Bioimage Informatics

Lecture 8, Spring 2012

Bioimage Data Analysis (II): Point Feature Detection

Outline

- Project assignment 02
- Comments on reading assignment 01

- Review: pixel resolution point feature detection
- Subpixel resolution point feature detection
- Reproducible research in computational science
- Other point/particle feature detection techniques

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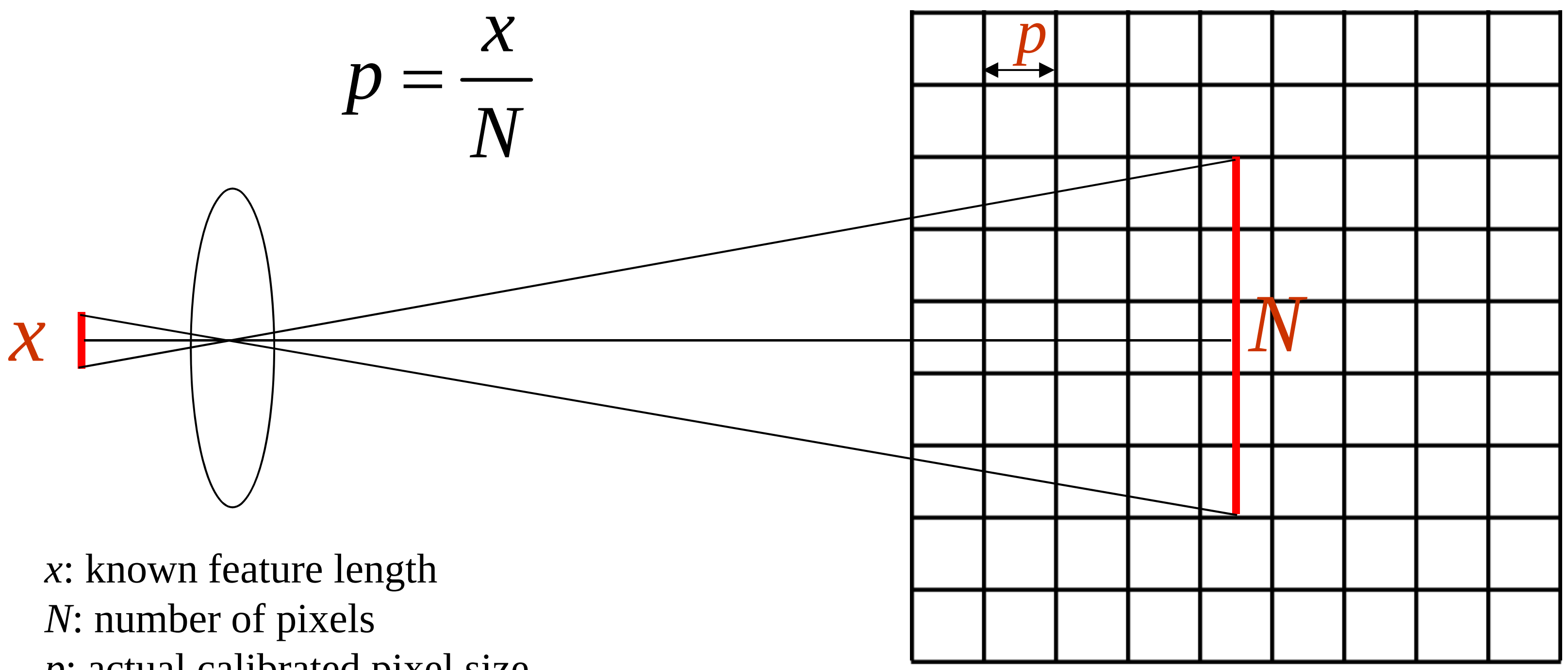
Microscope Camera Pixel Size Calibration

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

- Image features are first measured in pixel coordinates

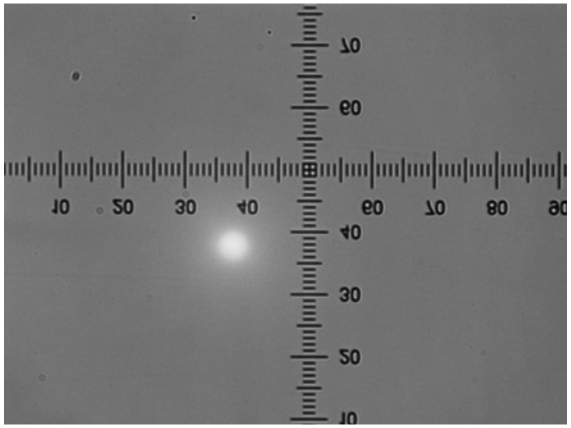
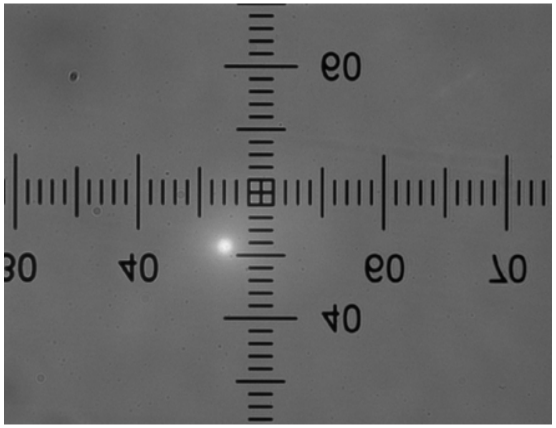
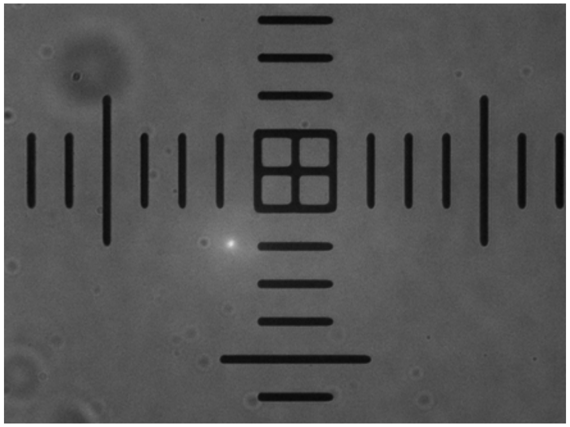
$$p = \frac{x}{N}$$



x: known feature length
N: number of pixels
p: actual calibrated pixel size

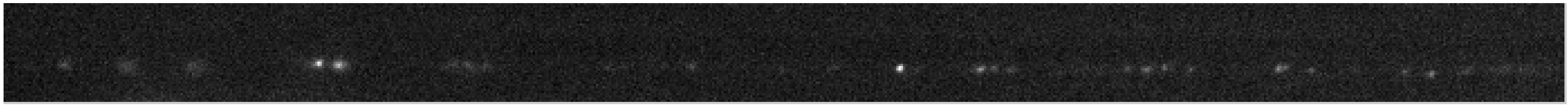
Project Assignment 02 (I)

- Camera calibration

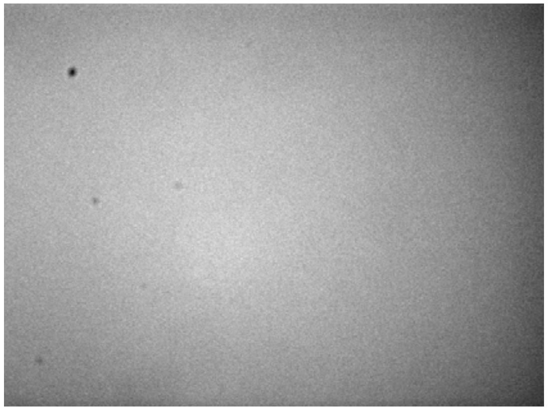
Project Assignment 02 (II)

- Background noise characterization



Vesicle transport in *Drosophila* nerve axons

- Illumination uniformity validation



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Reading Assignment 01 (I)

- General comments
  - Do not forget to put your name on your report.
  - There are no standard solutions.
  - Principle of due diligence.
- Detailed comments

“Seeing is believing is the fundamental strategy in biology research. But interpreting images by human eyes is always qualitative and laborious. ”

“Bioimage informatics studies biological images for mainly scientific research, while medical image analysis focuses mainly on medical images for direct clinical usage.”

