Advantages of super-resolution of point source detection beyond Rayleigh criterion

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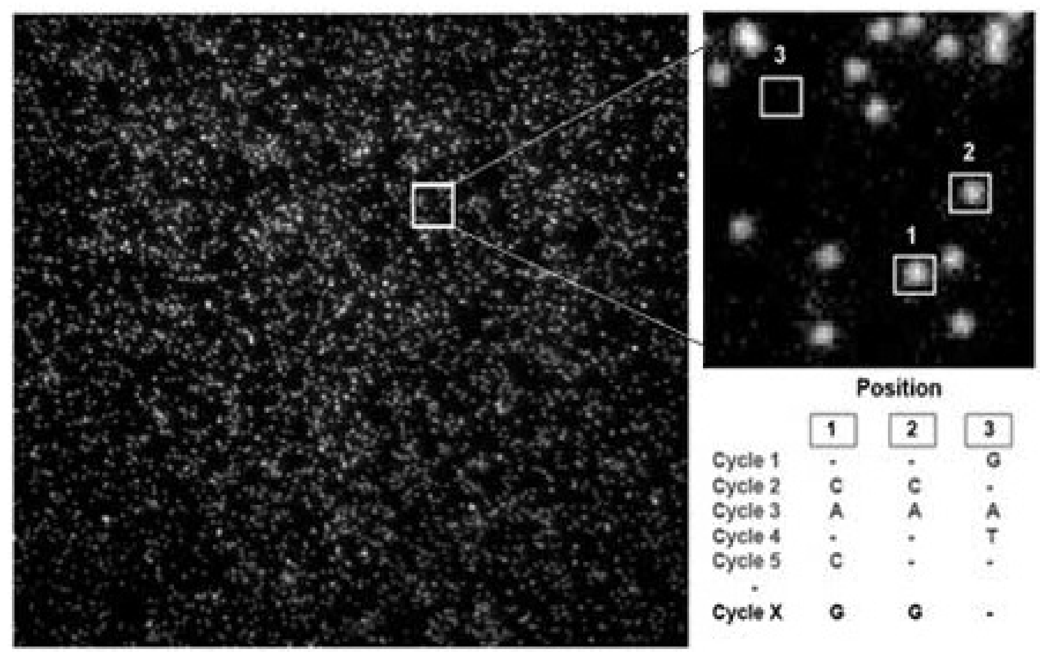
Bio-tech applications monitor point sources of light (aka quantum dots)

Examples of entities marked with light sources:

- DNK deciphering – DNA strands, nucleotides, instances of incorporation (process shown in the picture)
- Chemical pathogen detection
- Microbial, bacterial or viral contamination



The image is quoted from "Single Molecule DNA Sequencing of a Viral Genome", Timothy D. Harris, Phillip R. Buzby, Hazen Babcock, Eric Beer, Jayson Bowers, Ido Braslavsky, Marie Causey, Jennifer Colonell, James DiMeo, J. William Efcavitch, Eldar Giladi, Jaime Gill, John Healy, Mirna Jarosz, Dan Lapen, Keith Moulton, Stephen R. Quake, Kathleen Steinmann, Edward Thayer, Anastasia Tyurina, Rebecca Ward, Howard Weiss, Zheng Xie, Science, April 4, 2008, Vol. 320 pp. 106-109

Increase productivity of the instrument up to 10 X

Productivity of the instrument is driven by the amount of useful objects in the image. Useful means either properly counted or properly positioned (depending on the goals of the application).

Issue: Blending of proximate objects due to their non-zero size (determined by the optics)

Problems:

1) For object detection and counting applications:

