Bioimage Informatics

Lecture 7, Spring 2012

Bioimage Data Analysis (II): Point Feature Detection

Outline

- Overview of image feature detection
- Point/particle feature detection
- Demonstration
- Reproducible research in computational science
- Other point/particle feature detection techniques

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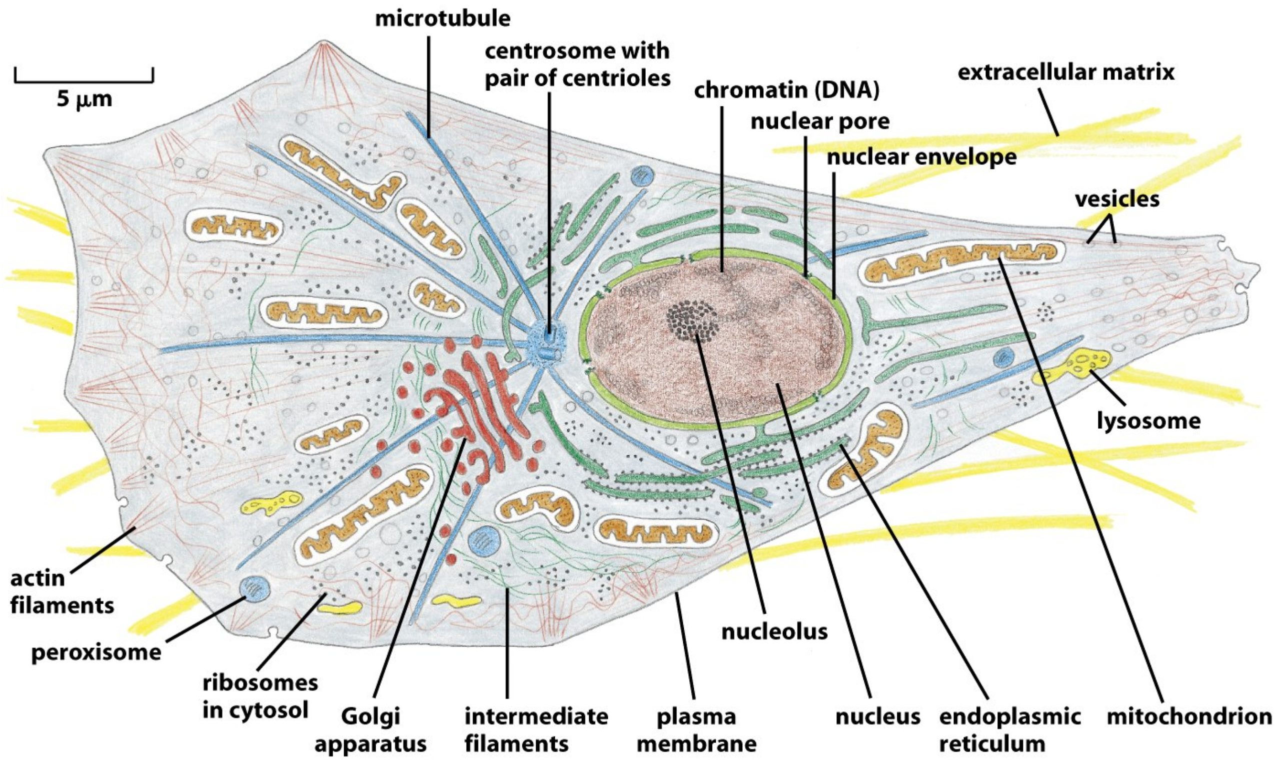
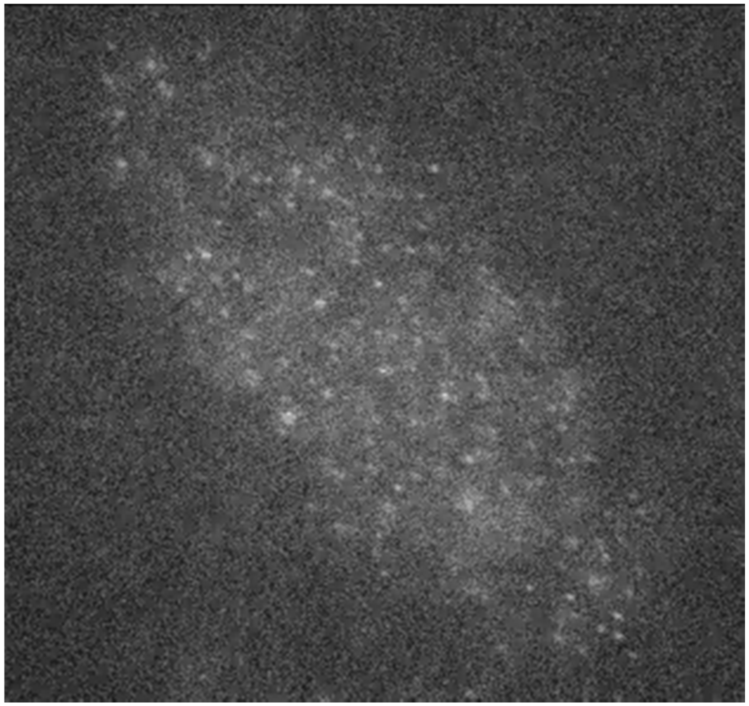
Image Feature Detection

- Point/particle features: diffraction-limited features
- Line/curve features
- Region/blob features
- Feature detection is often the first step in image data analysis.

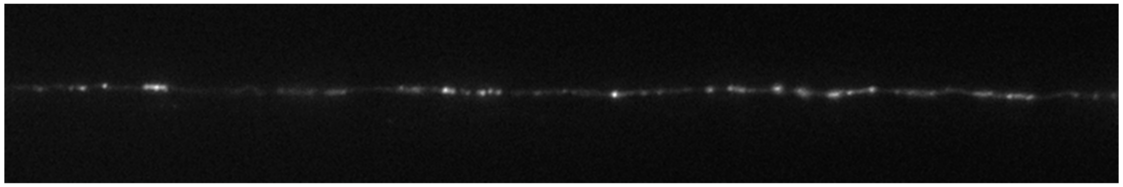


Alberts et al, MBoC, 5/e

Feature Detection: Points/Particles



Fluorescent speckles in a Xenopus extract spindle



Vesicles transported in a Drosophila motor neuron

- Overview of image feature detection
- Point/particle feature detection
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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 research articles use "particle detection" and "particle tracking" interchangeably. This may cause confusion.

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

Point Feature Detection (II)

- 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 main purpose of point detection, and bioimage analysis in general, is to get accurate and precise measurements.