Reading Assignment 01 (II)

- "This is high time we realize that computers are better than humans in analyzing and recognizing patterns and bioimage informatics will lay the foundation for automation and precision."

- "The techniques deciphered till date mainly flows the standard work pipeline starting from feature selection, segmentation, registration, clustering, information retrieval and lastly visualization."

- "In order to yield accurate and robust results, image analysis algorithms must be firmly grounded by knowledge of cellular and molecular processes."

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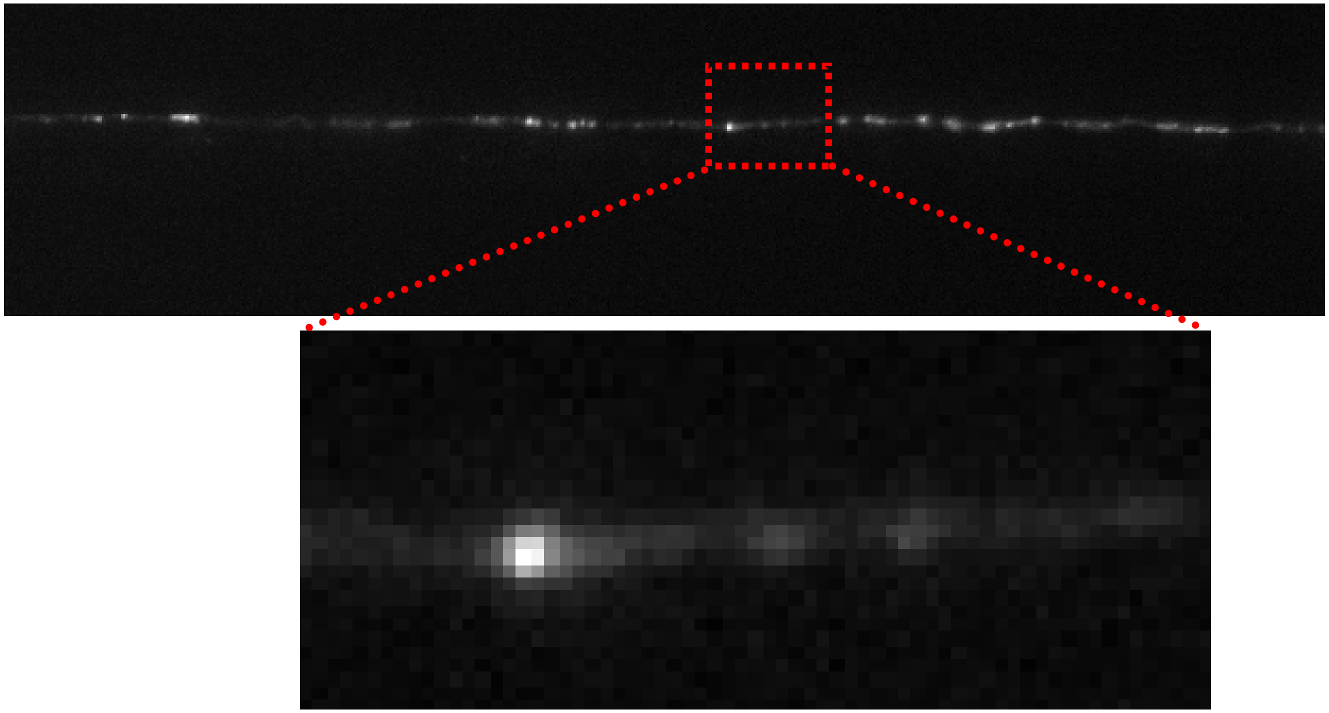
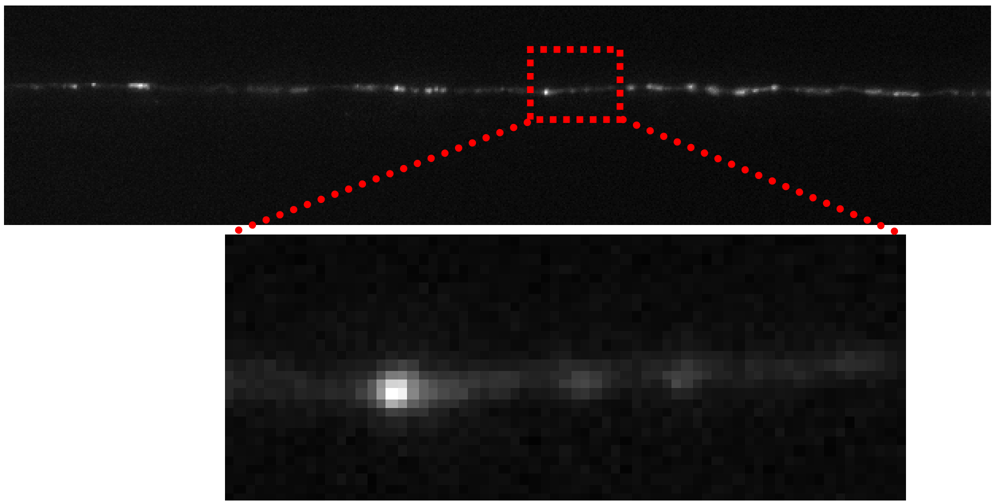
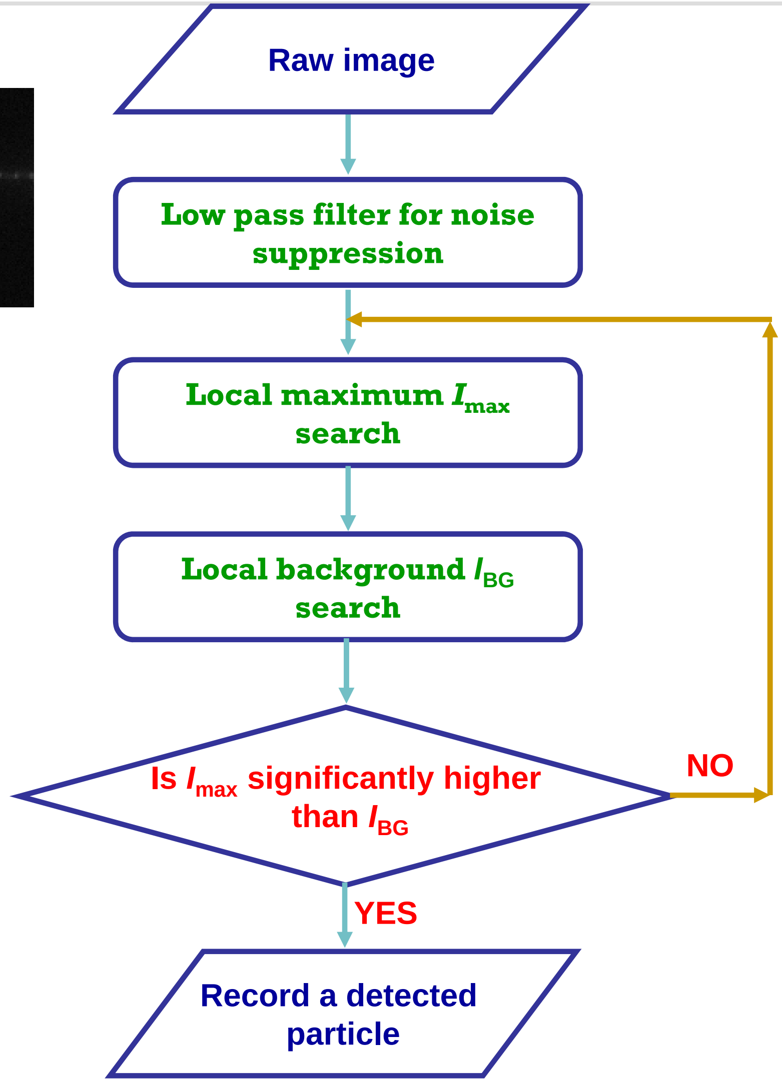
What is a Particle?