Gross under-count as multiple objects are counted as singles

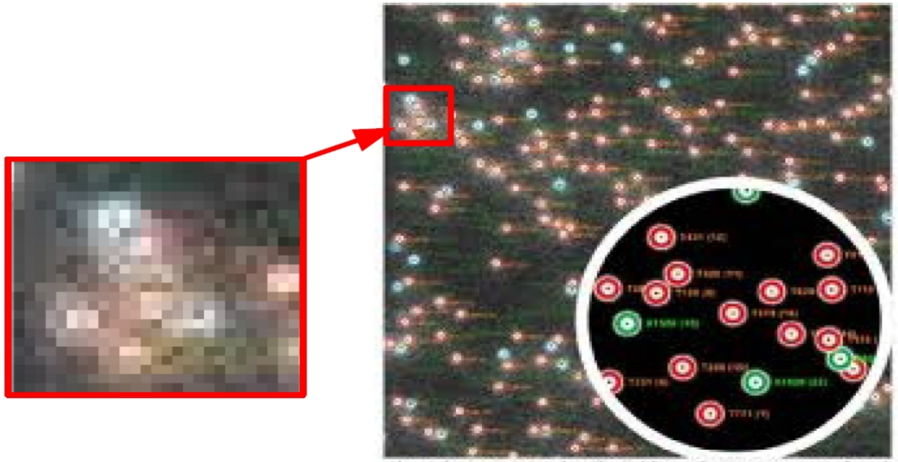
2) For object detection and location applications:

Blended objects are under-counted

and mis-positionned

Note: The image (right) is high quality synthetic

Problems in positioning of point source objects: points have physical size – determined by optics



Above: Images captured on the HeliScope Sequencer are transferred to the Analysis Engine, which identifies nucleotide incorporations and pinpoints their locations.

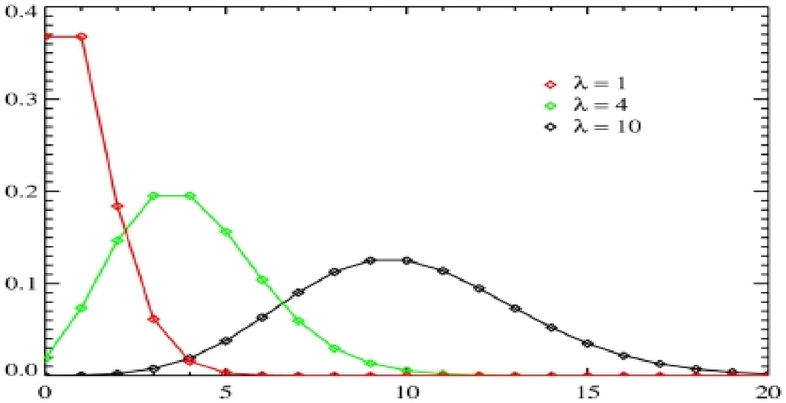
Random distribution of the Objects in the Field of View – recall the properties of Poisson distribution

If objects are distributed over the filed of view randomly and uniformly how many objects are expected to be found in an Area A?

Reminiscent of an Old Problems:

