Bioimage Informatics

Lecture 6, Spring 2012

Bioimage Data Analysis (I): Basic Operations

Bioimage Data Analysis (II): Feature Detection

Outline

- Basic image analysis: image filtering
- Basic image analysis: image intensity derivative calculation
- Project assignment 1

- Overview of image feature detection
- Point/particle feature detection
- Reproducible research in computational science

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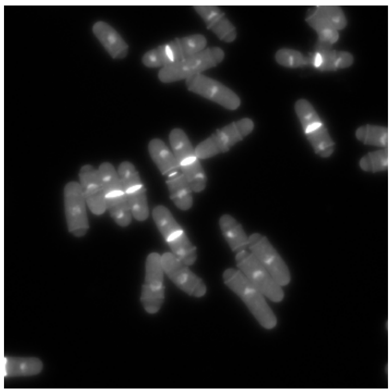
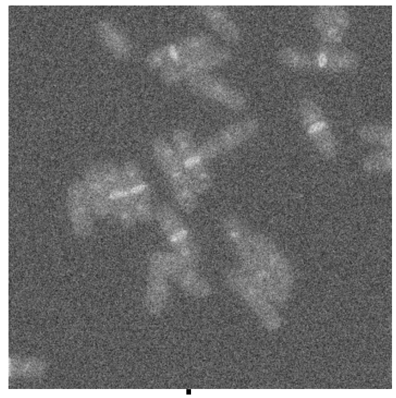
Basic Concept of Image Filtering (I)

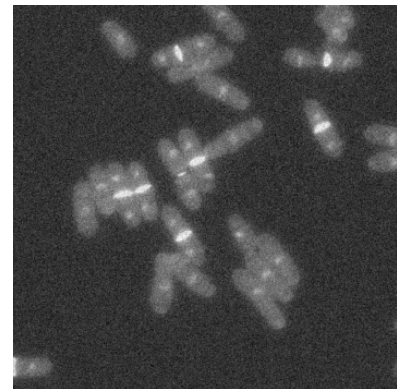
- Application I: noise suppression



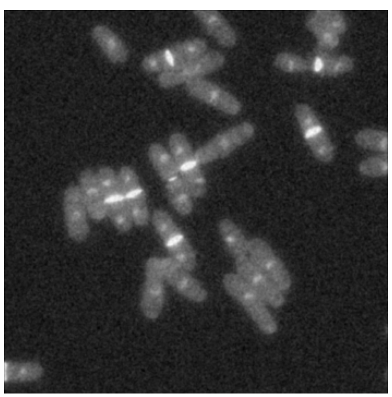
original



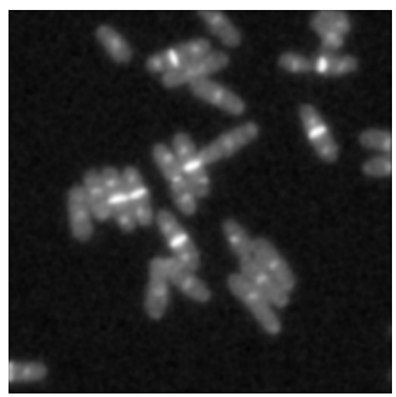
noise added



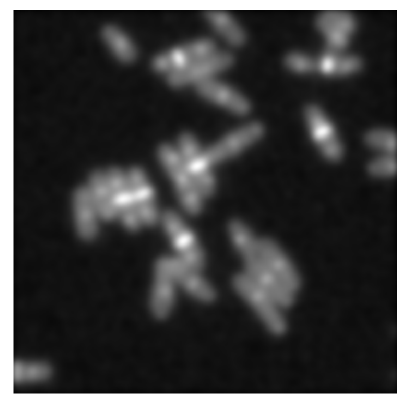
$\sigma=1$



$\sigma=2$



$\sigma=5$



$\sigma=10$

Basic Concept of Image Filtering (II)

- Application II: image conditioning

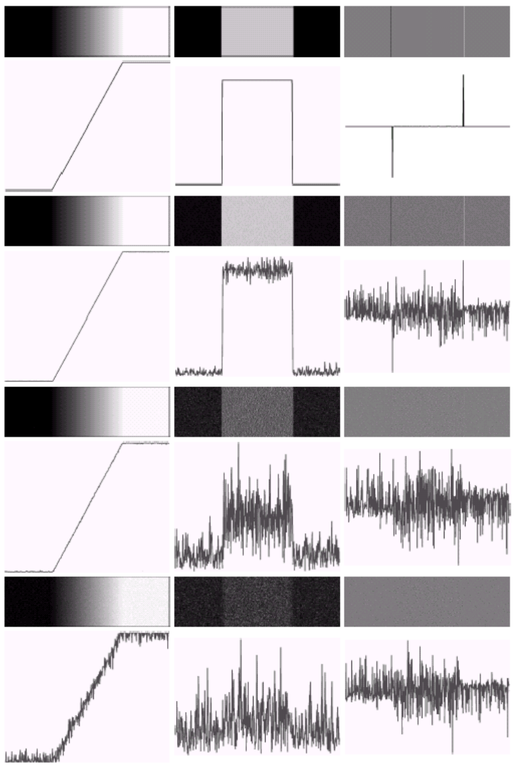


FIGURE 10.7 First column: images and gray-level profiles of a ramp edge corrupted by random Gaussian noise of mean 0 and $\sigma = 0.0, 0.1, 1.0$, and 10.0, respectively. Second column: first-derivative images and gray-level profiles. Third column: second-derivative images and gray-level profiles.

a
b
c
d

Gonzalez & Woods, DIP 2/e

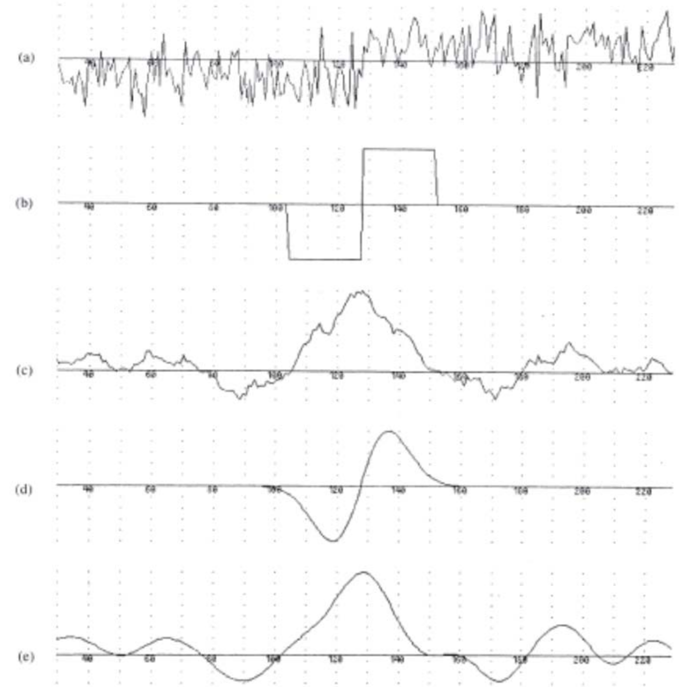


Fig. 1. (a) A noisy step edge. (b) Difference of boxes operator. (c) Difference of boxes operator applied to the edge. (d) First derivative of Gaussian operator. (e) First derivative of Gaussian applied to the edge.

Canny, J., *A Computational Approach To Edge Detection*, IEEE Trans. Pattern Analysis and Machine Intelligence, 8(6):679–698, 1986.

Basic Concept of Image Filtering (III)

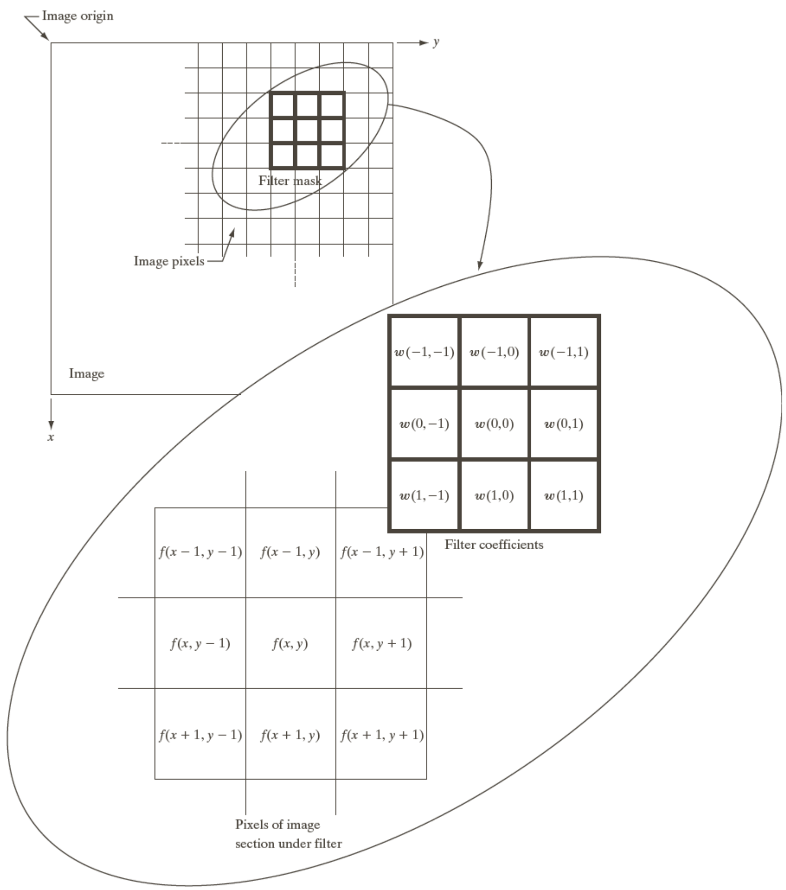


FIGURE 3.28 The mechanics of linear spatial filtering using a 3 × 3 filter mask. The form chosen to denote the coordinates of the filter mask coefficients simplifies writing expressions for linear filtering.