- 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)

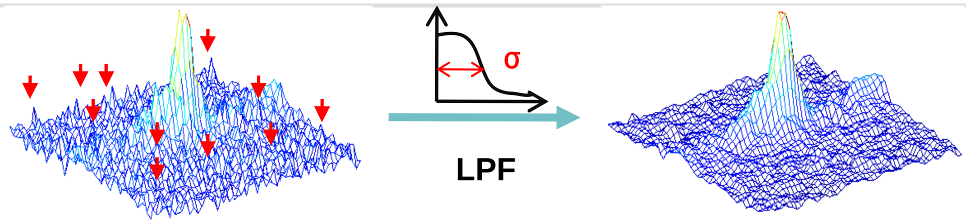
- The Fourier transform of a Gaussian kernel is Gaussian.

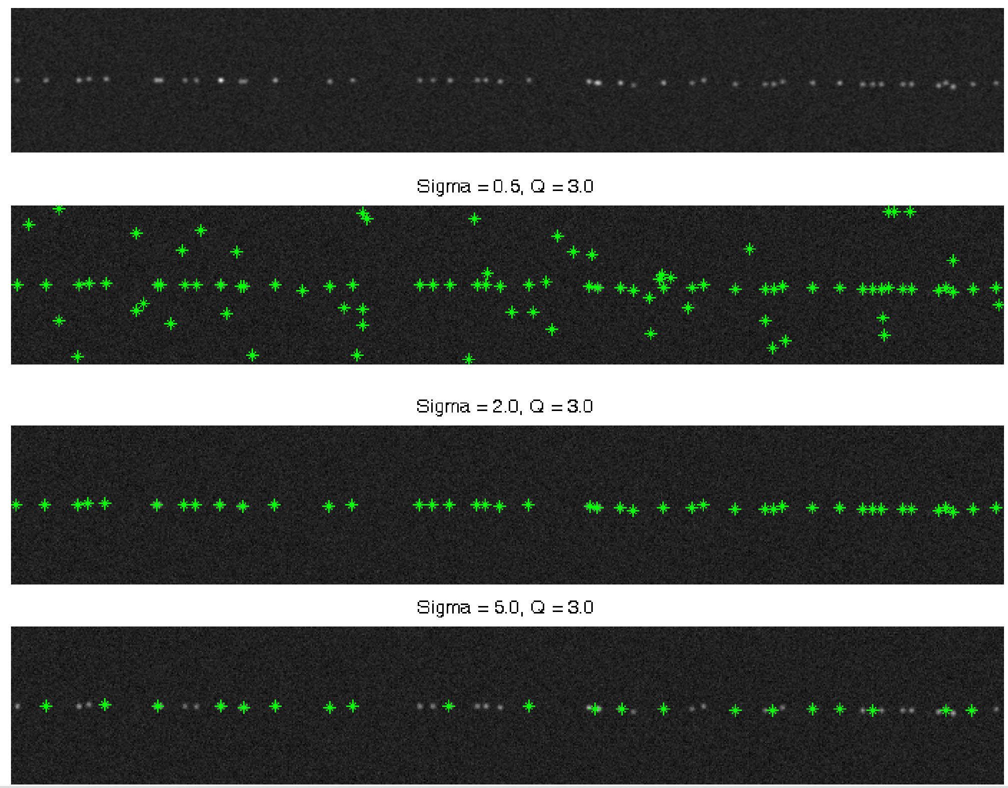
$$\frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} \frac{e^{-\frac{\sigma^2\omega^2}{2}}}{\sqrt{2\pi}}$$

- 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.

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

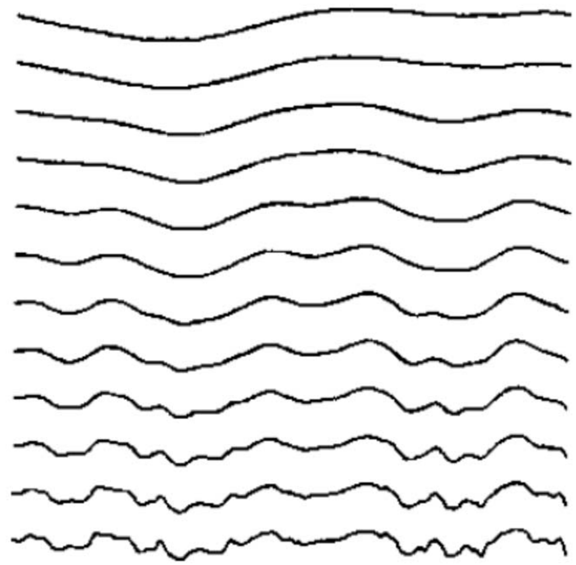
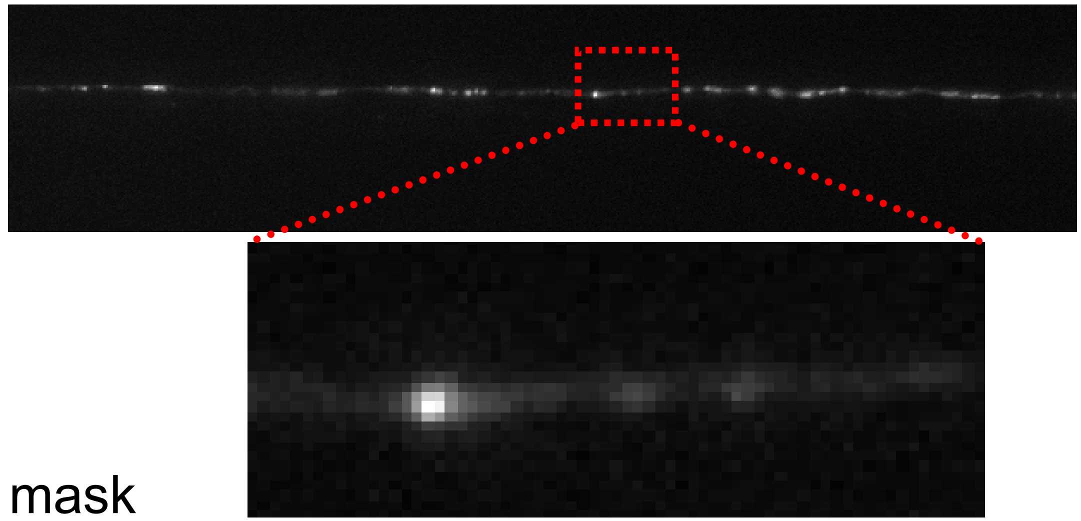


Figure 1. A sequence of gaussian smoothings of a waveform, with σ decreasing from top to bottom. Each graph is a constant-σ profile from the scale-space image.