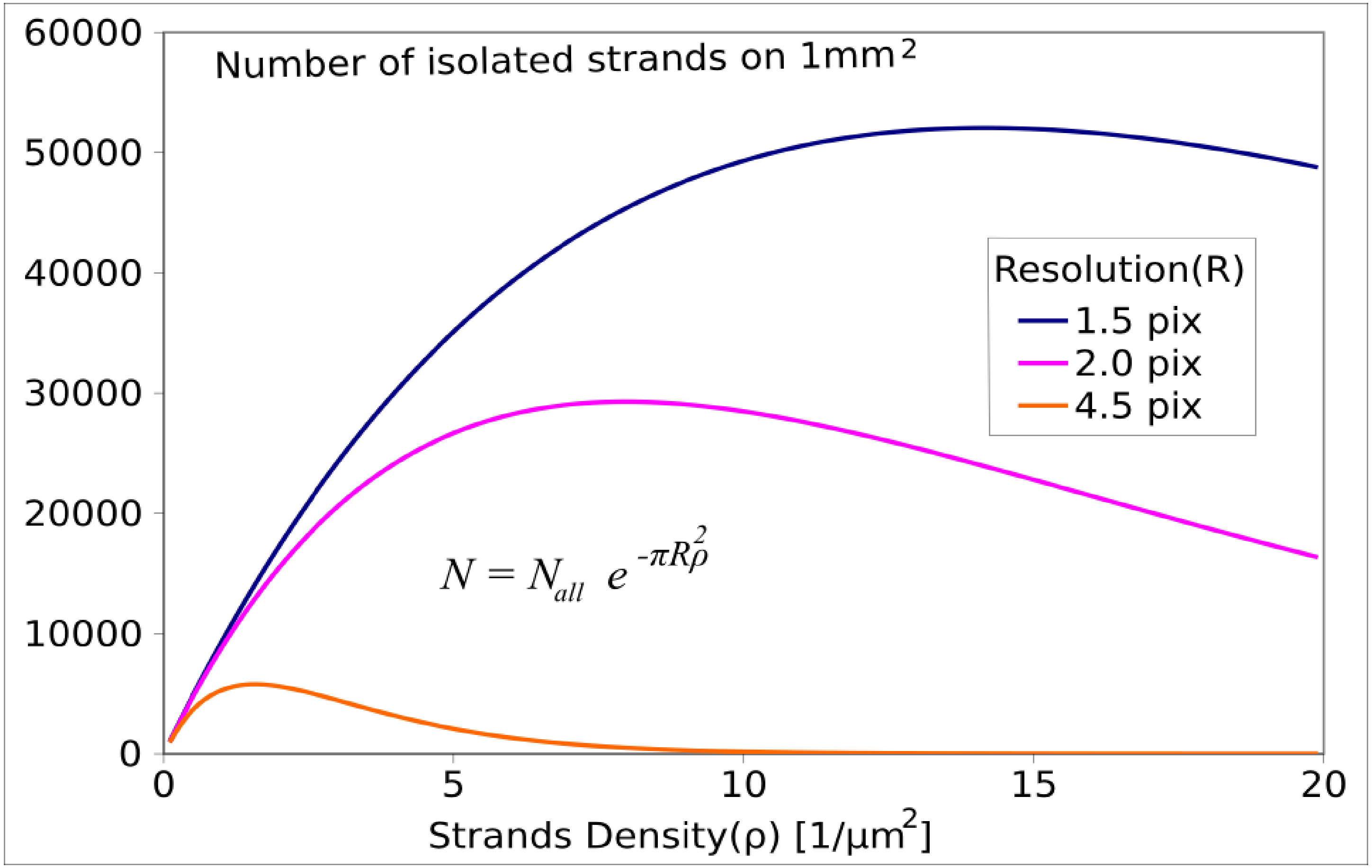
If the average density of raisin in the dough is D what is the expected number of raisins in a bun of a volume V?

$$p(n) = P(X = n) = e^{-\lambda} \frac{\lambda^n}{n!}$$



Number of useful (non-blended) point sources depends on Object density and Optical resolution

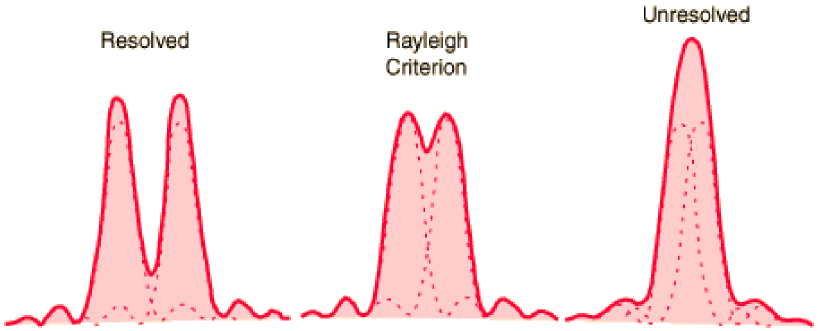
We show dependence of a **number of useful (detected as single) objects** on density of the objects in the field of view for three sample values of the **resolution of the instrument** (4.5 pixels, 2.0 pixels and 1.5 pixels).



Note that for each value of the resolution the number of useful objects reaches its maximum value at some value of density of the objects and then declines with higher density. The better the **resolution of the instrument** the higher the value of the useful objects, taht the tool can detect in its field of view.

Rayleigh criterion

The Rayleigh criterion is the generally accepted criterion for the minimum resolvable detail. For a closely positioned pair of objects it means that a minimum is seen in the image grey-level values between the two objects:

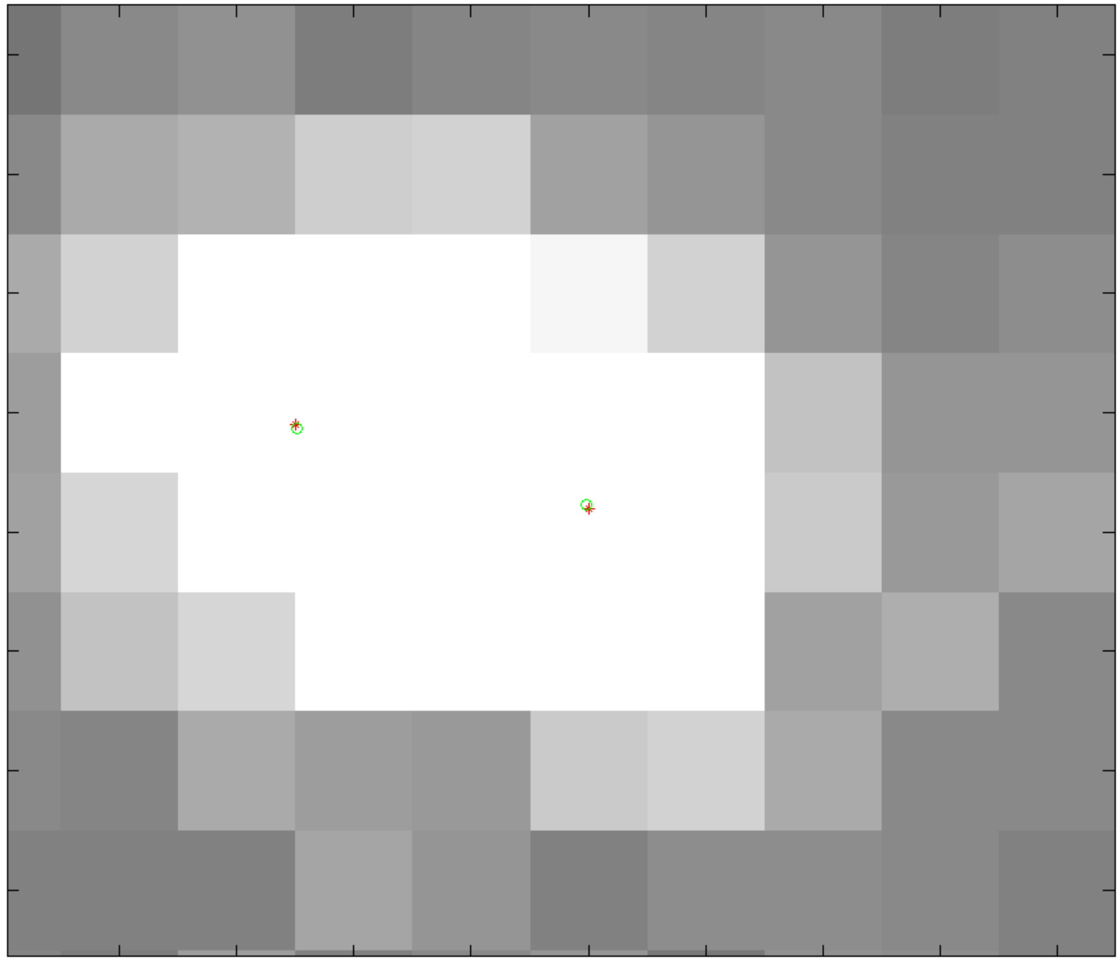


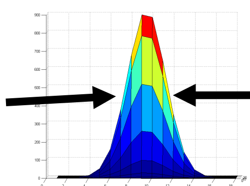
$$\sin(\Theta) = 1.22\ \lambda / D$$

Resolution beyond Rayleigh Criterion

The method allowed to shorten separation distance needed for resolution over 3 times (4.5 pix to 1.5 pix for the real images).

Resolution is driven by SNR and goes beyond Rayleigh Microscope Resolution Criterion of a half – wave distance.