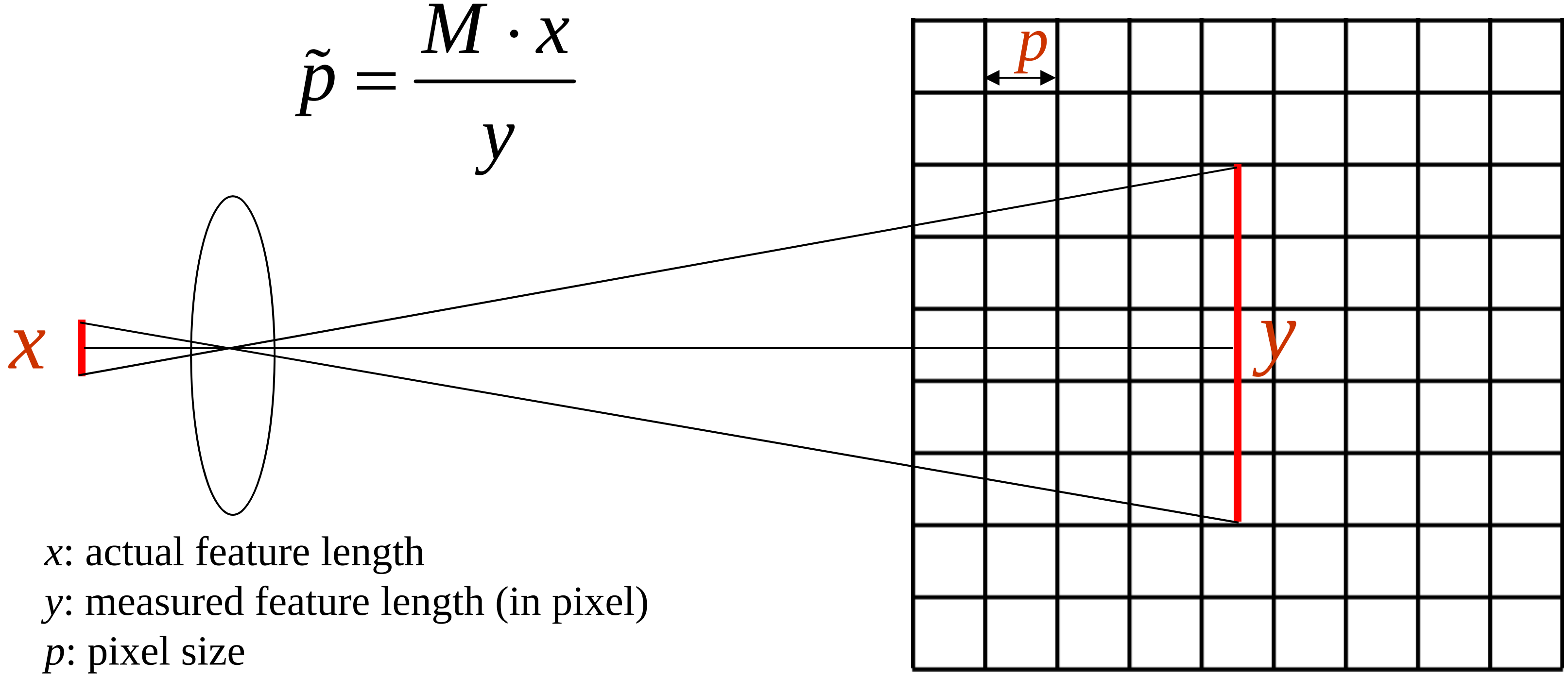
Microscope Camera Pixel Size Calibration

- Example: Photometrics CoolSnap HQ2

  http://www.photomet.com/products/ccdcams/coolsnap_hq2.php

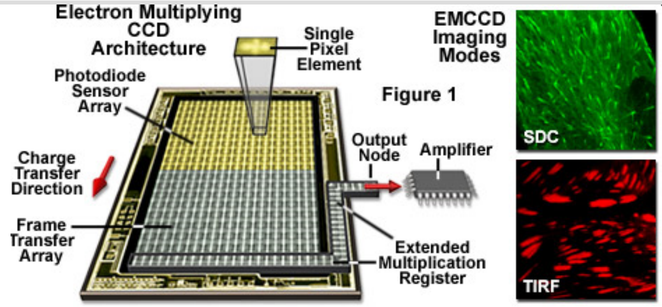
- Image features are first measured in pixel coordinates