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

Step 2: Local Maximum Detection

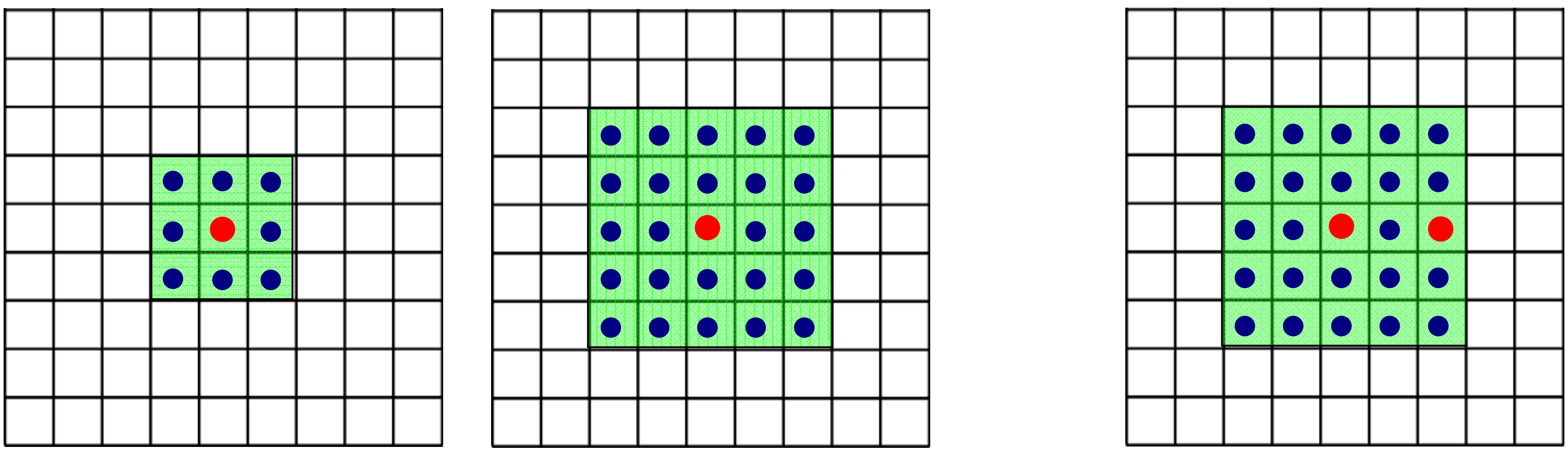
- A local maxima has an intensity that is no less than those of its neighbors.