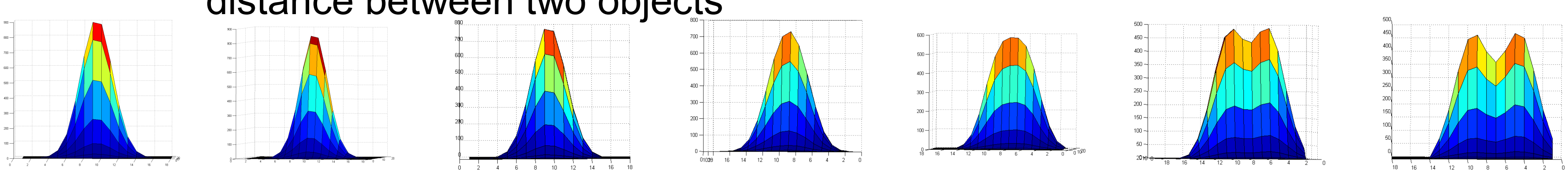


FWHM

3.0 pix

1 pix 1.5 pix 2 pix 2.5 pix 3.5 pix 4.5 pix 5.0 pix

distance between two objects

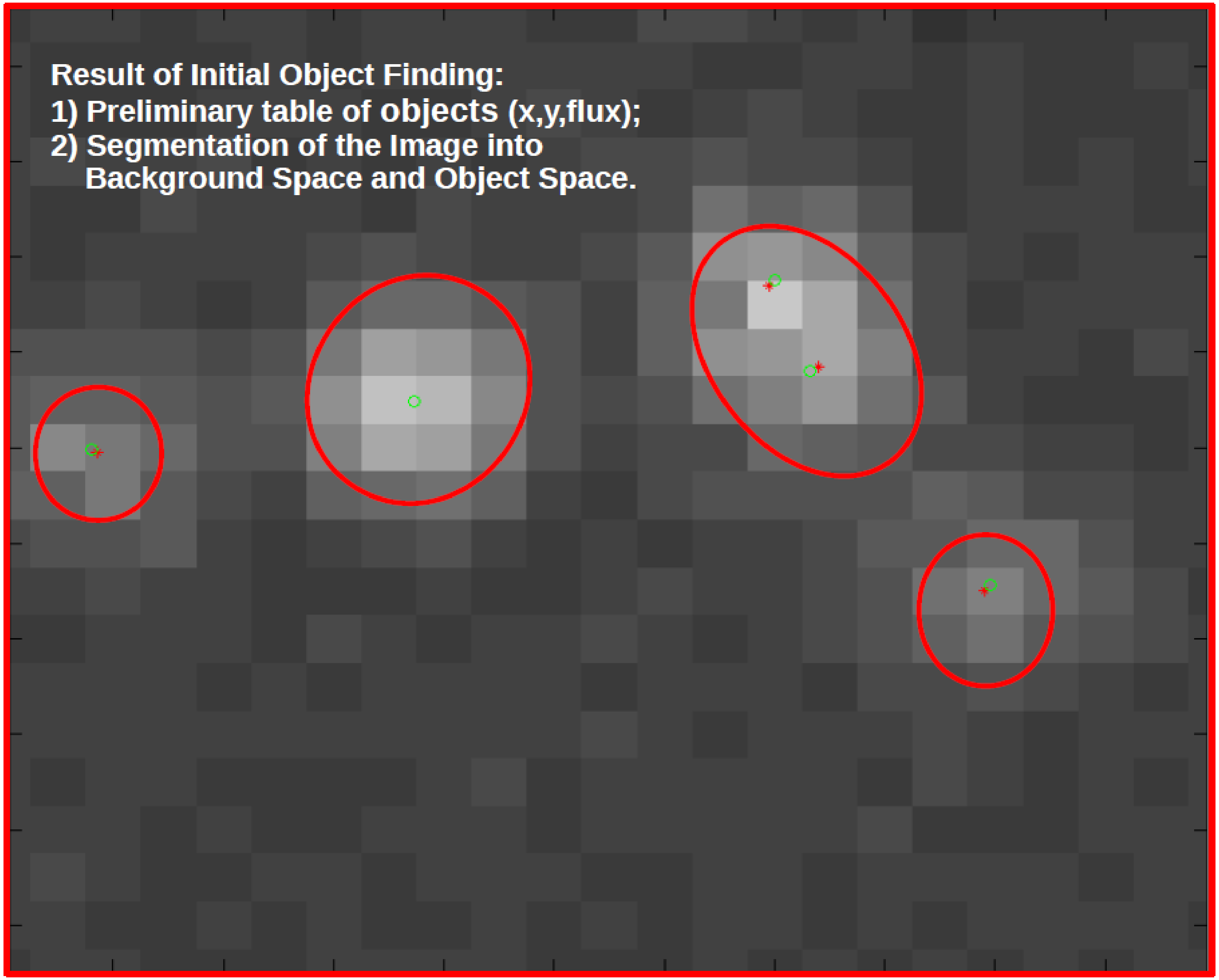


Regular object detection – detects a few blended objects

Solution:

Blended objects are resolved and positioned with the accuracy determined by SNR (Signal to Noise Ratio) in the Images like Single standing Point Objects.