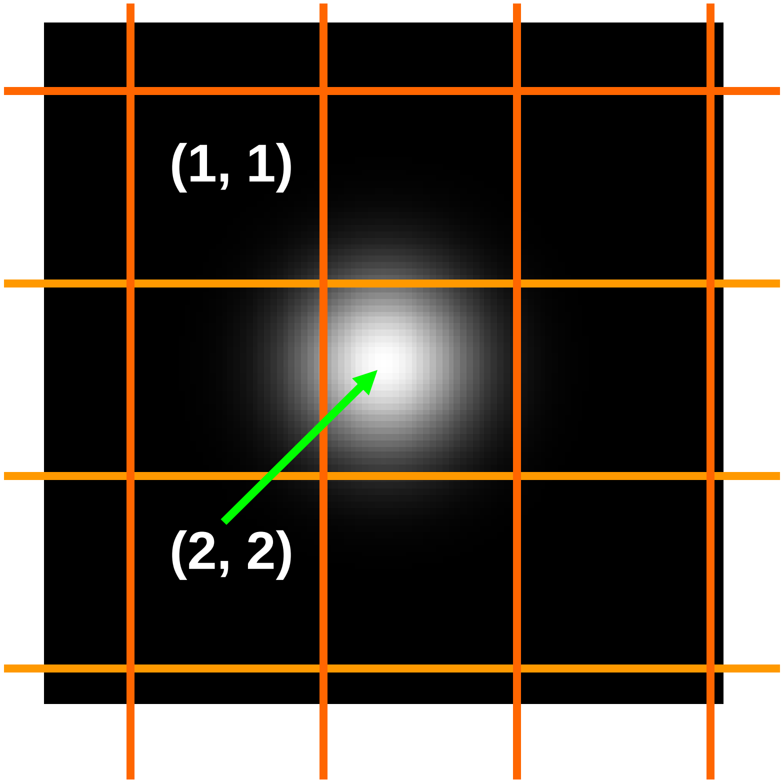
$$\tilde{p} = \frac{M \cdot x}{y}$$

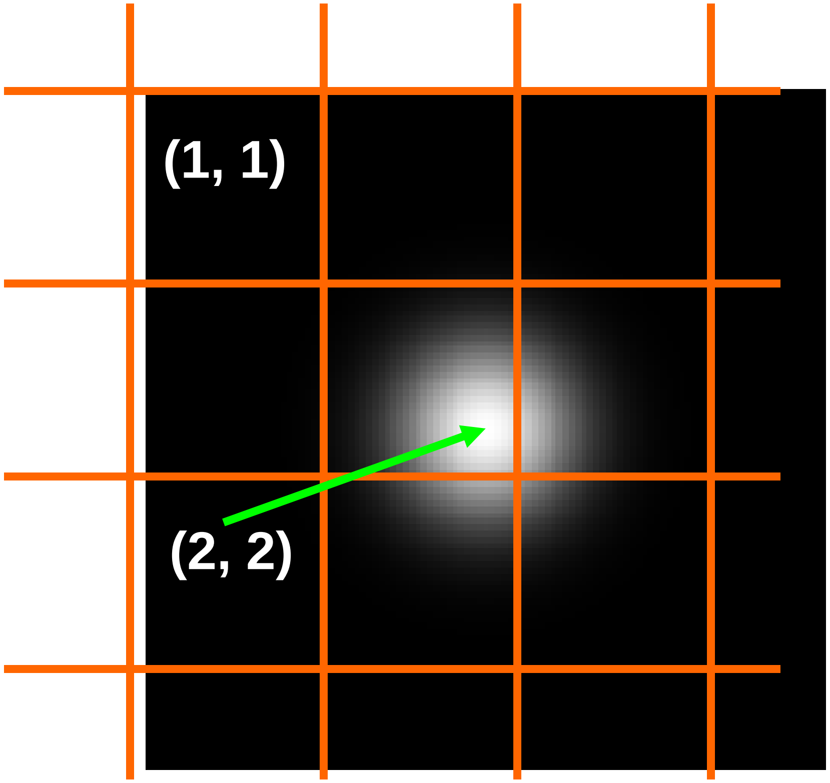


x: actual feature length
y: measured feature length (in pixel)
p: pixel size
M: magnification

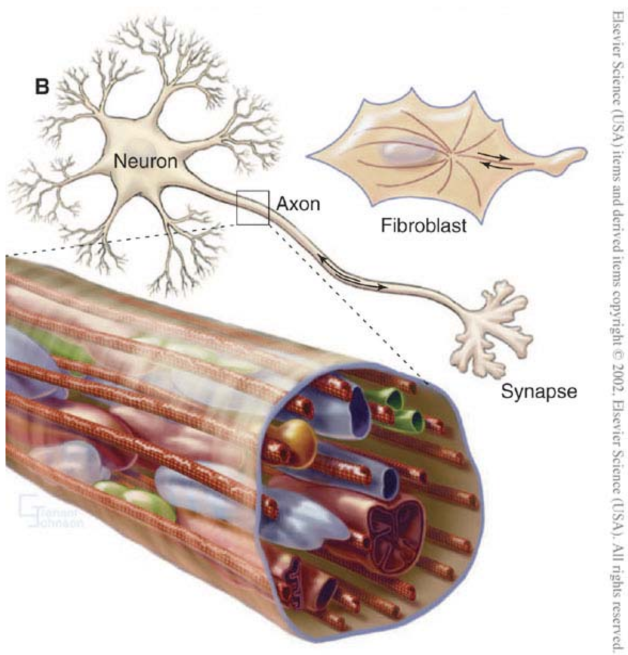
Pixel Resolution Limit in Point Detection