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

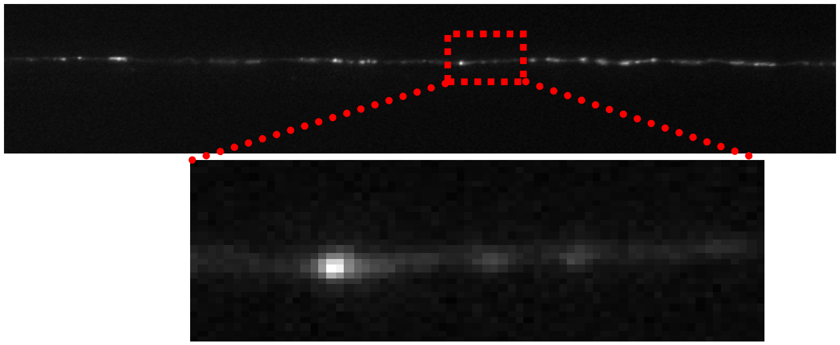
3X3 mask

5X5 mask

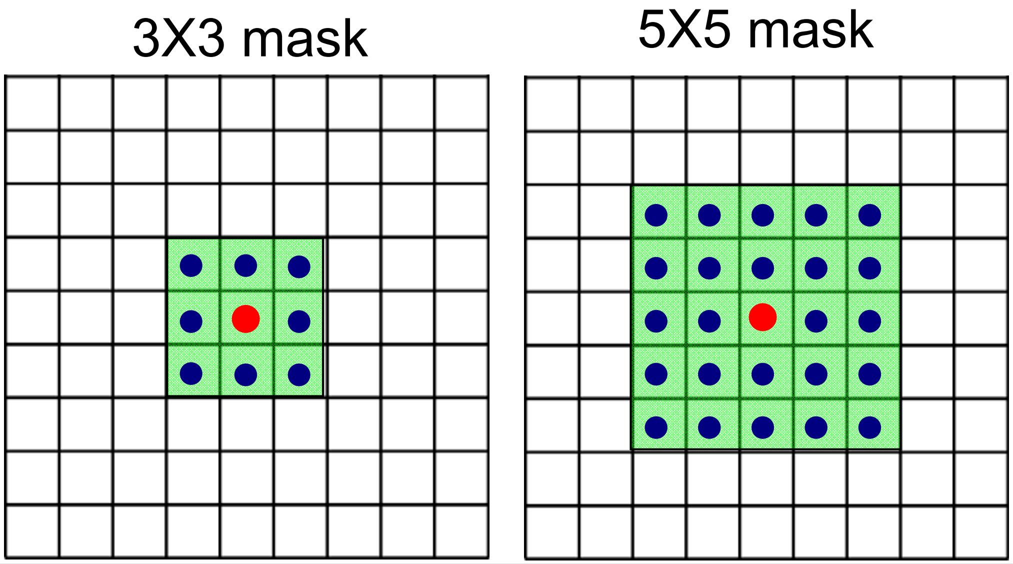


Step 3: Local Background Detection

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

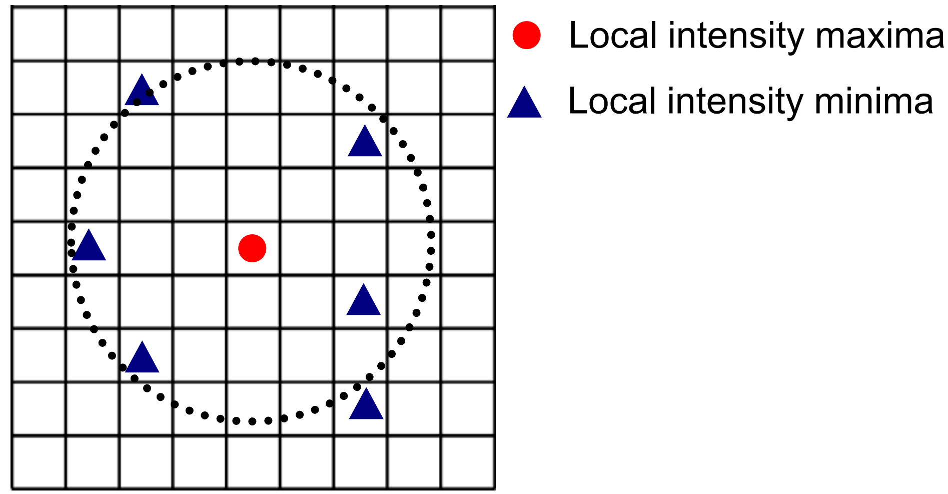


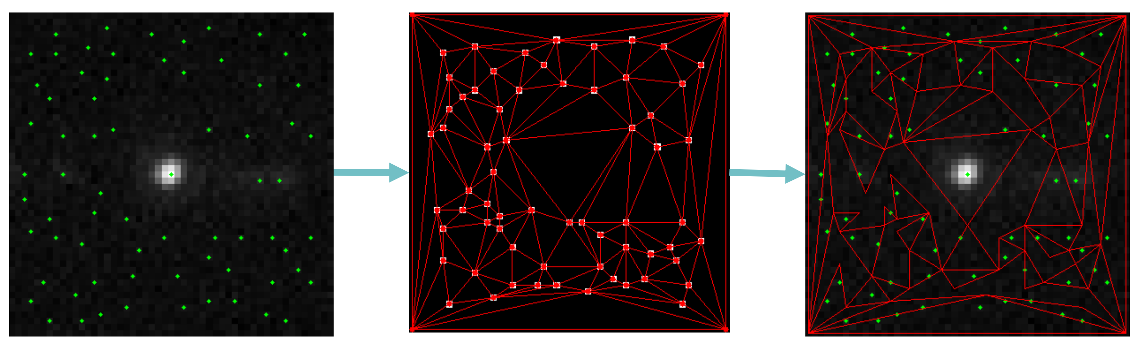
- Local background is detected through detection of local intensity minima.



Step 3: Establishing Corresponding Between Local Maxima and Local Minima

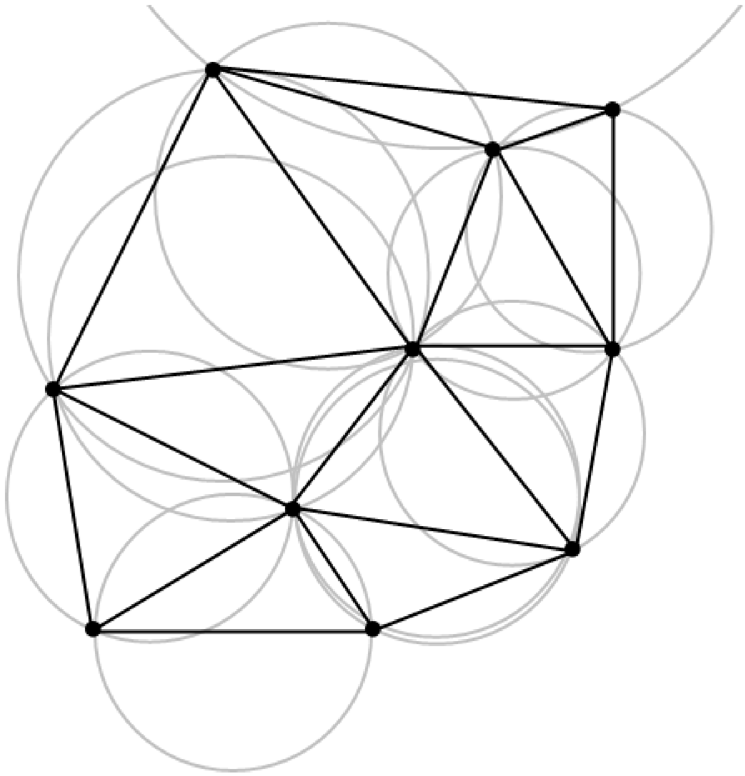
- Different approaches can be used to establish correspondence between local maxima and local minima.
    - Nearest neighbor
    - Delaunay triangulation





Delaunay Triangulation

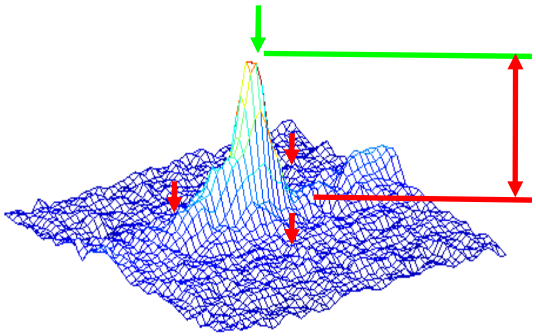
- For a given set of points in a plane, its Delaunay triangulation satisfies the condition that every circumcircle of a triangle is empty.

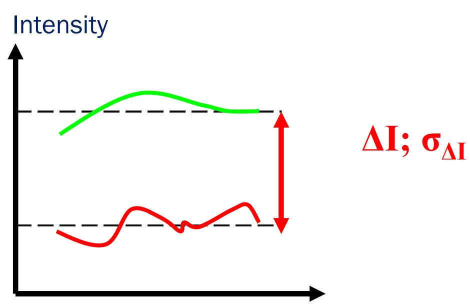


- Some nice properties of Delaunay triangulation
  - It favors large internal angles.
  - It links points in a nearest neighbor manner.

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

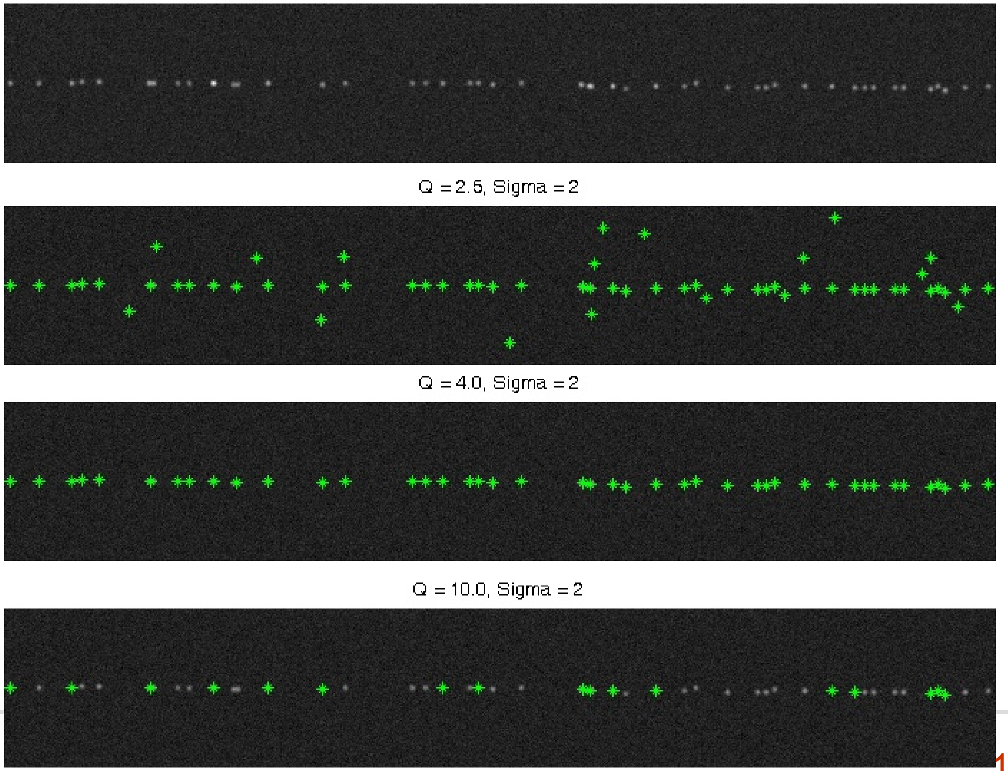
Step 4: Statistical Selection of Features





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

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^*.