Gonzalez & Woods, DIP 3/e http://www.imageprocessingplace.com/

Basic Concept of Image Filtering (IV)

- Image filtering in the spatial domain

$$\sum_{s=-a}^{a}\sum_{t=-b}^{b} w(s,t)\, f(x+s, y+t) = \sum_{s=-a}^{a}\sum_{t=-b}^{b} w(-s,-t)\, f(x+s, y+t) = w(x,y) \otimes f(x,y)$$

$$f(x,y) \longrightarrow \boxed{w(x,y)} \longrightarrow g(x,y)$$

$$g(x,y) = w(x,y) \otimes f(x,y)$$

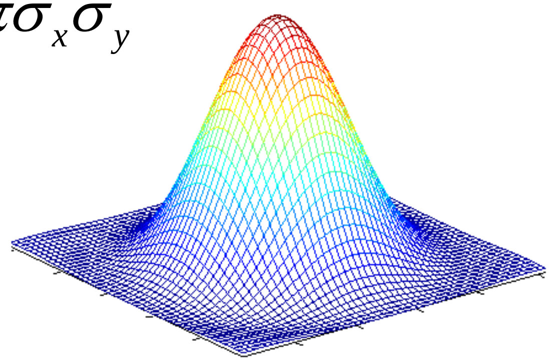
$$G(u,v) = W(u,v) \cdot F(u,v)$$

Gaussian Filter (I)

- Gaussian kernel in 1D

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

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



- 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 Filters (II)

- 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

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

  - It provides a good approximation of Airy disk

- Basic image analysis: image filtering
- **Basic image analysis: image intensity derivative calculation**
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Combination of Noise Suppression and Gradient Estimation (I)

- Implementation

$$I_x(i,j) = \frac{I(i+1,j) - I(i-1,j)}{2}$$

$$I_y(i,j) = \frac{I(i,j+1) - I(i,j-1)}{2}$$

- Notation:

  J: raw image;

  I: filtered image after convolution with Gaussian kernel G.

- A basic property of convolution

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

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Basic Image Operations

- Reading an imaging
- Accessing individual pixels
- Setting a region of interest (ROI)
- Writing an image

- Basic image analysis: image filtering
- Basic image analysis: image intensity derivative calculation
- Project assignment 1

- **Overview of image feature detection**
- Point/particle feature detection
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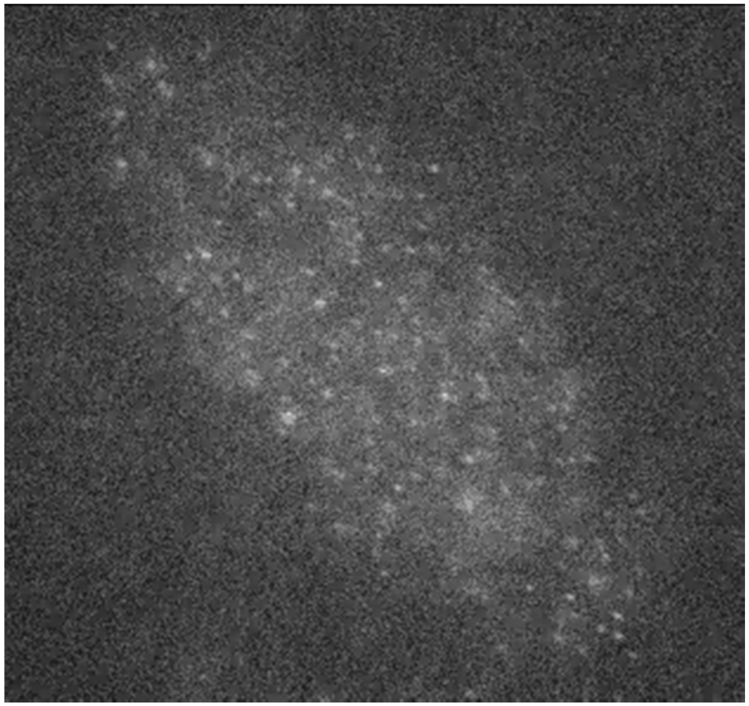
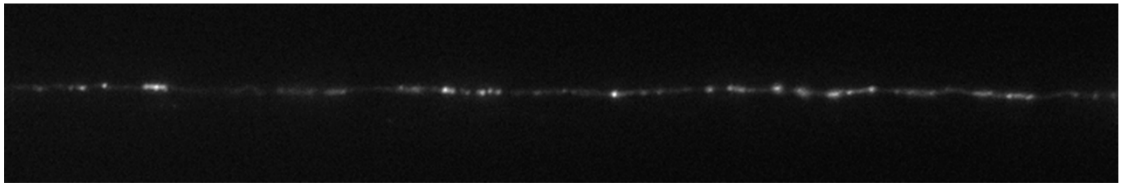
Feature Detection: Points/Particles

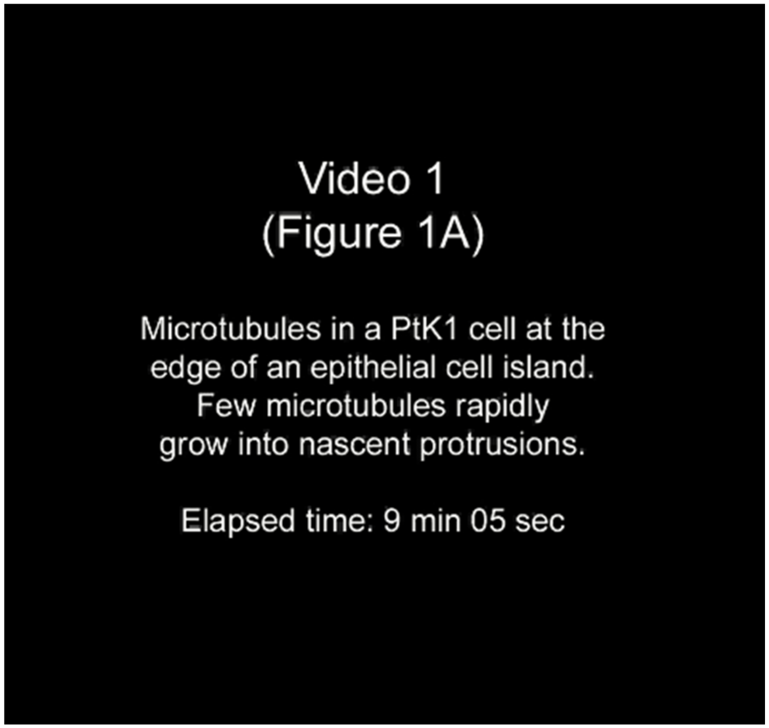


Fluorescent speckles in a Xenopus extract spindle



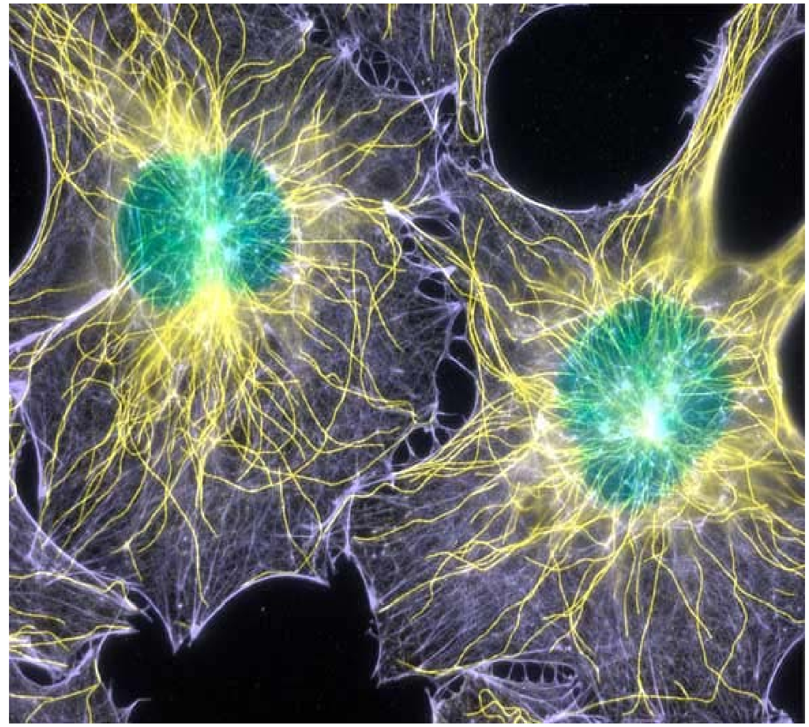
Vesicles transported in a Drosophila motor neuron