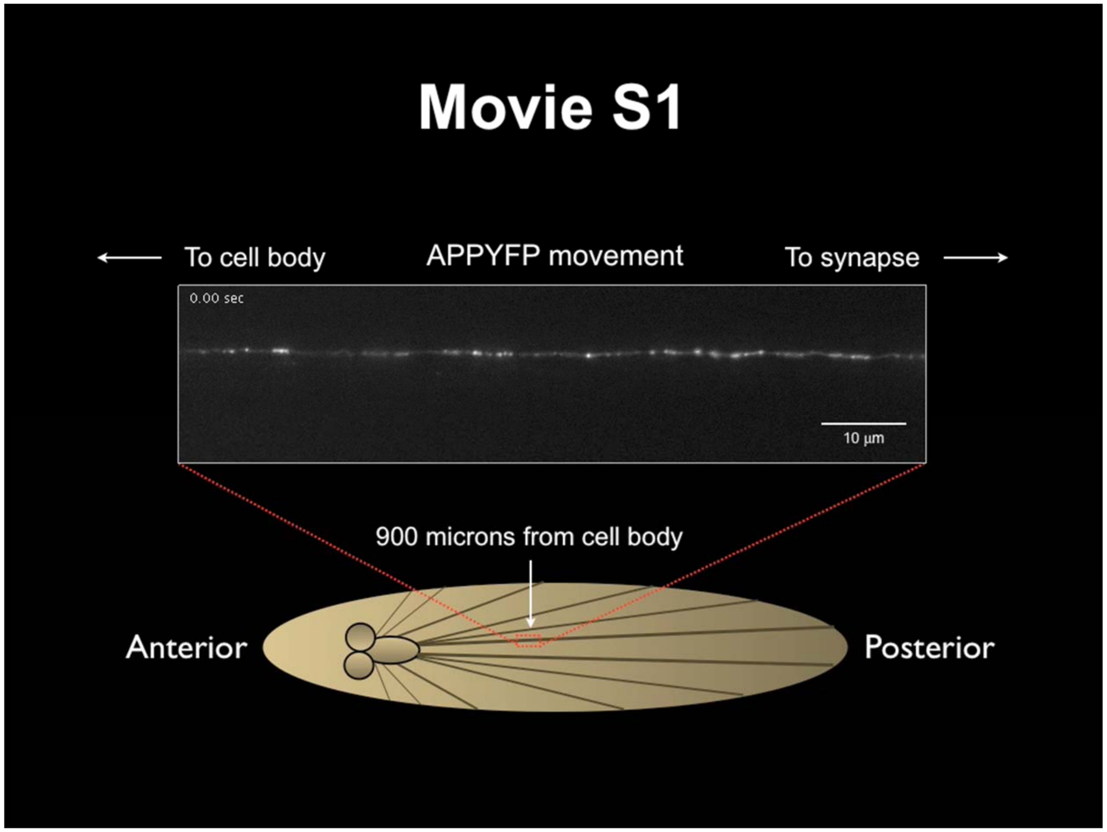




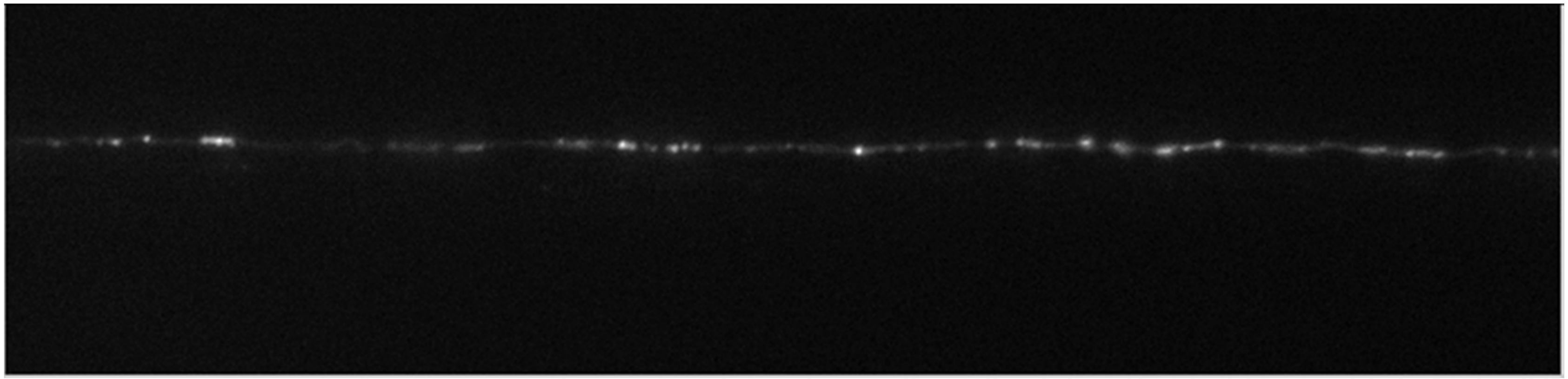


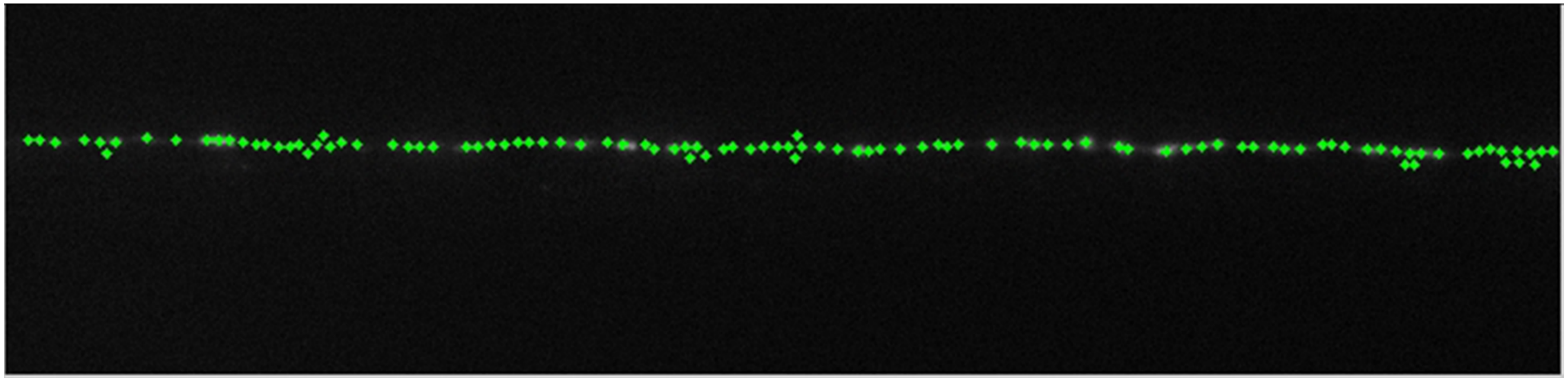
Application: Axonal Cargo Transport





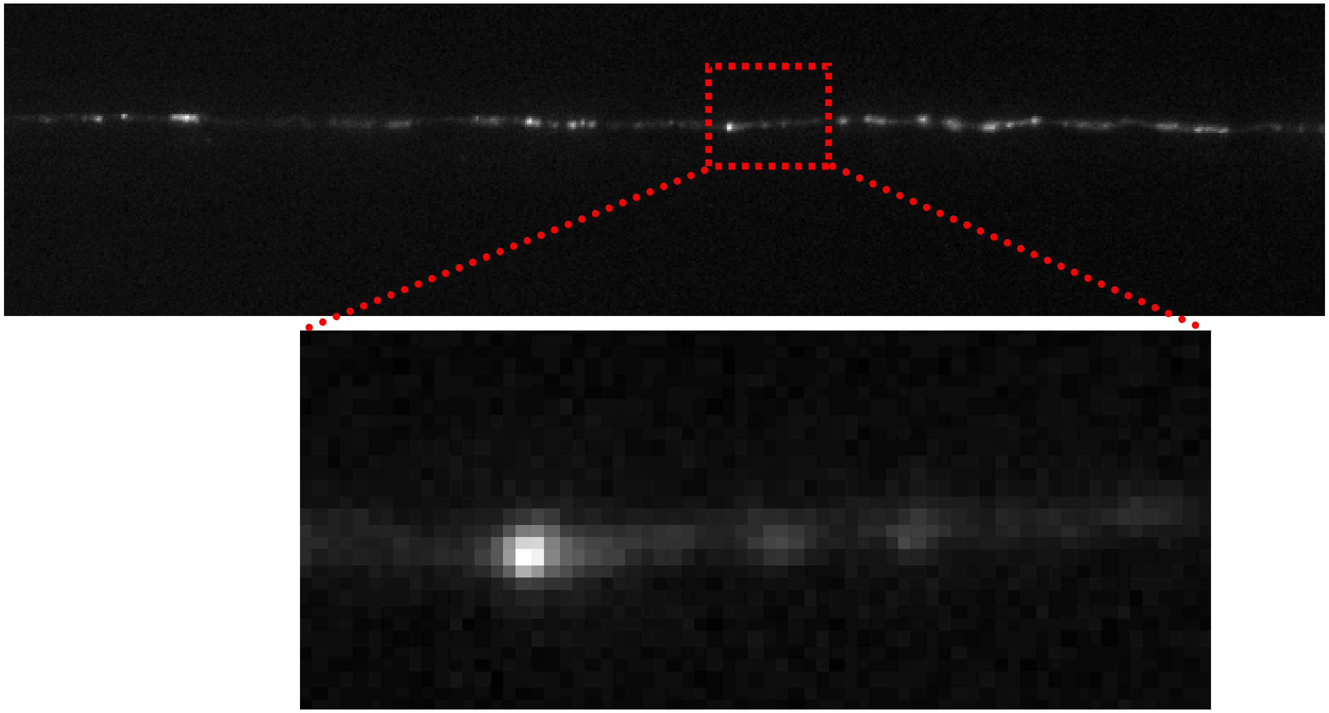
Particle Detection Result



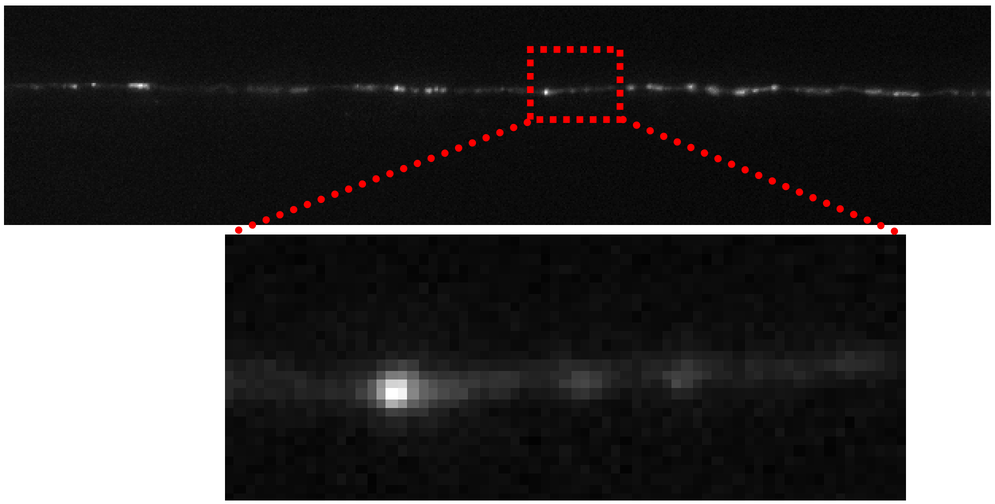


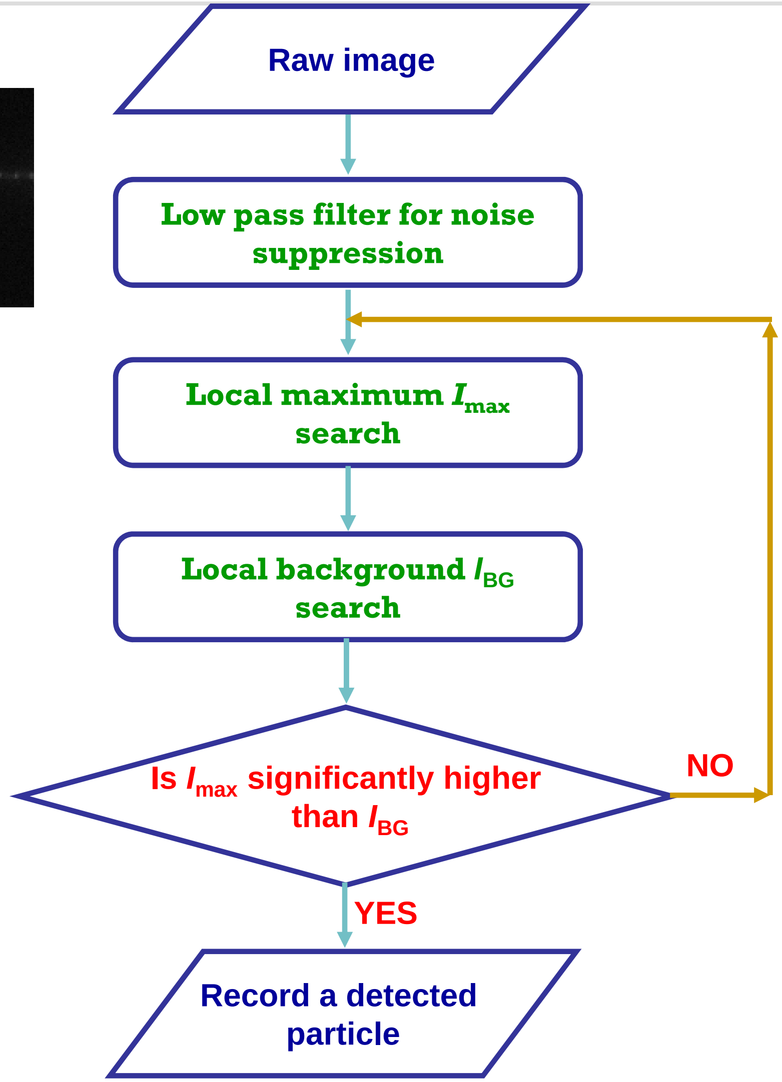
What is a Particle?

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



Analysis Procedure of Particle Detection