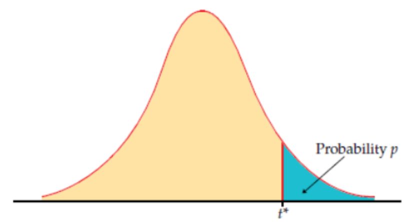


TABLE D

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											

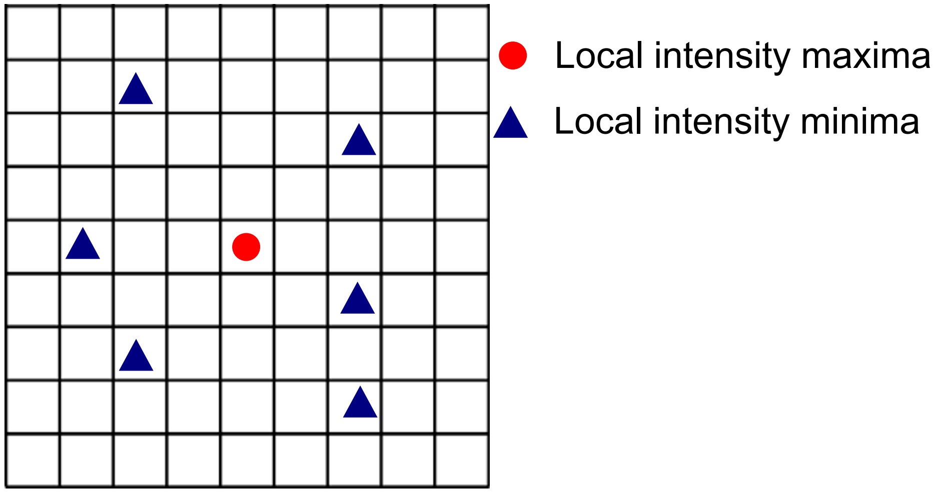
Feature Intensity Measurement

- 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 $N_{dark} = \sqrt{D \cdot T}$ $N_{camera} = \sqrt{N_{read}^2 + N_{dark}^2}$
- 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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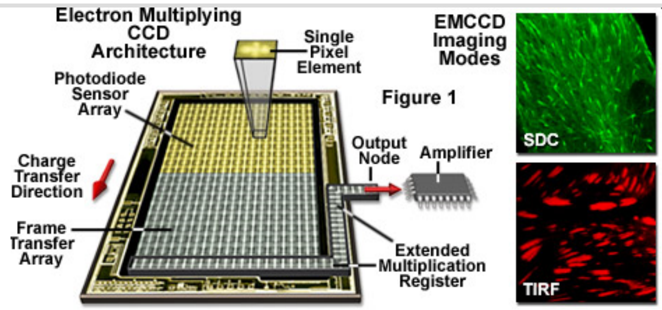
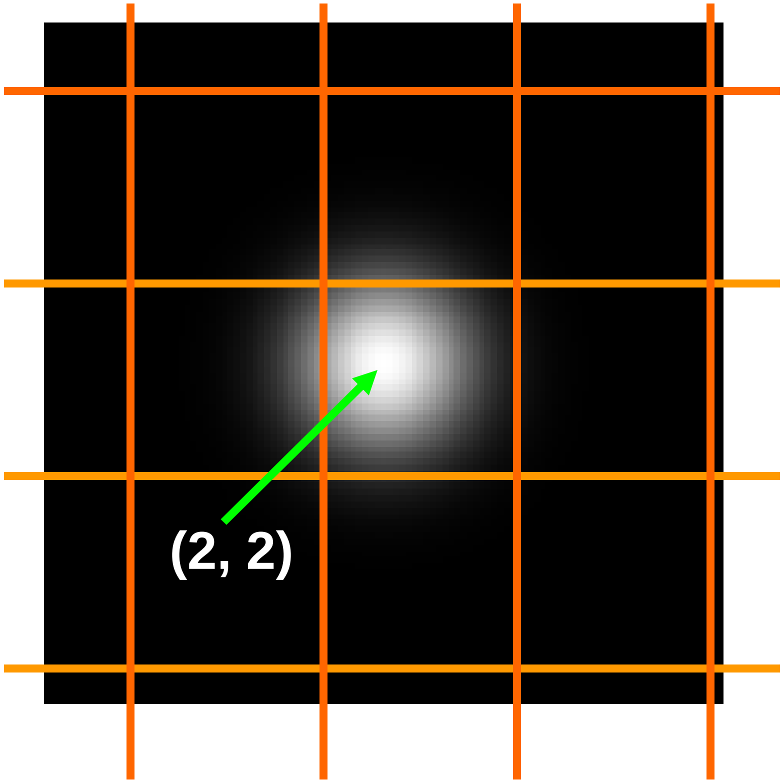
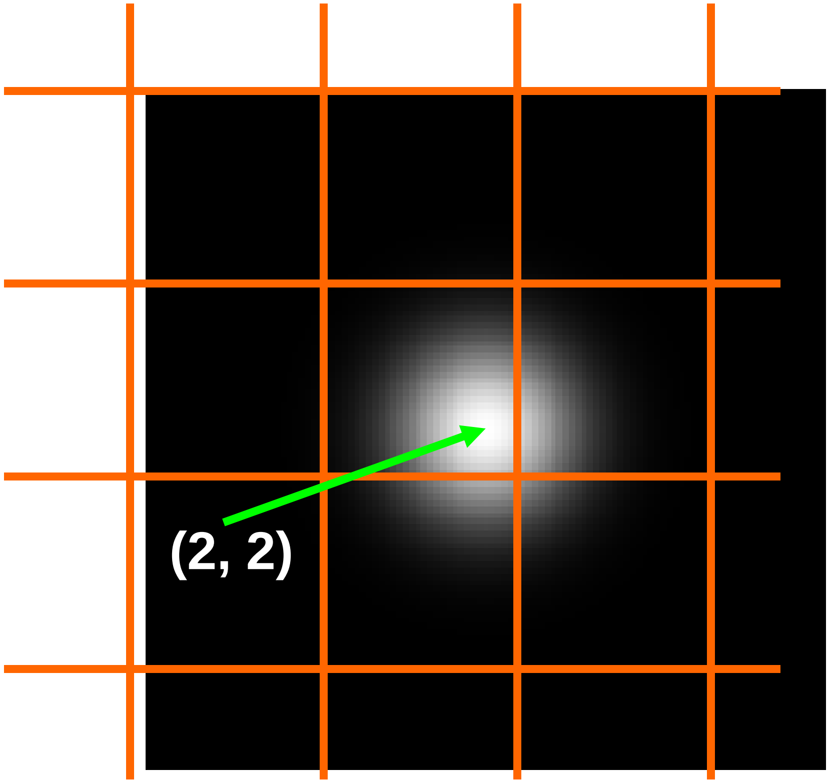
Sub-Pixel Resolution Point Detection