Feature Detection: Lines/Curves



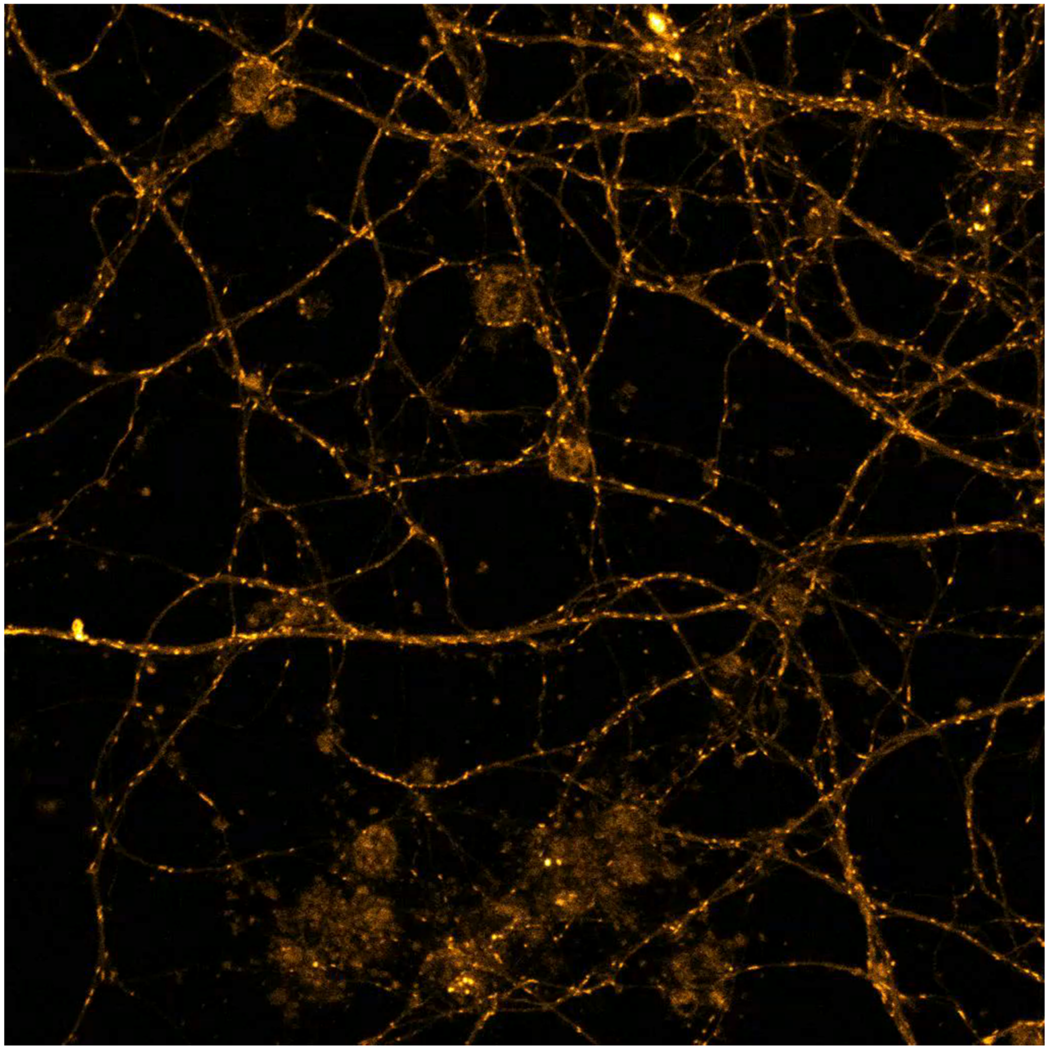
T. Wittmann et al, *J. Cell Biol.*, 161:845, 2003.

http://www.cell.com/cell_picture_show

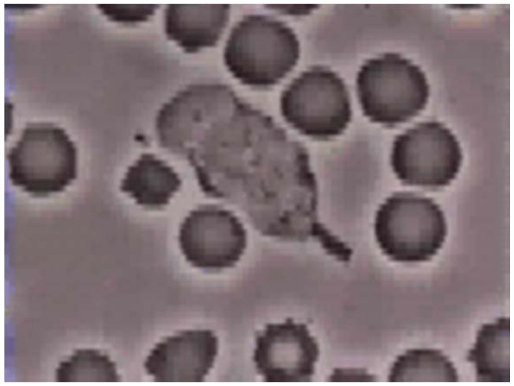


Nikon Small World, 2003
Torsten Wittmann, UCSF
Filamentous actin and microtubules (structural proteins) in mouse fibroblasts (cells) (1000x)

Feature Detection: Regions



Mitochondria in mouse hippocampal neuron, James Lim, LBNL



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

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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)

- What information is extracted from feature detection:
  - <u>point position</u>: sub-pixel resolutions are often required.

  - <u>point intensity</u>: may contain information about the number of molecules within the diffraction limit.

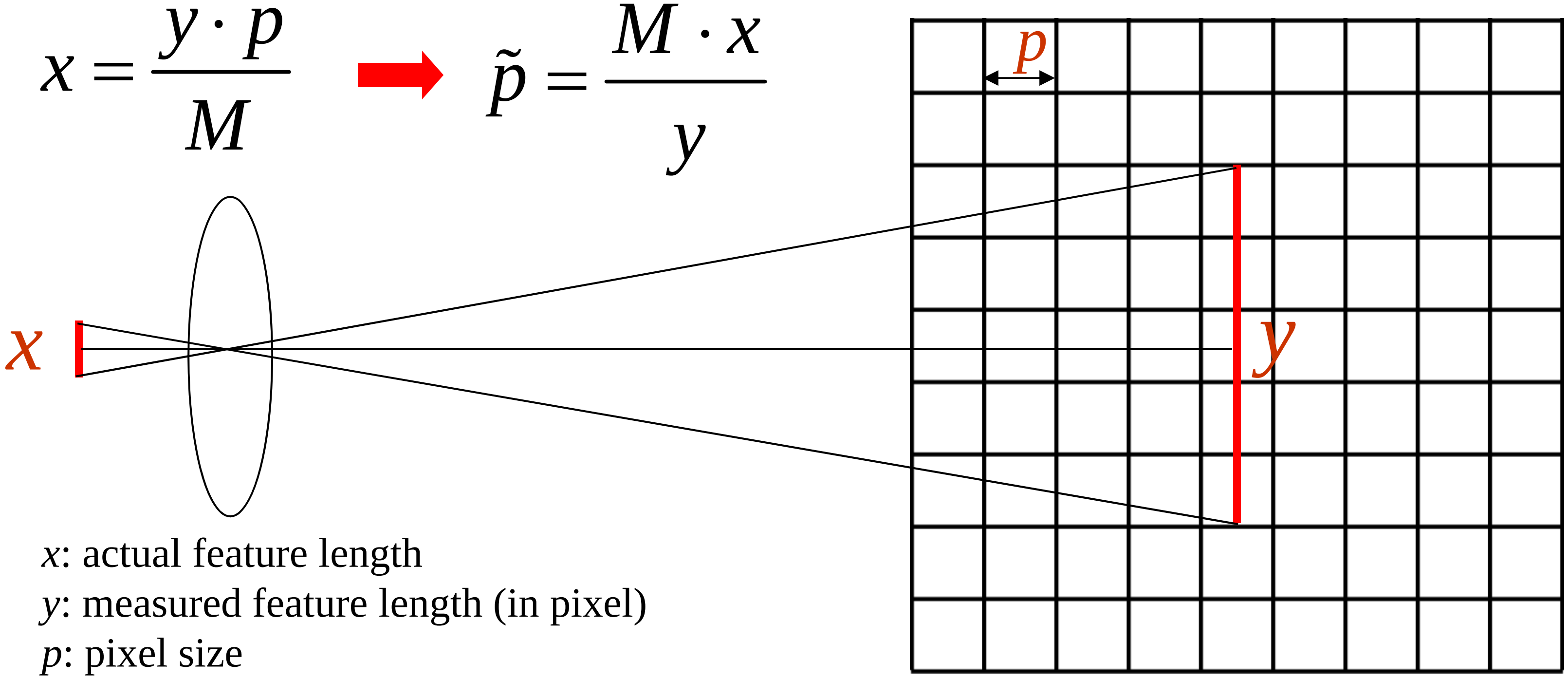
- The main purpose of point detection, and bioimage analysis in general, <u>is to get accurate and precise measurements</u>.