The Simulated image (right) shows objects (green) and their detected positions (red).



About the Method

The super-resolution method is based on computation of the higher order moments in the space of Hermitian functions.

The method was patented, the details can be seen in:

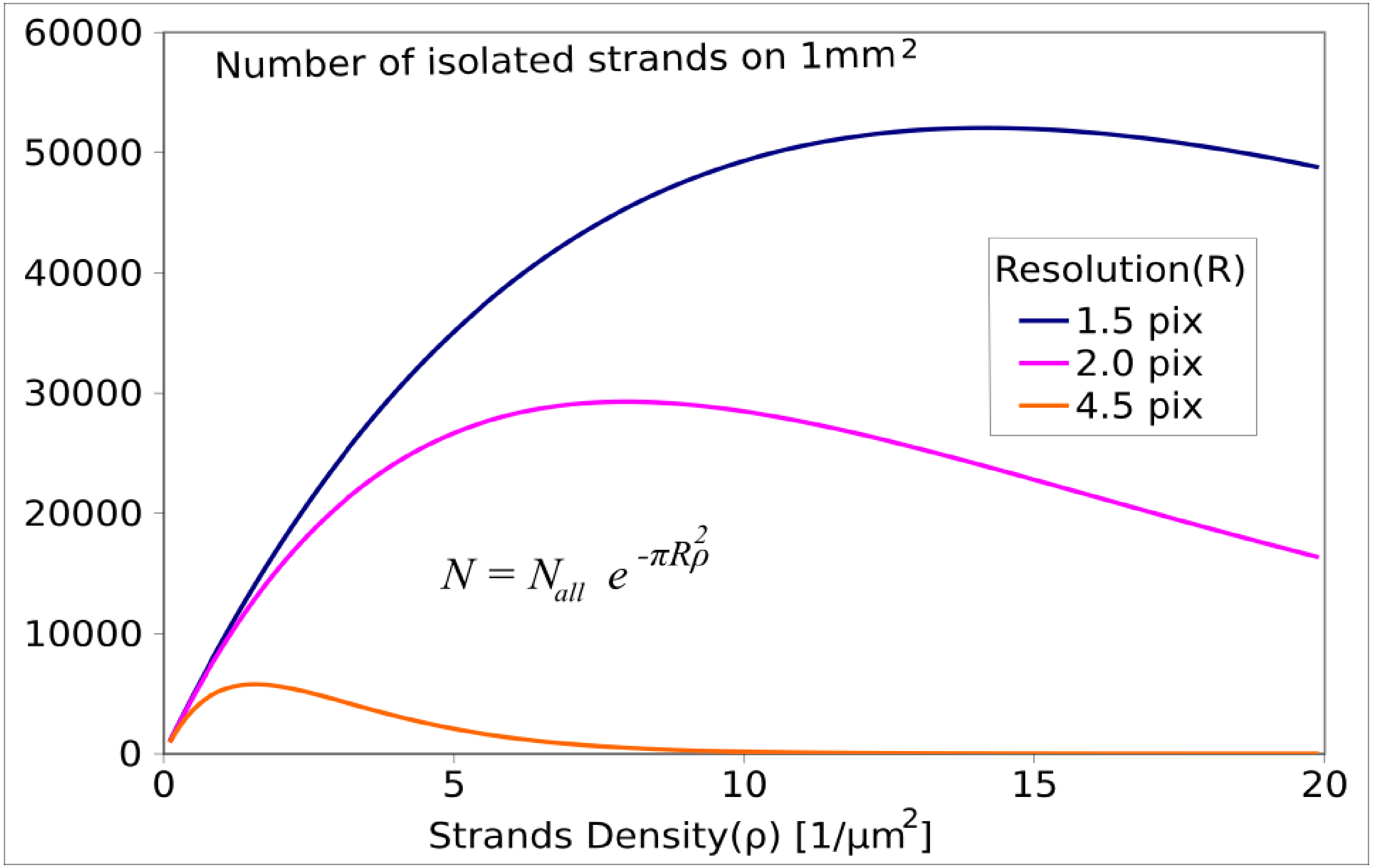
http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8831316.PN.&OS=PN/8831316&RS=PN/8831316