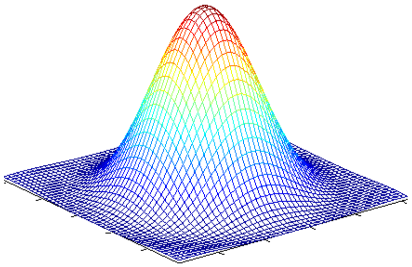
Sub-pixel Detection by Gaussian Fit

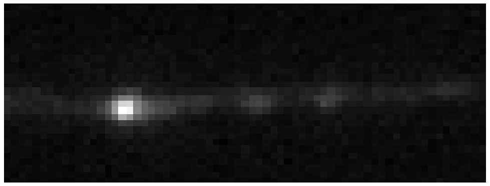
- Fit using a Gaussian kernel, which represents the ideal image of a point

$$K(x,y;x_0,y_0) = K(x-x_0,y-y_0) = A \cdot exp\left[-\frac{(x-x_0)^2+(y-y_0)^2}{B}\right]$$

- Problem formulation: to minimize the difference between the translated kernel and the image

$$\min_{(x_0,y_0)\in R^2} \left| I(x,y) - K(x,y;x_0,y_0) \right|$$





Gaussian Fitting Implementation Details (I)

- Calculation of $\left| I(x,y) - K(x,y;x_0,y_0) \right|$

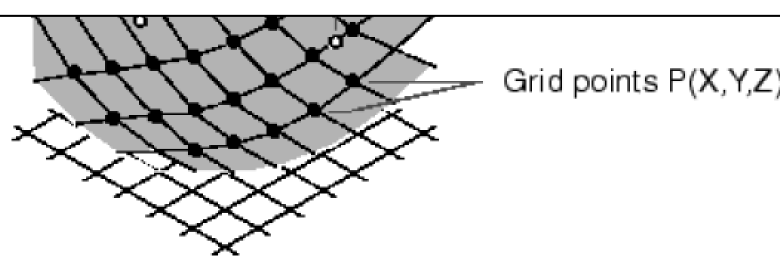
$$E(x_0,y_0) = \sum_{i=1}^{M} \sum_{j=1}^{N} \left| I(i,j) - K\left(\underline{i - x_0, j - y_0}\right) \right|$$

May not be integer coordinates

Two ways to look at an image

- Discrete view: It is a matrix
- Continuous view: It is a surface

•



Gaussian Fitting Implementation Details (II)

- How to set the Gaussian kernel
  - By fitting the Airy Disk using a Gaussian

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

  - By fitting measured PSF (from beads) using a Gaussian

- A can be matched with the local maximum.

Gaussian Fitting Implementation Details (III)

- Spatial sampling: three-times oversampling of Airy disk

$$\frac{\text{Airy disk radius}}{\text{pixel size}} \geq 3$$

- Under high SNR, spatial sampling may be relaxed to 2~2.5.

Gaussian Fitting Implementation Details (IV)

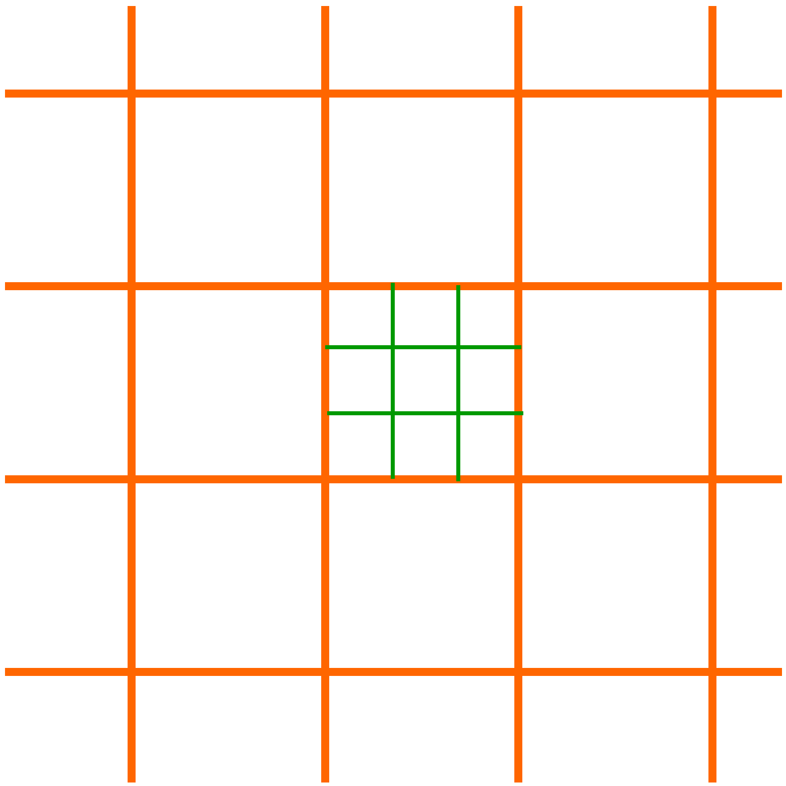
- Optimization strategy I: exhaustive search

- Implementation of exhaustive search

  - Oversampling the kernel and images

  $\rightarrow$ use a small pixel size: e.g. 10nm

  - If multiple minima were identified, use their average position



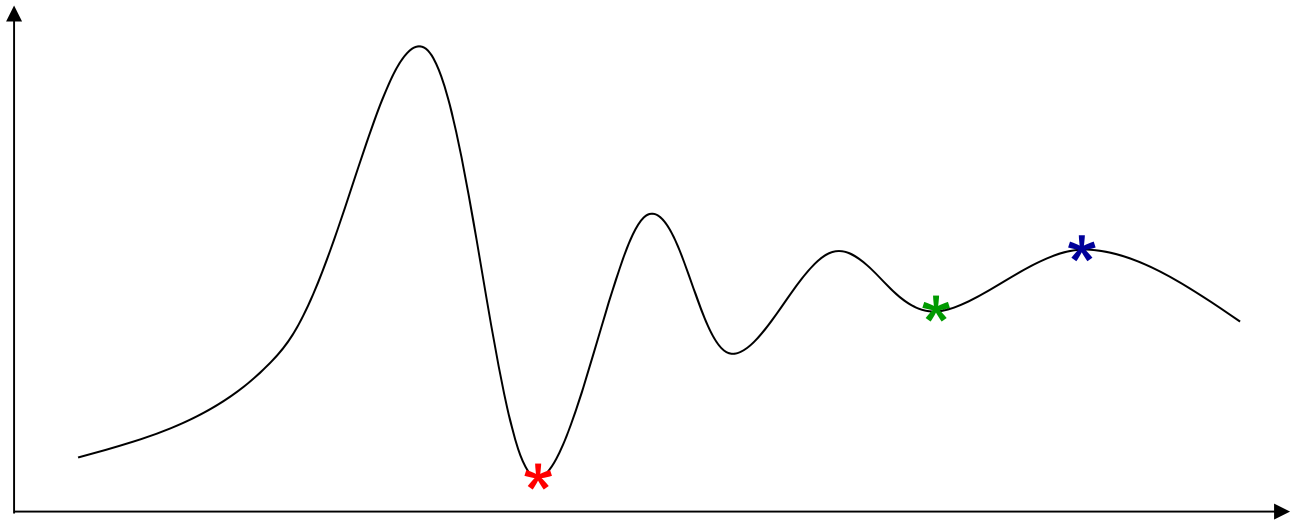
Gaussian Fitting Implementation Details (V)