Microscope Camera Pixel Size Calibration

- Example: Photometrics CoolSnap HQ2
  http://www.photomet.com/products/ccdcams/coolsnap_hq2.php

- Image features are first measured in pixel coordinates

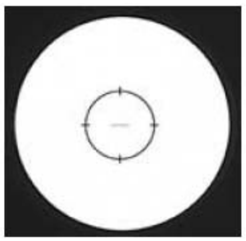
$$x = \frac{y \cdot p}{M} \Rightarrow \tilde{p} = \frac{M \cdot x}{y}$$



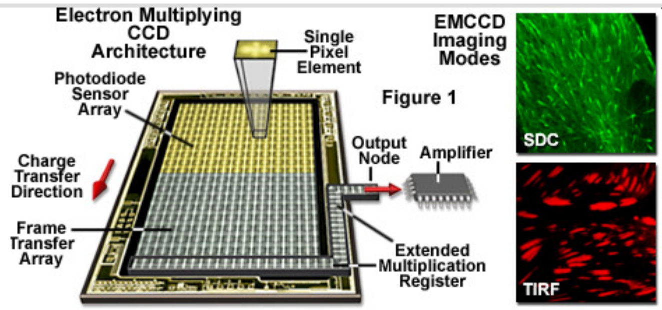
x: actual feature length
y: measured feature length (in pixel)
p: pixel size
M: magnification

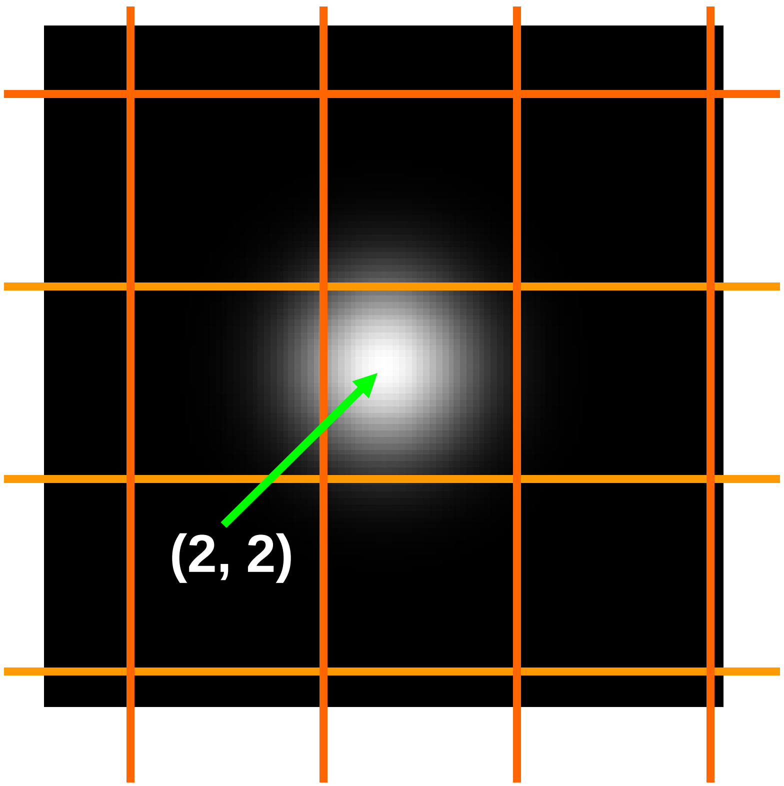

0
10
20
30
40
60
70
80
90
100

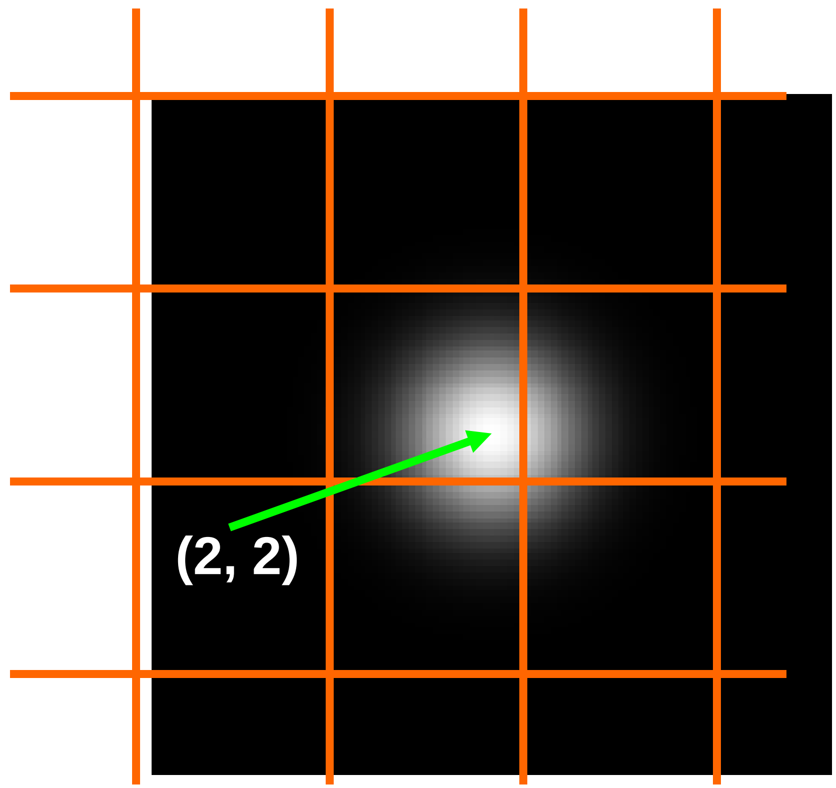

100x0.01=1mm



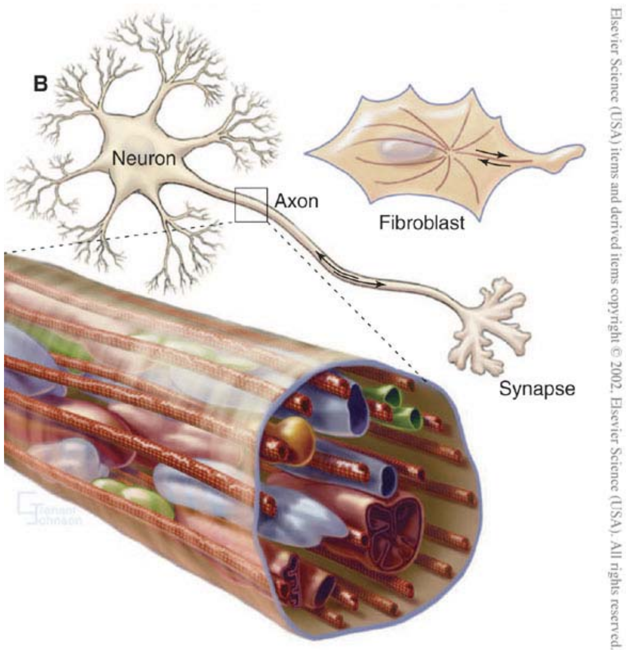
Pixel Resolution Limit in Point Detection

