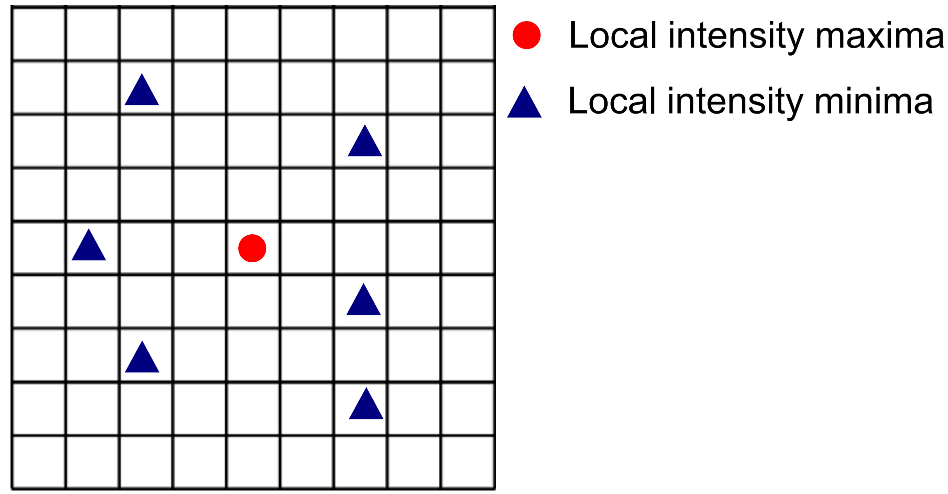
  - Nearest neighbor
  - Delaunay triangulation





Feature Intensity Measurement

- Intensity calculation with background subtraction

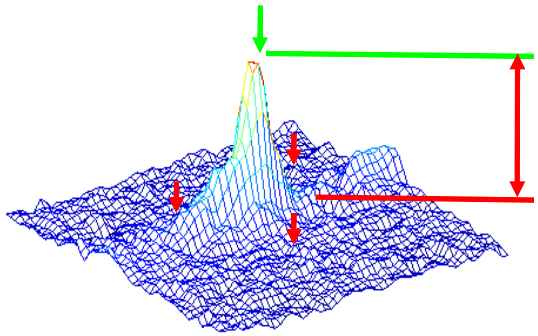


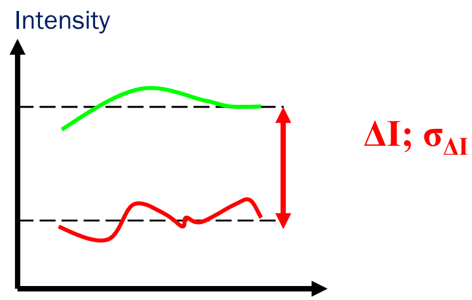
$$I_{net} = I_{max} - \frac{1}{N}\sum_{i=1}^{N} I_{BG}^{i}$$

N: number of local minima used to calculate background

I_{net}: net intensity

Step 4: Statistical Selection of Features





$$I_{max} - I_{BG} \geq Q \cdot \sigma_{\Delta I}?$$

Q: selection quantile