- Initialization:
  - Use detected local maxima to localize the search

- Strengths and weaknesses
  - Good (not necessarily optimal) solution guaranteed
  - Computationally expensive, slow

Gaussian Fitting Implementation Details (VI)

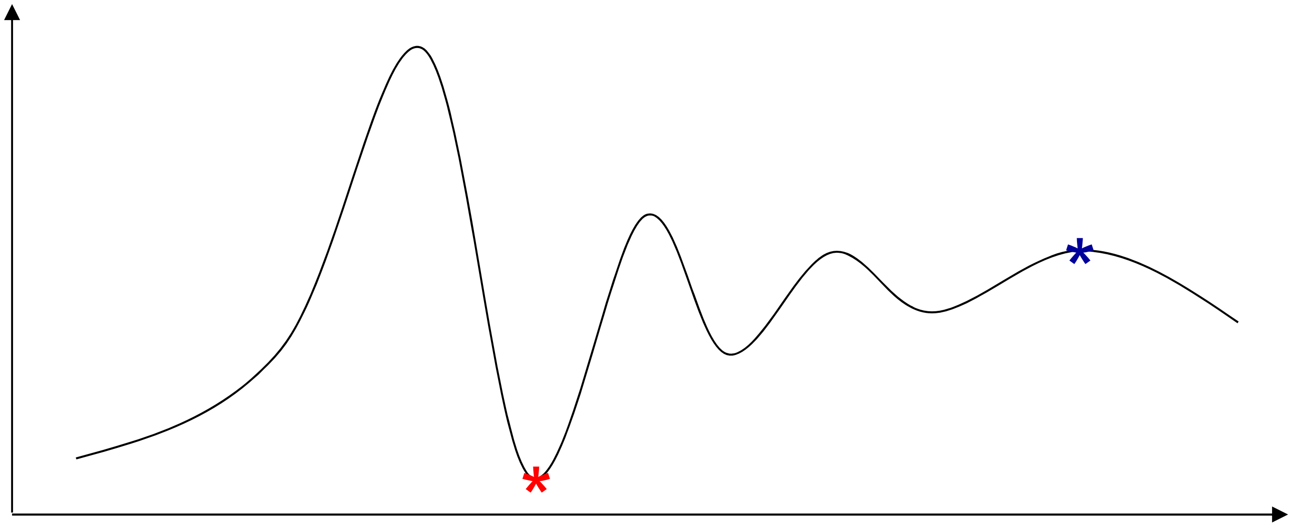
- Optimization strategy II: optimization search
  - Many optimization techniques can be applied



- Initialization:
  - Use detected local maxima
  - Use multiple randomly selected initiation points

Gaussian Fitting Implementation Details (VII)

- Strengths and weaknesses
  - Not limited by oversampling rate
  - May be trapped in local minimum



Sub-pixel Detection by Correlation

- Detection by maximization correlation

$$C_{x_0,y_0} = \sum_{i=1}^{M} \sum_{j=1}^{N} I_{i,j} K\left(i - x_0, j - y_0\right)$$

- Often the correlation function is normalized

$$C_{x_0,y_0} = \frac{\sum_{i=1}^{M} \sum_{j=1}^{N} I_{i,j} K\left(i - x_0, j - y_0\right)}{\sqrt{\sum_{i=1}^{M} \sum_{j=1}^{N} I_{i,j}^2} \cdot \sqrt{\sum_{i=1}^{M} \sum_{j=1}^{N} K^2\left(i - x_0, j - y_0\right)}}$$

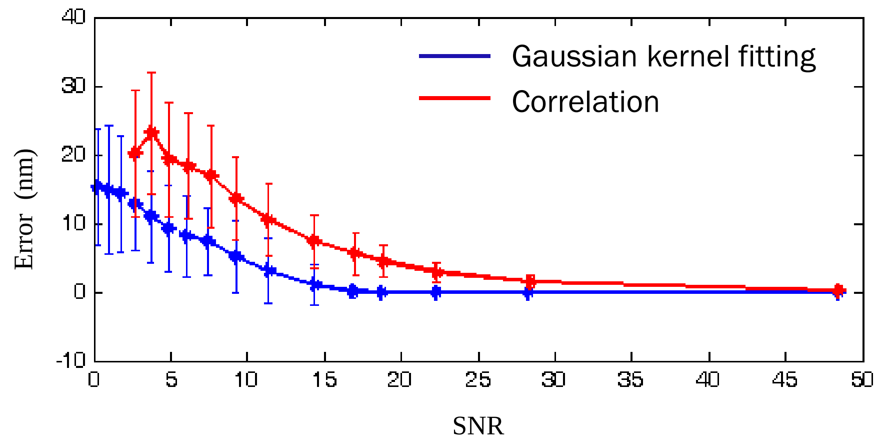
Implementation Strategy

- The same strategy as in Gaussian fitting as the only difference is the cost function

  - Strategy I: exhaustive search
  - Strategy II: optimization search

Gaussian Fitting vs Correlation

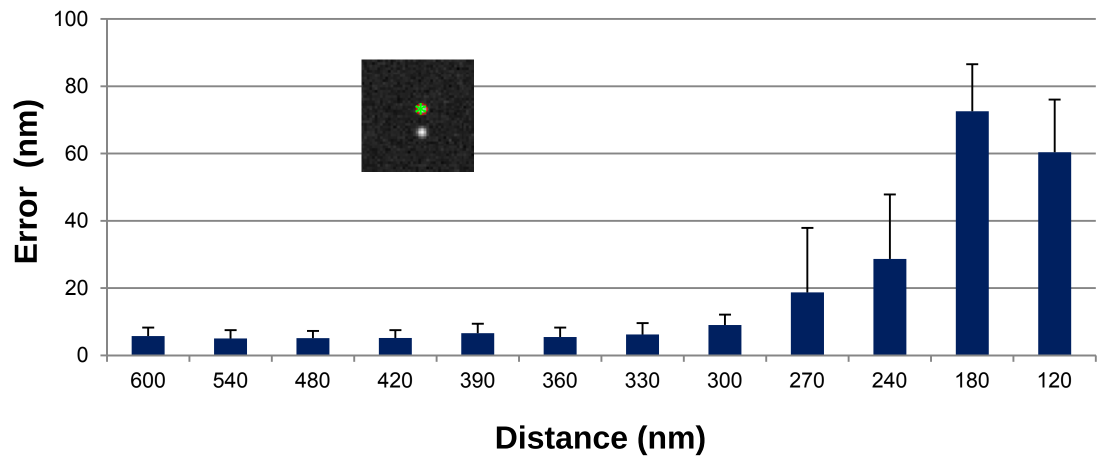
- For point features, Gaussian fitting is the best method overall.



- For larger non-diffraction limited features, correlation gives higher resolution.

Limitations of Sub-Pixel Detection

- When the distance between the two point features goes below the Rayleigh limit, they can no longer be resolved <u>reliably</u> unless under very high SNR.



- Project assignment 02
- Comments on reading assignment 01

- Review: pixel resolution point feature detection
- Subpixel resolution point feature detection
- Reproducible research in computational science
- Other point/particle feature detection techniques

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

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Questions?