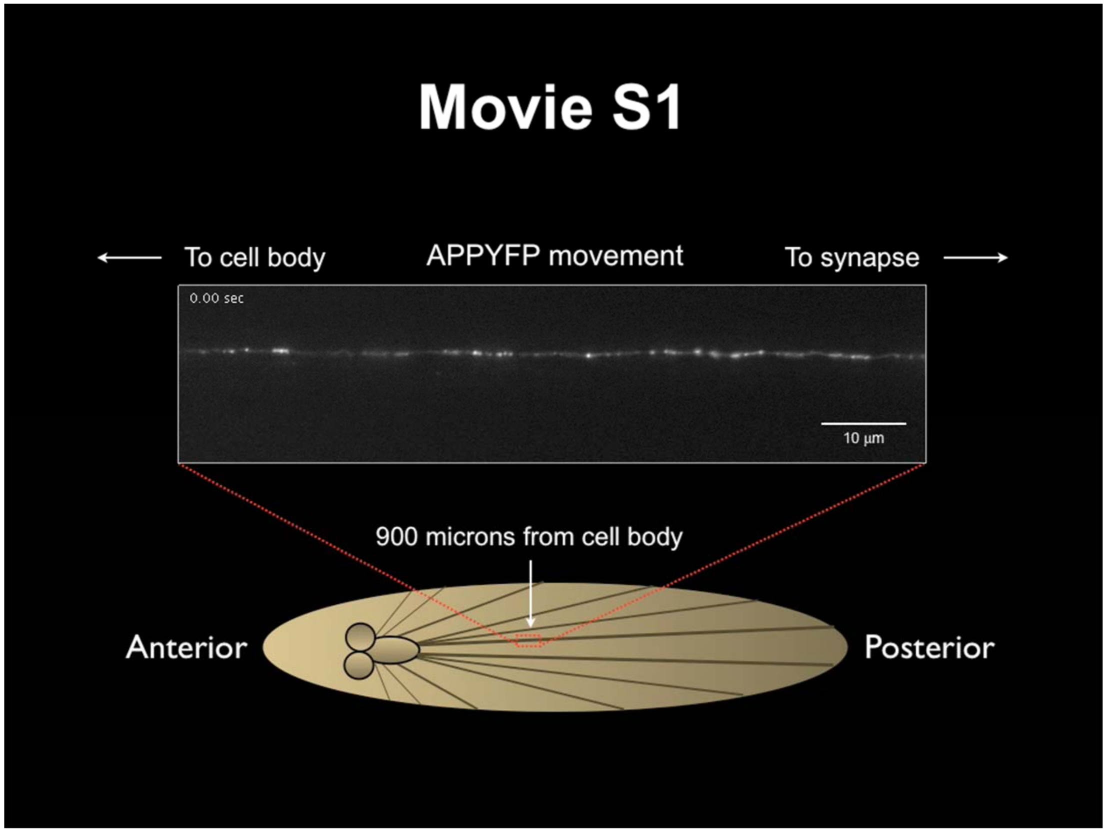




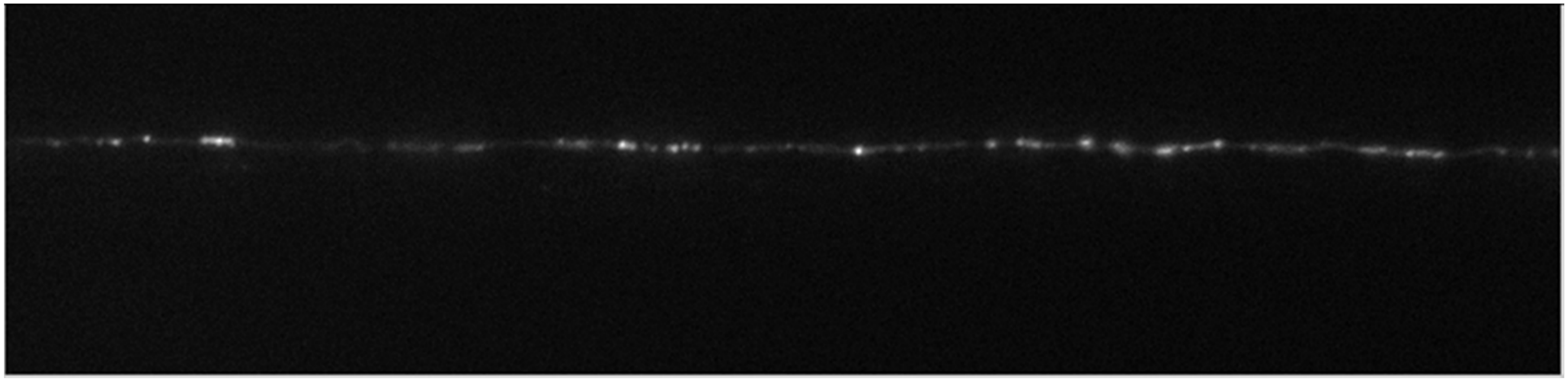


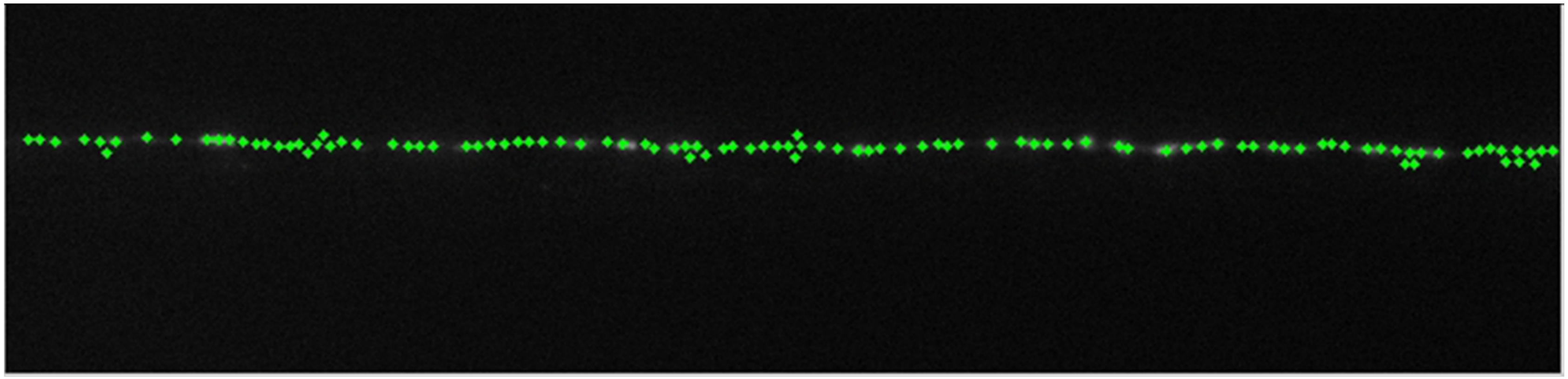
Application: Axonal Cargo Transport





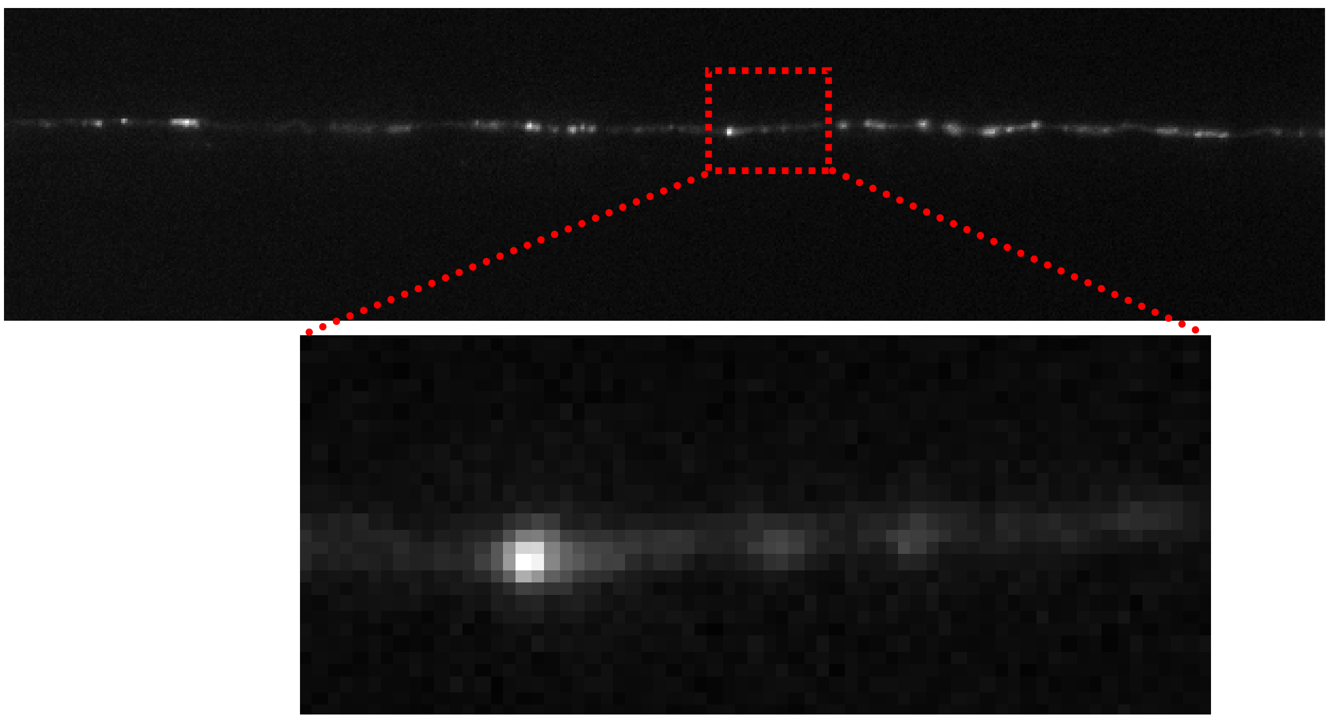
Particle Detection Demo



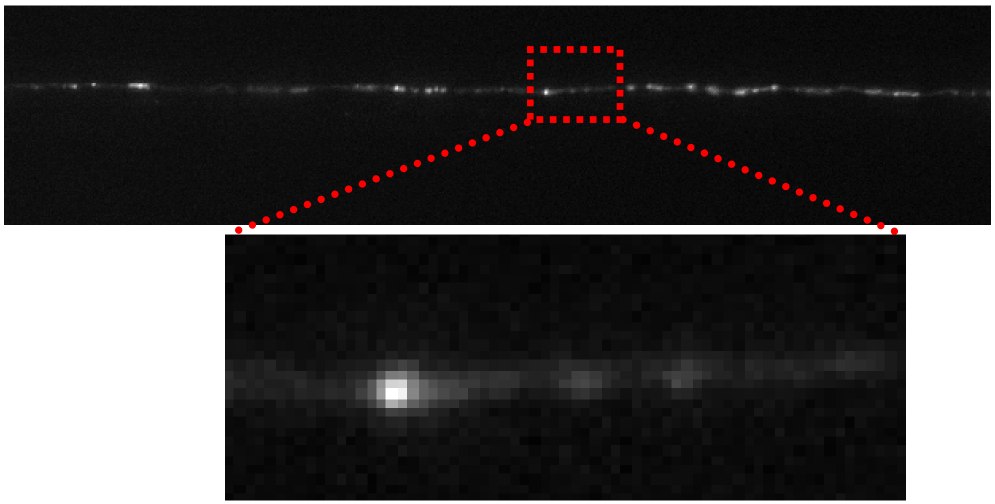


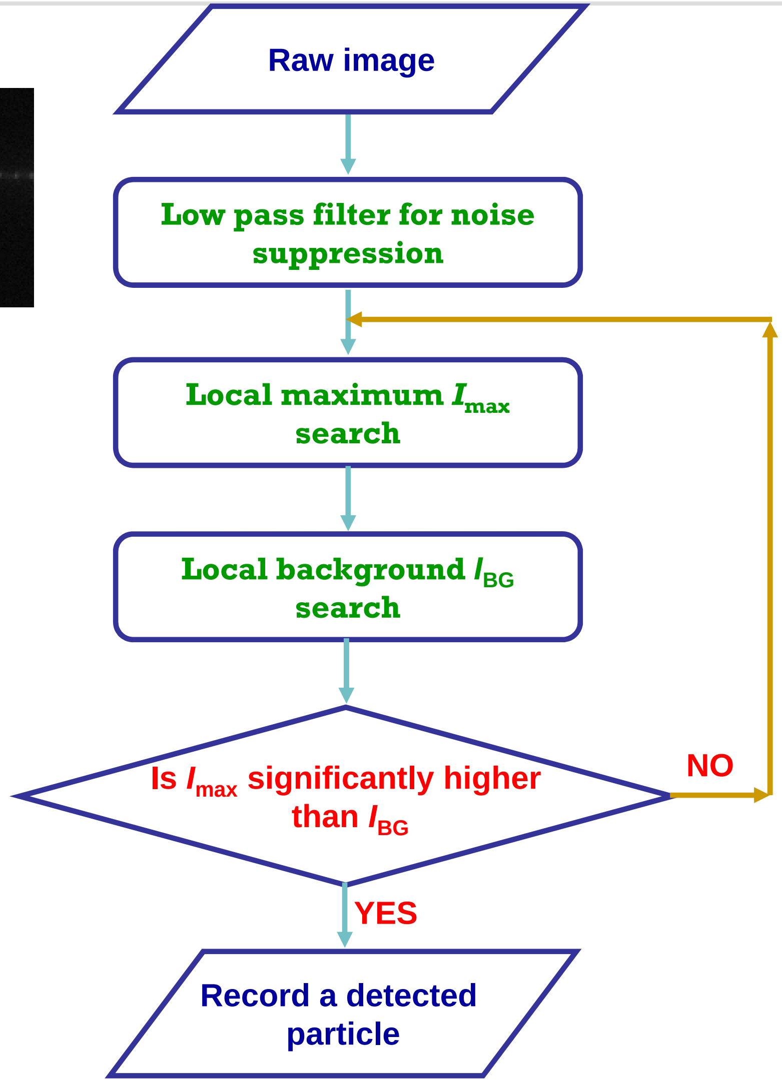
What is a Particle?