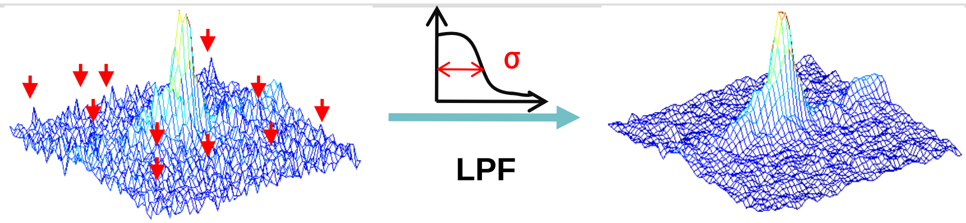




Step 1: Low Pass Filter (I)

- The Fourier transform of a Gaussian kernel is Gaussian.

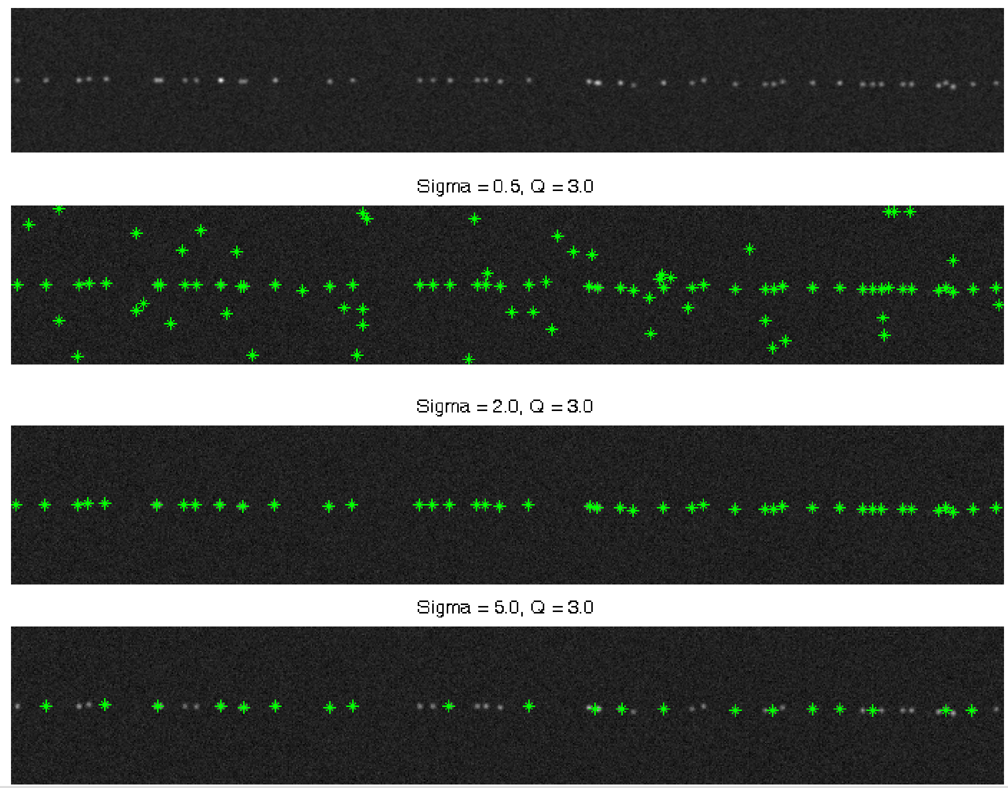
$$\frac{1}{\sqrt{2\pi}\sigma}e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} \frac{e^{-\frac{\sigma^2\omega^2}{2}}}{\sqrt{2\pi}}$$



- 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 is to set σ as a third of the Rayleigh limit.