Advantages of super-resolution processing and accurate object detection

1) Our tool provides accurate object count, while State of the art methods grossly underestimate
2) Our tool provides increased productivity of the instrument up to 10 times (measured in a maximum number of useful objects one can fit in a FOV (field of view) of the instrument)
3) Accurate positioning of the objects enables monitoring of development in time
4) Accurate object detection leads to reliable confidence metric
5) Accurate object detection can be used for diagnostics of the instruments imaging quality (optical system state) based on object distribution analysis
6) Accurate object detection can be used for chem-bio trouble shooting – accurate density and state of the reagents measures

1) Accurate object count

State of the art methods without the super-resolution tend to grossly underestimate the object count

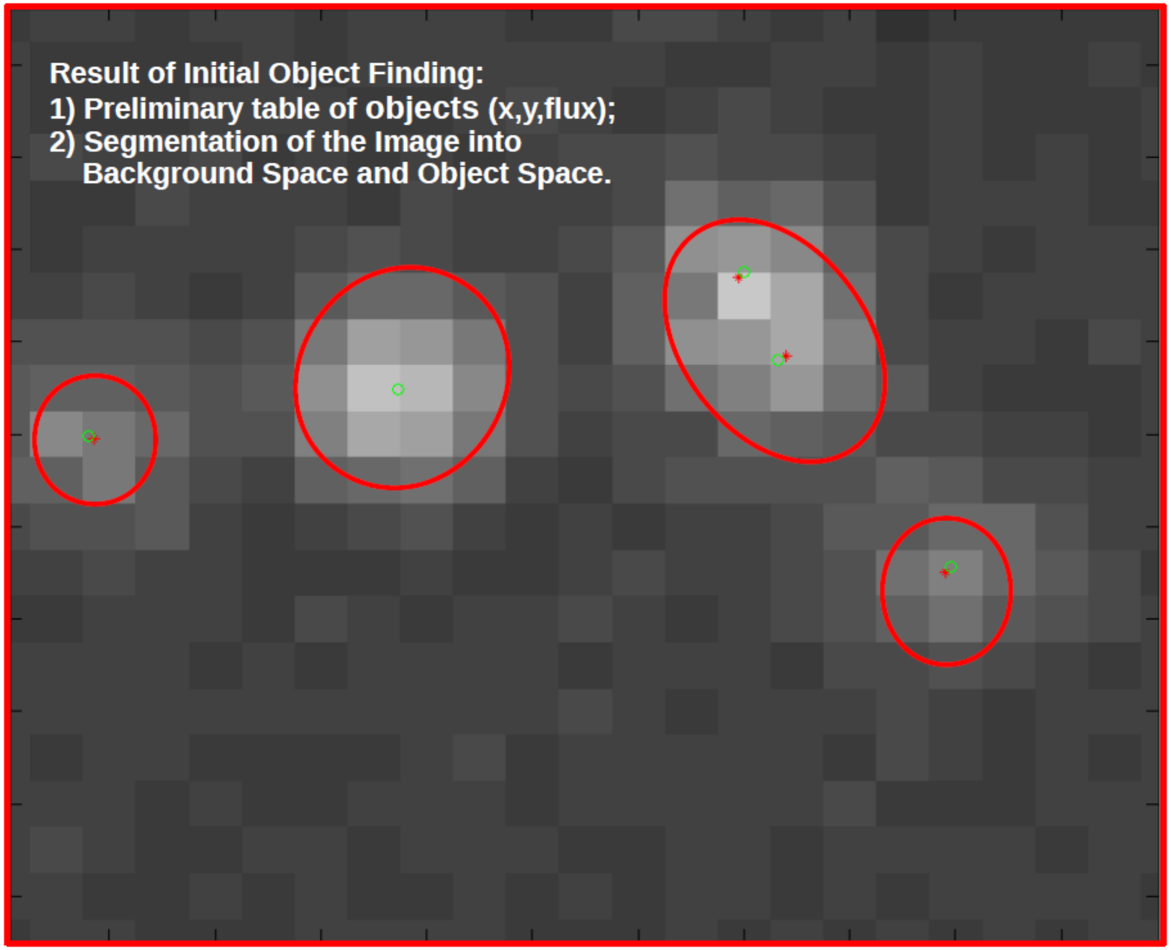


2) Productivity of the instrument = = number of useful objects in FOV

Productivity of the instrument (measure in a maximum number of useful objects on can fit in a FOV (field of view) of the instrument is increased 10 X times. Obviously the speed of processing of the samples with one instrument can be correspondingly increased.