- 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)

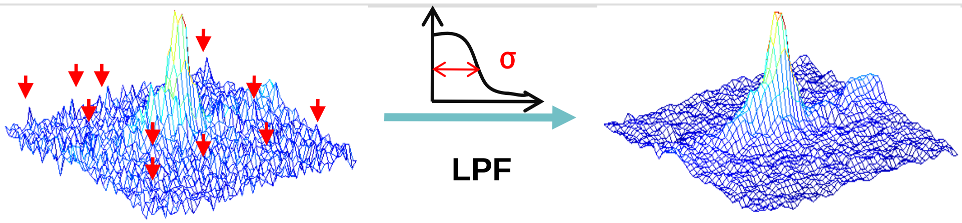
- The Fourier transform of a Gaussian kernel is Gaussian.

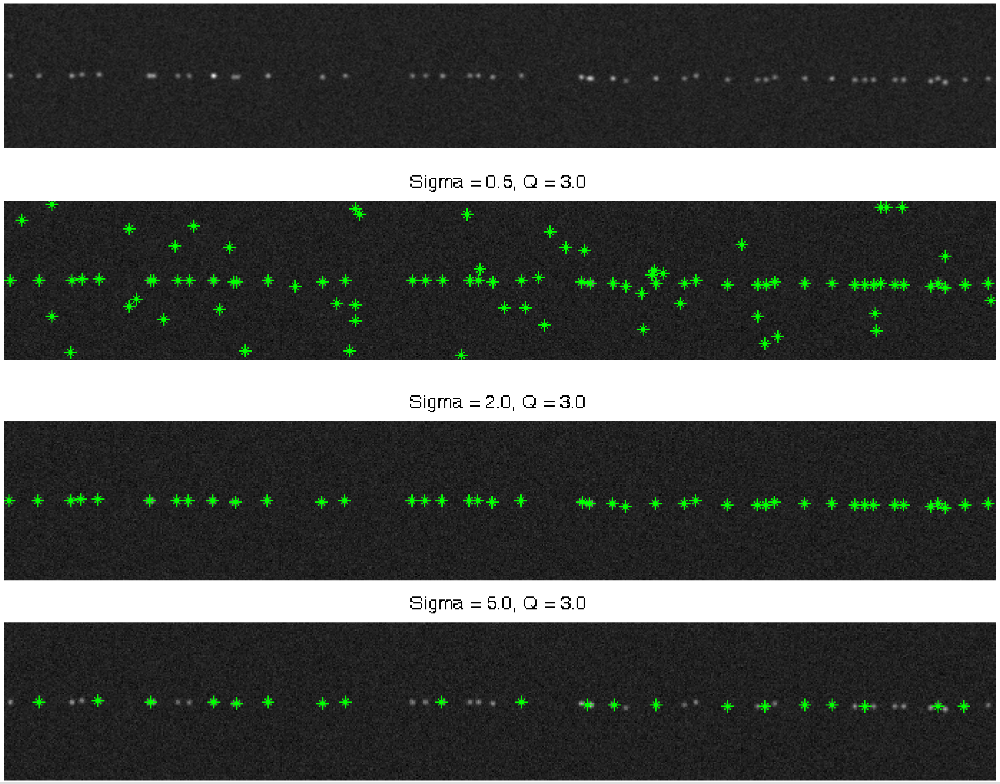
$$\frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} \frac{e^{-\frac{\sigma^2\omega^2}{2}}}{\sqrt{2\pi}}$$

- 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.

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

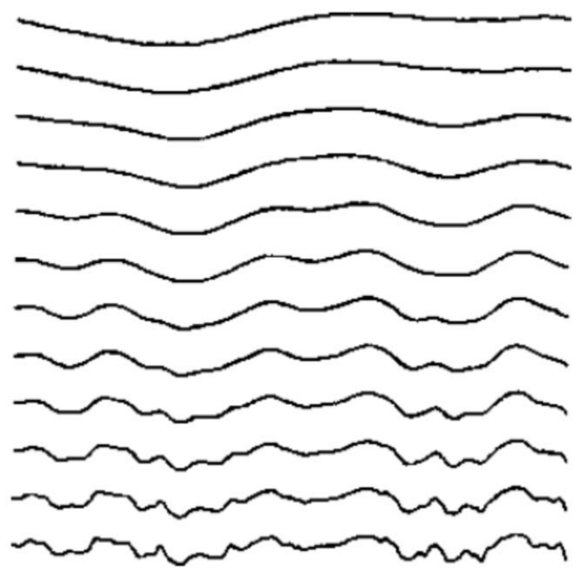
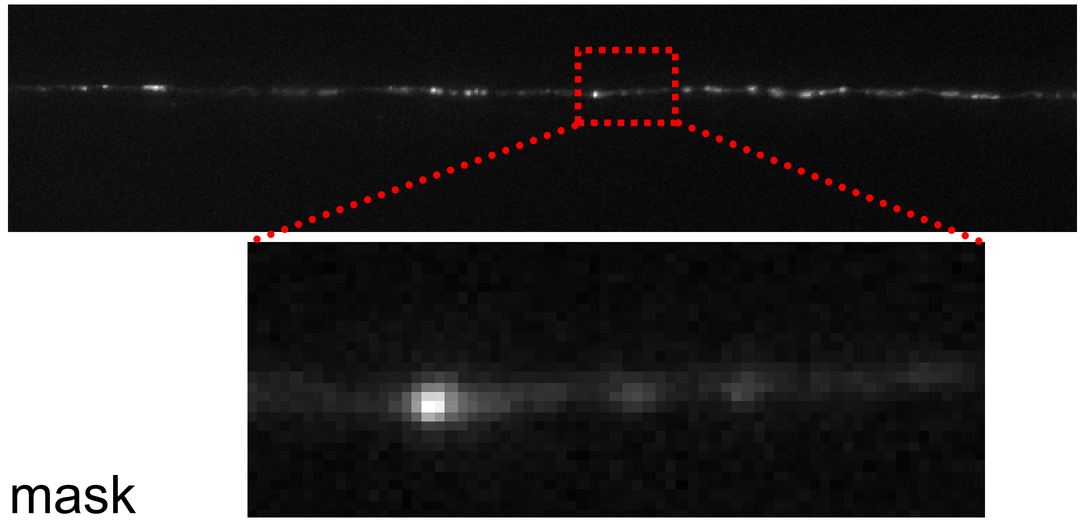


Figure 1. A sequence of gaussian smoothings of a waveform, with σ decreasing from top to bottom. Each graph is a constant-σ profile from the scale-space image.

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

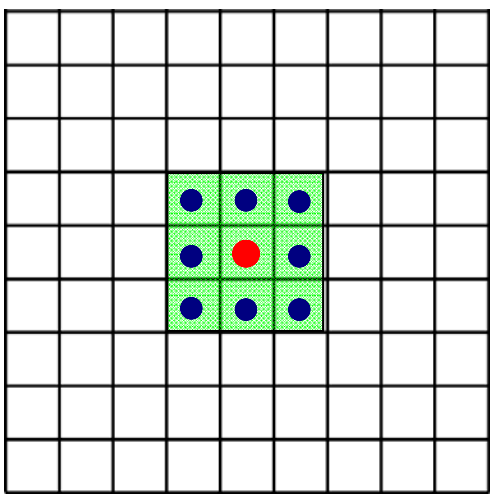
Step 2: Local Maximum Detection

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

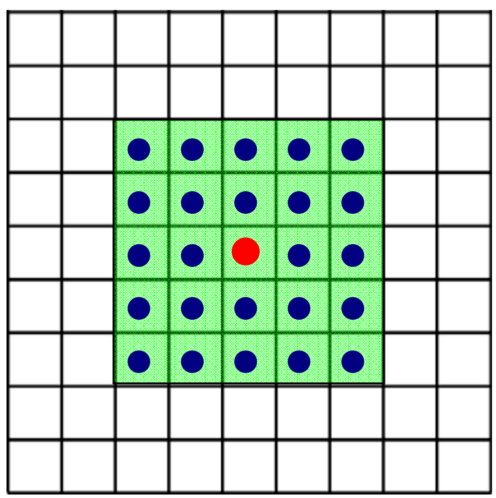


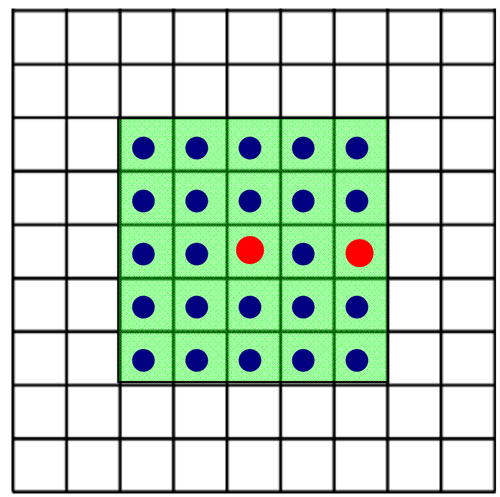
- Large masks give more stable results but lower detection resolution.