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

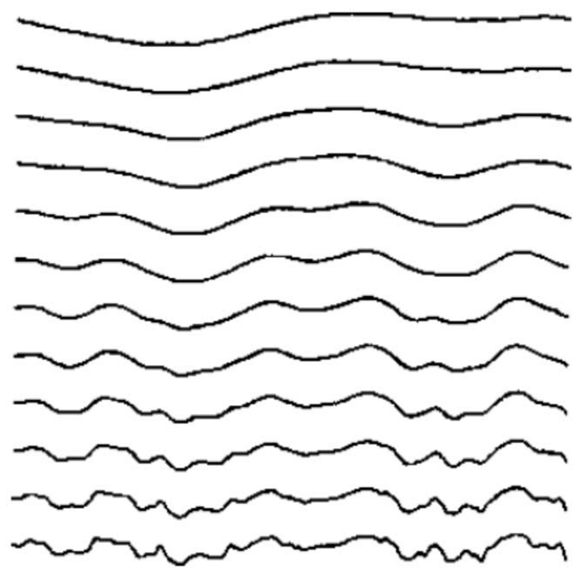
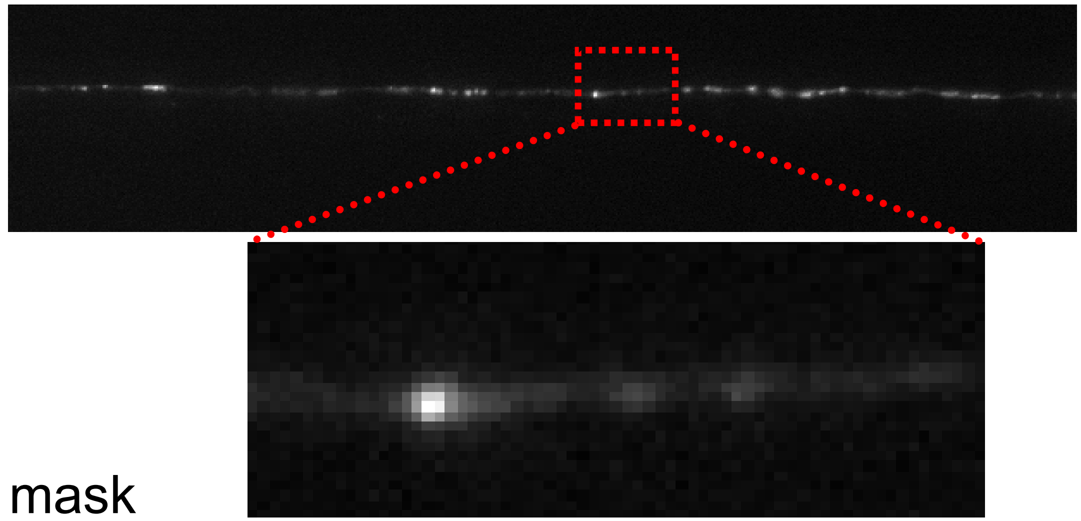


Figure 1. A sequence of gaussian smoothings of a waveform, with σ decreasing from top to bottom. Each graph is a constant-σ profile from the scale-space image.

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

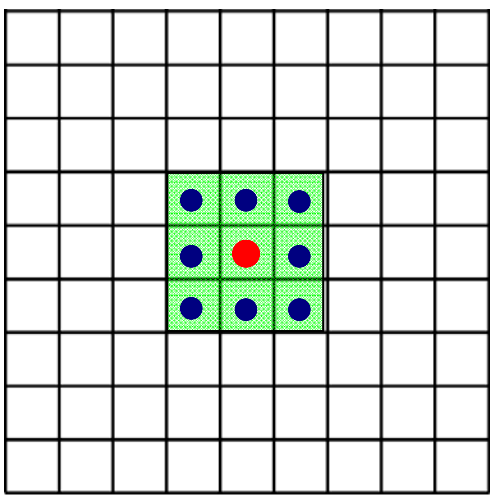
Step 2: Local Maximum Detection

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

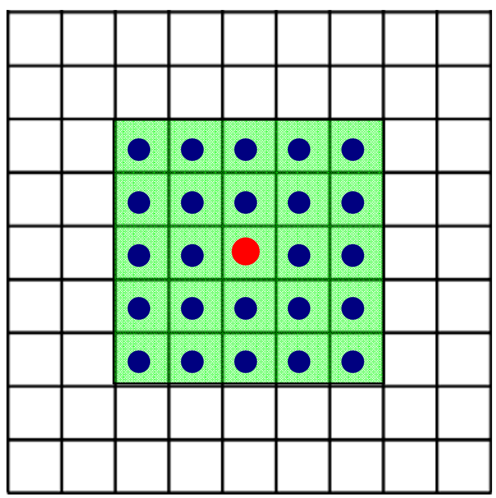


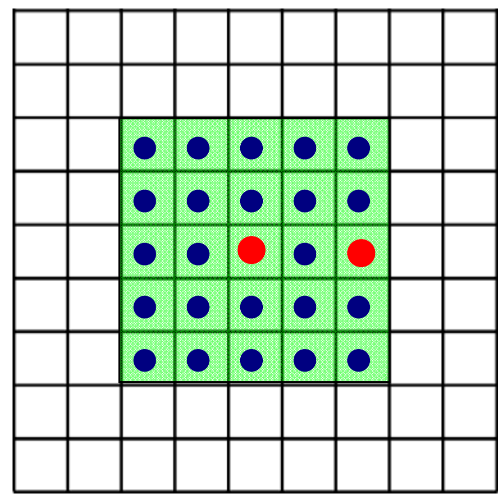
- Large masks give more stable results but lower detection resolution.

3X3 mask



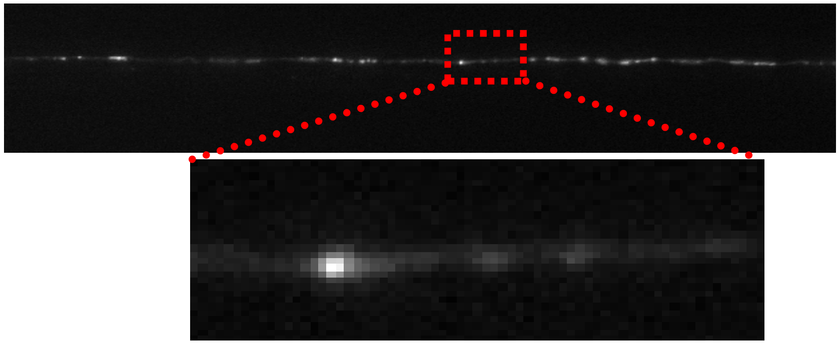
5X5 mask



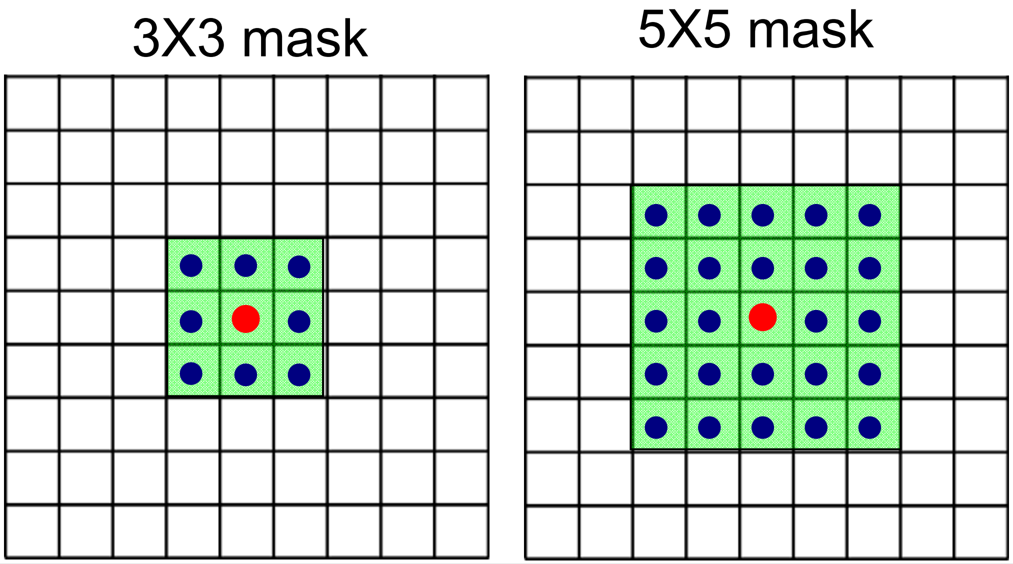


Step 3: Local Background Detection

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

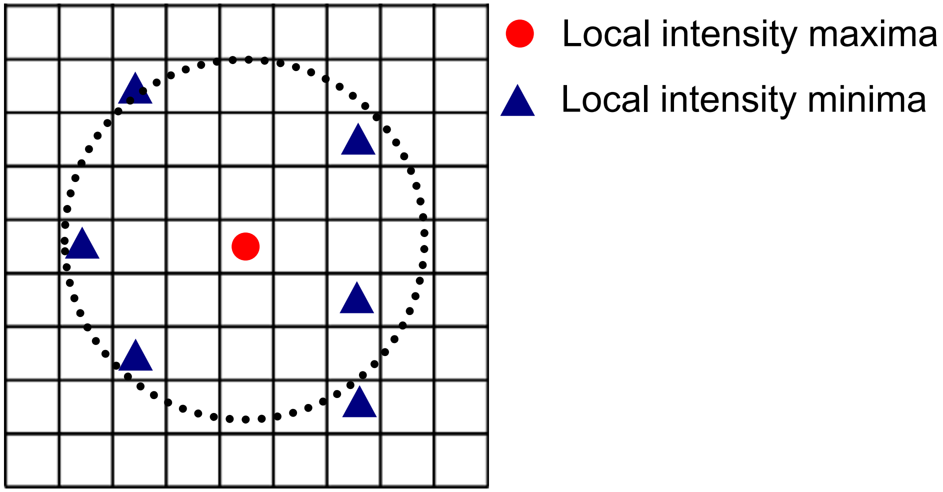


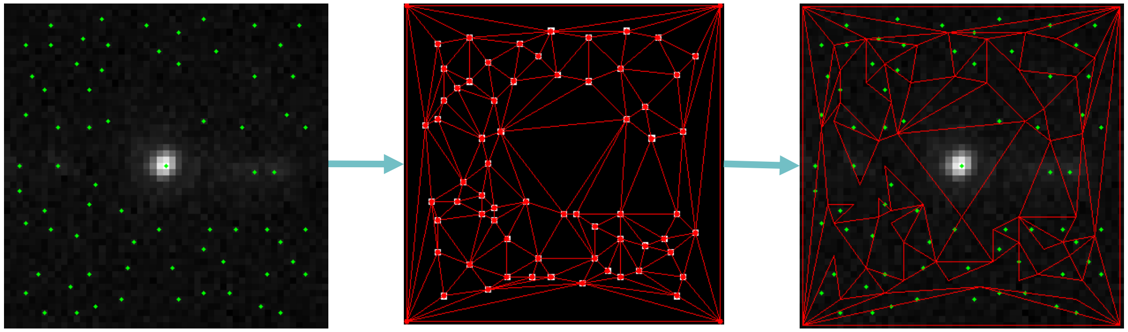
- Local background is detected through detection of local intensity minima.



Step 3: Establishing Corresponding Between Local Maxima and Local Minima

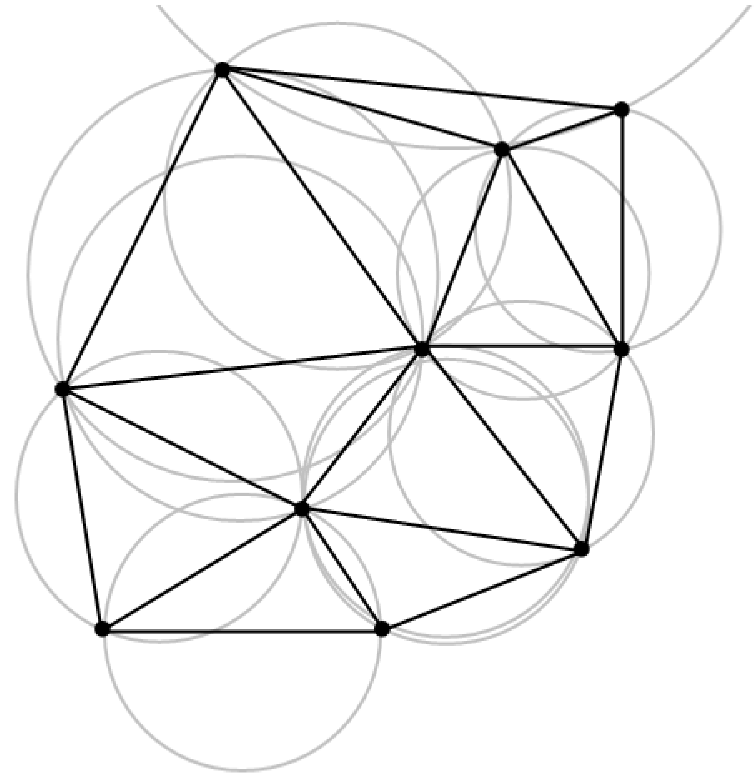
- Different approaches can be used to establish correspondence between local maxima and local minima.
  - Nearest neighbor
  - Delaunay triangulation