3X3 mask



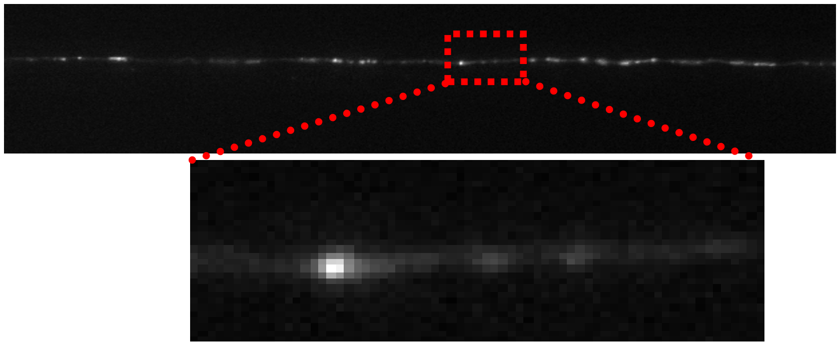
5X5 mask



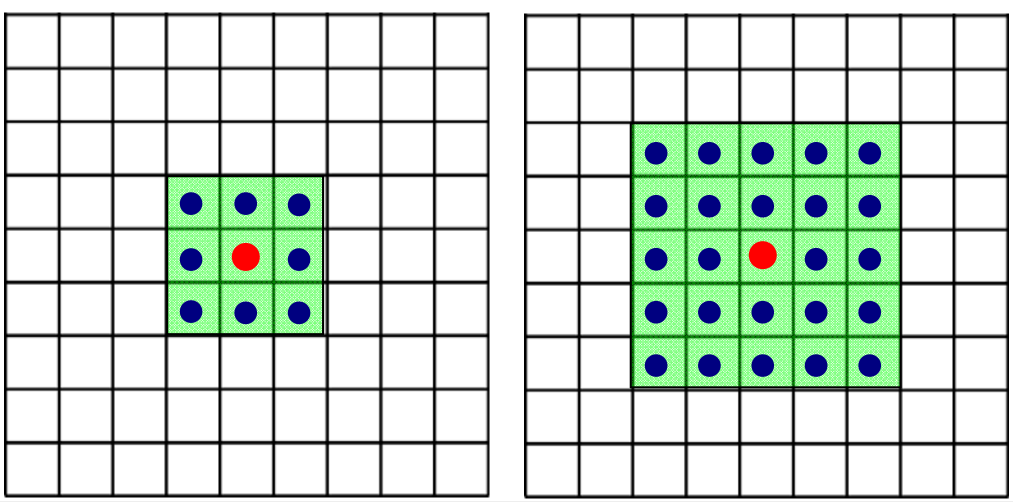


Step 3: Local Background Detection

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

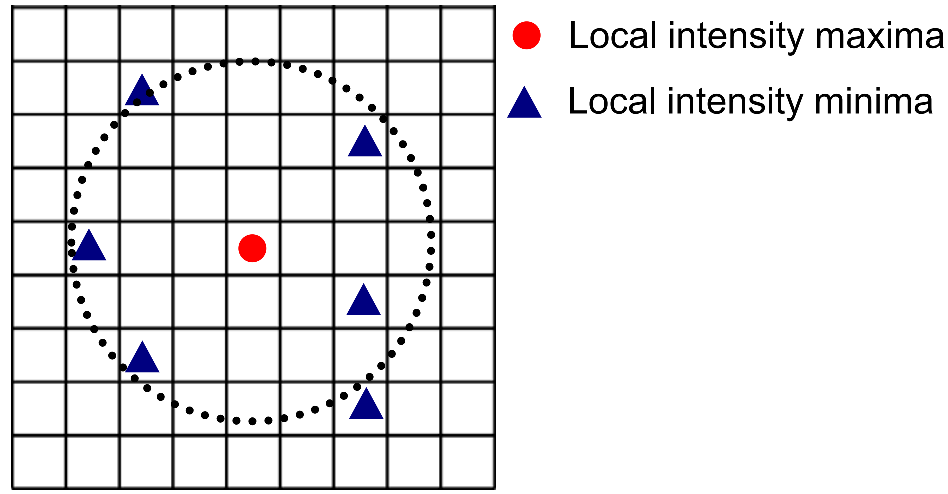


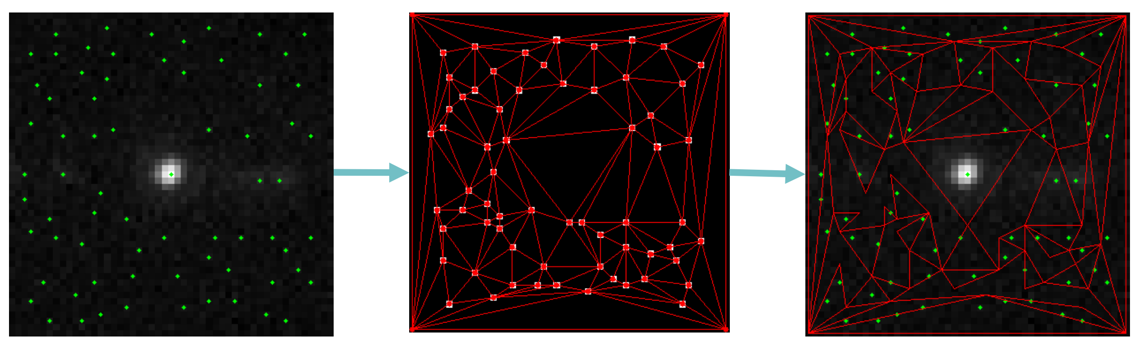
- Local background is detected through detection of local intensity minima.



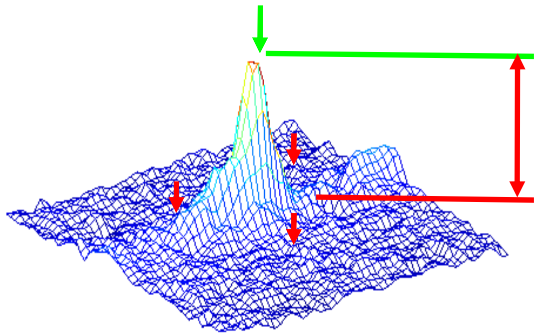
Step 3: Establishing Corresponding Between Local Maxima and Local Minima

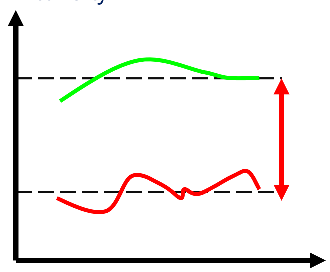
- Different approaches can be used to establish correspondence between local maxima and local minima.
  - - Nearest neighbor
  - - Delaunay triangulation





Step 4: Statistical Selection of Features

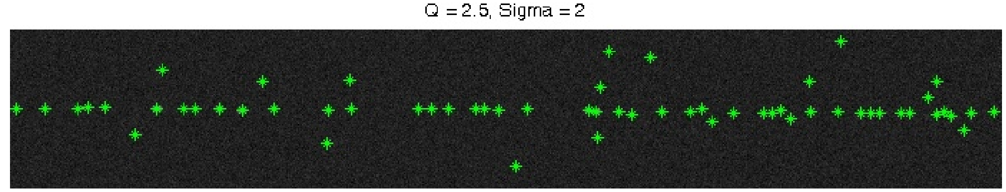




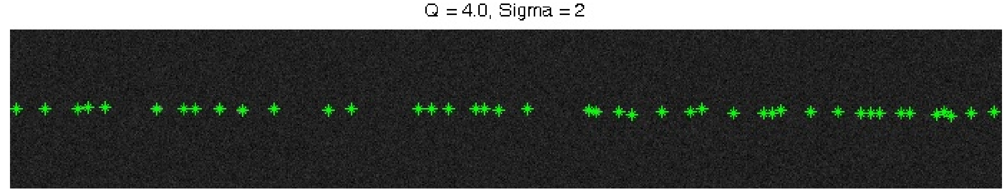
$$I_{max} - I_{BG} \geq Q \cdot \sigma_{\Delta I} ?$$

Q: selection quantile

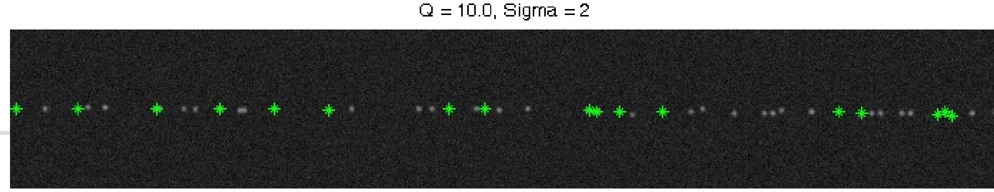
Q = 2.5, Sigma = 2



Q = 4.0, Sigma = 2



Q = 10.0, Sigma = 2



Introduction to the t-distribution

- For a normally distributed variable x~N(μ; σ), the mean of n samples follows a normal distribution

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \sim N\left(\mu, \frac{\sigma}{\sqrt{n}}\right)$$

- The normalized

$$\frac{x-\mu}{\sigma/\sqrt{n}} \sim N(0,1)$$

- The t statistic defined by

$$\frac{x-\mu}{s/\sqrt{n}} \text{ where } s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}$$

A Review of Two Sample t-test

- Two-sample t significance test

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\dfrac{s_1^2}{n_1} + \dfrac{s_2^2}{n_2}}}$$

Table entry for p and C is the critical value t^* with probability p lying to its right and probability C lying between $-t^*$ and t^*.