Delaunay Triangulation

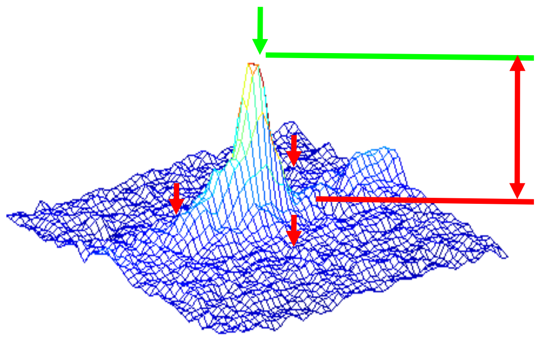
- For a given set of points in a plane, its Delaunay triangulation satisfies the condition that every circumcircle of a triangle is empty.

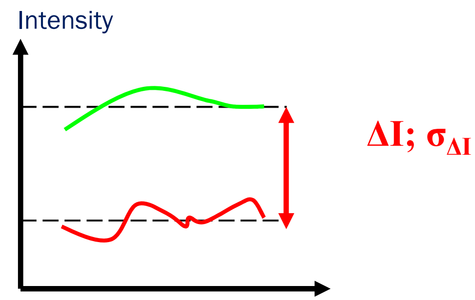


- Some nice properties of Delaunay triangulation
  - It favors large internal angles.
  - It links points in a nearest neighbor manner.

http://www.cs.cornell.edu/home/chew/Delaunay.html

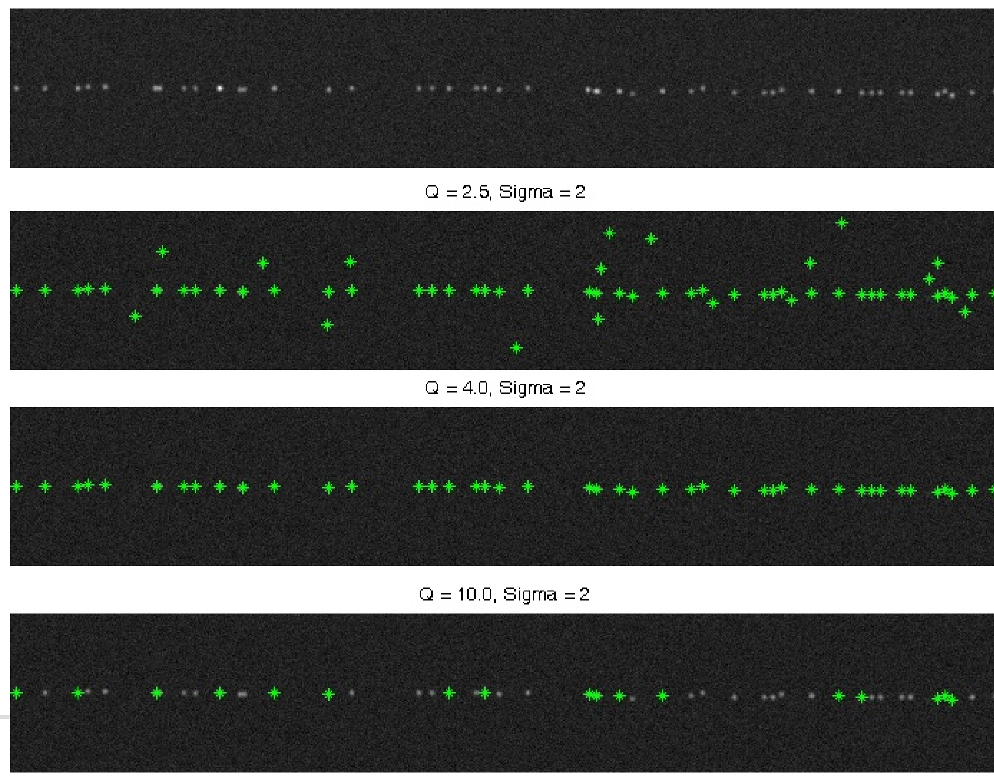
Step 4: Statistical Selection of Features





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

Q: selection quantile



Introduction to the t-distribution

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

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

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

- When we substitute standard deviation for σ, we get the t-distribution with n-1 degrees of freedom

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

A Review of Two Sample t-test

- Two sample t statistic

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{\dfrac{s_1^2}{n_1} + \dfrac{s_2^2}{n_2}}}$$

- Two-sample t-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^*.

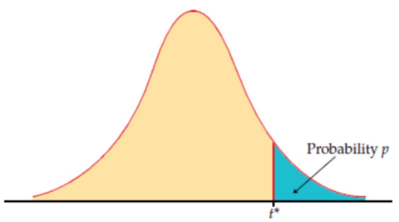


TABLE D

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											

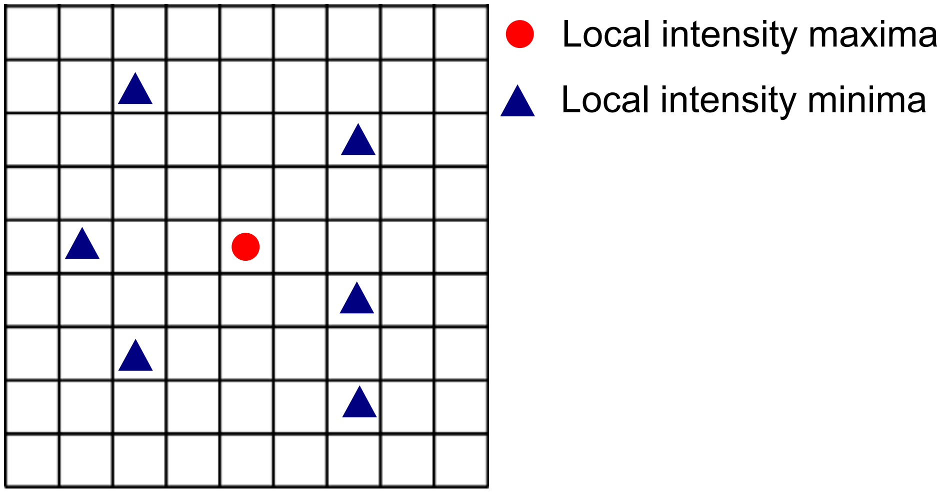
Feature Intensity Measurement

- 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.

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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

Questions?