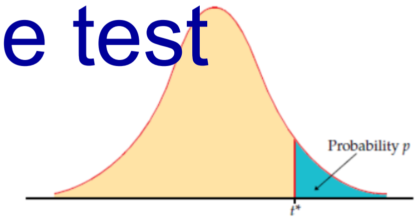


TABLE D

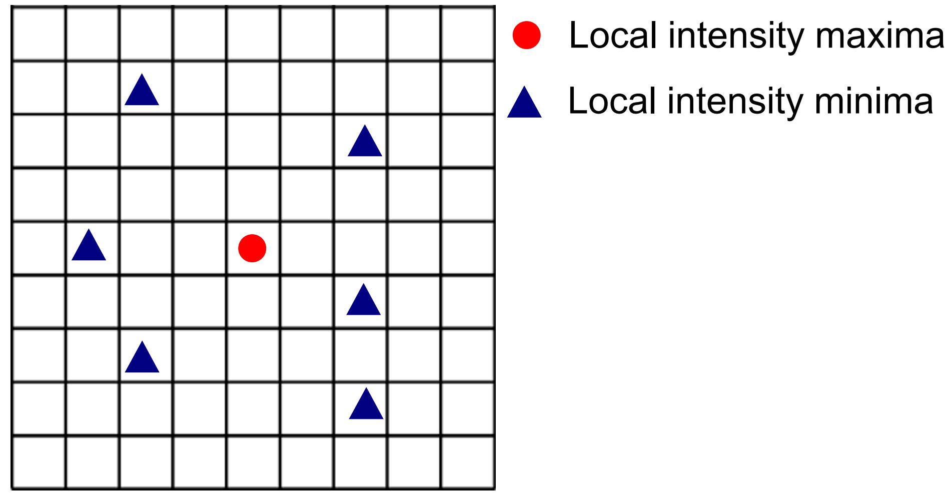
t distribution critical values

	Upper-tail probability *p*											
df	.25	.20	.15	.10	.05	.025	.02	.01	.005	.0025	.001	.0005
1	1.000	1.376	1.963	3.078	6.314	12.71	15.89	31.82	63.66	127.3	318.3	636.6
2	0.816	1.061	1.386	1.886	2.920	4.303	4.849	6.965	9.925	14.09	22.33	31.60
3	0.765	0.978	1.250	1.638	2.353	3.182	3.482	4.541	5.841	7.453	10.21	12.92
4	0.741	0.941	1.190	1.533	2.132	2.776	2.999	3.747	4.604	5.598	7.173	8.610
5	0.727	0.920	1.156	1.476	2.015	2.571	2.757	3.365	4.032	4.773	5.893	6.869
6	0.718	0.906	1.134	1.440	1.943	2.447	2.612	3.143	3.707	4.317	5.208	5.959
7	0.711	0.896	1.119	1.415	1.895	2.365	2.517	2.998	3.499	4.029	4.785	5.408
8	0.706	0.889	1.108	1.397	1.860	2.306	2.449	2.896	3.355	3.833	4.501	5.041
9	0.703	0.883	1.100	1.383	1.833	2.262	2.398	2.821	3.250	3.690	4.297	4.781
10	0.700	0.879	1.093	1.372	1.812	2.228	2.359	2.764	3.169	3.581	4.144	4.587
11	0.697	0.876	1.088	1.363	1.796	2.201	2.328	2.718	3.106	3.497	4.025	4.437
12	0.695	0.873	1.083	1.356	1.782	2.179	2.303	2.681	3.055	3.428	3.930	4.318
13	0.694	0.870	1.079	1.350	1.771	2.160	2.282	2.650	3.012	3.372	3.852	4.221
14	0.692	0.868	1.076	1.345	1.761	2.145	2.264	2.624	2.977	3.326	3.787	4.140
15	0.691	0.866	1.074	1.341	1.753	2.131	2.249	2.602	2.947	3.286	3.733	4.073
16	0.690	0.865	1.071	1.337	1.746	2.120	2.235	2.583	2.921	3.252	3.686	4.015
17	0.689	0.863	1.069	1.333	1.740	2.110	2.224	2.567	2.898	3.222	3.646	3.965
18	0.688	0.862	1.067	1.330	1.734	2.101	2.214	2.552	2.878	3.197	3.611	3.922
19	0.688	0.861	1.066	1.328	1.729	2.093	2.205	2.539	2.861	3.174	3.579	3.883
20	0.687	0.860	1.064	1.325	1.725	2.086	2.197	2.528	2.845	3.153	3.552	3.850
21	0.686	0.859	1.063	1.323	1.721	2.080	2.189	2.518	2.831	3.135	3.527	3.819
22	0.686	0.858	1.061	1.321	1.717	2.074	2.183	2.508	2.819	3.119	3.505	3.792
23	0.685	0.858	1.060	1.319	1.714	2.069	2.177	2.500	2.807	3.104	3.485	3.768
24	0.685	0.857	1.059	1.318	1.711	2.064	2.172	2.492	2.797	3.091	3.467	3.745
25	0.684	0.856	1.058	1.316	1.708	2.060	2.167	2.485	2.787	3.078	3.450	3.725
26	0.684	0.856	1.058	1.315	1.706	2.056	2.162	2.479	2.779	3.067	3.435	3.707
27	0.684	0.855	1.057	1.314	1.703	2.052	2.158	2.473	2.771	3.057	3.421	3.690
28	0.683	0.855	1.056	1.313	1.701	2.048	2.154	2.467	2.763	3.047	3.408	3.674
29	0.683	0.854	1.055	1.311	1.699	2.045	2.150	2.462	2.756	3.038	3.396	3.659
30	0.683	0.854	1.055	1.310	1.697	2.042	2.147	2.457	2.750	3.030	3.385	3.646
40	0.681	0.851	1.050	1.303	1.684	2.021	2.123	2.423	2.704	2.971	3.307	3.551
50	0.679	0.849	1.047	1.299	1.676	2.009	2.109	2.403	2.678	2.937	3.261	3.496
60	0.679	0.848	1.045	1.296	1.671	2.000	2.099	2.390	2.660	2.915	3.232	3.460
80	0.678	0.846	1.043	1.292	1.664	1.990	2.088	2.374	2.639	2.887	3.195	3.416
100	0.677	0.845	1.042	1.290	1.660	1.984	2.081	2.364	2.626	2.871	3.174	3.390
1000	0.675	0.842	1.037	1.282	1.646	1.962	2.056	2.330	2.581	2.813	3.098	3.300
z^*	0.674	0.841	1.036	1.282	1.645	1.960	2.054	2.326	2.576	2.807	3.091	3.291
	50%	60%	70%	80%	90%	95%	96%	98%	99%	99.5%	99.8%	99.9%
	Confidence level *C*											

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

Camera Noise Model

- Signal $S = I \cdot QE \cdot T$

- Signal shot noise $N_{shot} = \sqrt{S}$

- Camera noise $N_{dark} = \sqrt{D \cdot T}$ $N_{dark} = \sqrt{N_{read}^2 + N_{dark}^2}$

- Total noise $N_{total} = \sqrt{N_{shot}^2 + N_{read}^2 + N_{dark}^2}$

References

- A. Ponti et al, Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy, *Biophysical Journal*, 84:3336-3352, 2003.

- Moore et al, *Introduction to the practice of statistics*, 6th ed., W. H. Freeman, 2009.

Open Source & Reproducible Research

An article about computational science in a scientific publication is not the scholarship itself, it is merely advertising of the scholarship. The actual scholarship is the complete software development environment and the complete set of instructions which generated the figures.

—D. Donoho (http://www-stat.stanford.edu/~donoho/)

- Jon Claerbout is often credited as the first who proposed reproducible research.

- There are challenges. But these challenges can be overcome.

- Methods for public-funded biological studies should be open-source.

http://reproducibleresearch.net/index.php/Main_Page

http://sepwww.stanford.edu/data/media/public/sep/jon/

Open Source & Reproducible Research (II)

- Current literatures of image processing and computer vision often are formulated mathematically and do not provide source code.

- Challenges
  - implementation (numerical issues)
  - parameter tuning
  - robustness a major performance issue

Some General Comments

- It is possible but limiting to consider bioimage analysis as just another application.

- Excellent research opportunities in bioimage informatics

- Challenges
    - Solid training in image processing and computer vision
    - Interdisciplinary background and thinking
        - For identifying and solving problems
        - For collaboration
    - "Non-traditional" career path

Questions?