3) Accurate positioning of objects

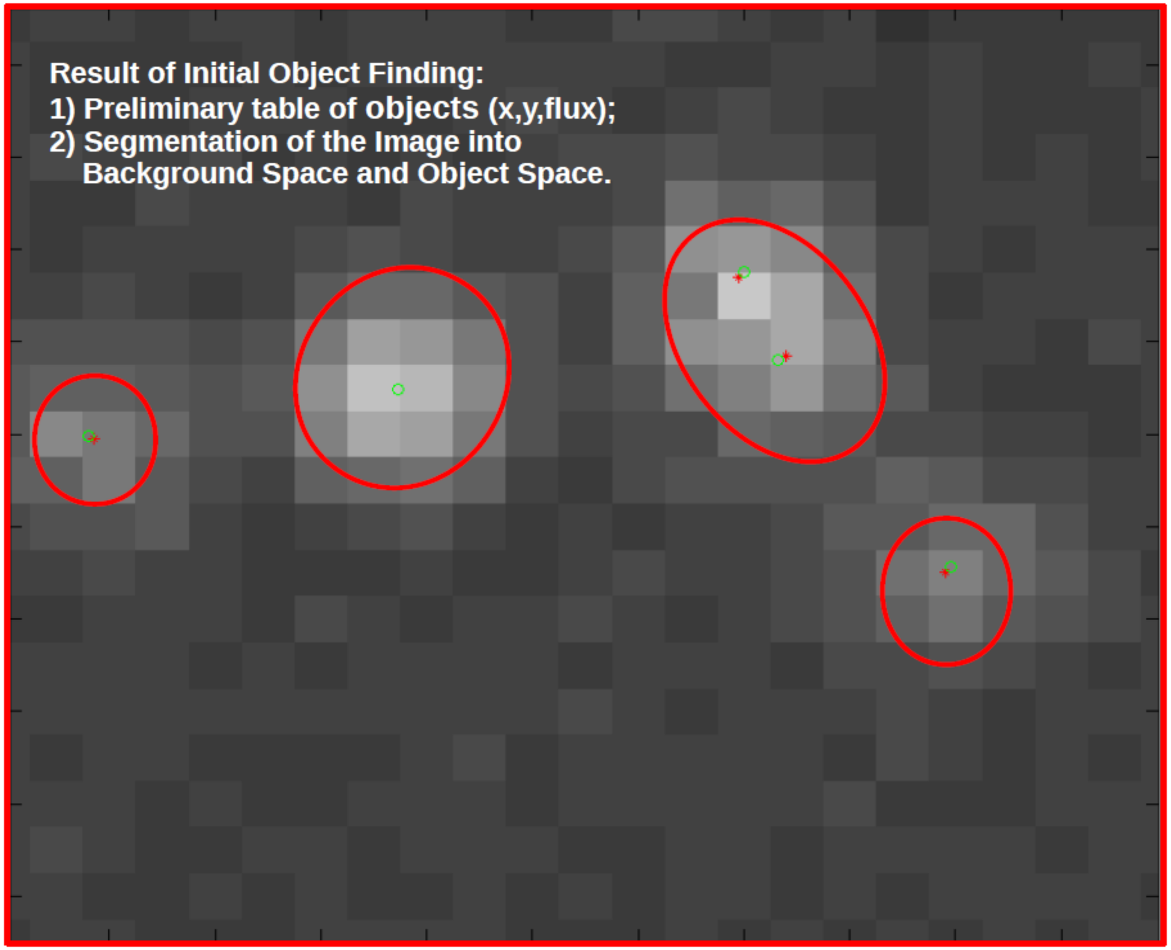
Accurate positioning: blended objects are resolved;
Position Accuracy ~ SNR (Signal to Noise Ratio) as is Accuracy of the placement of separately standing objects
Accurate object Positions are important for : object alignment.
If a history of the object is important (DNA strands being built, pathogen reaction followed etc)



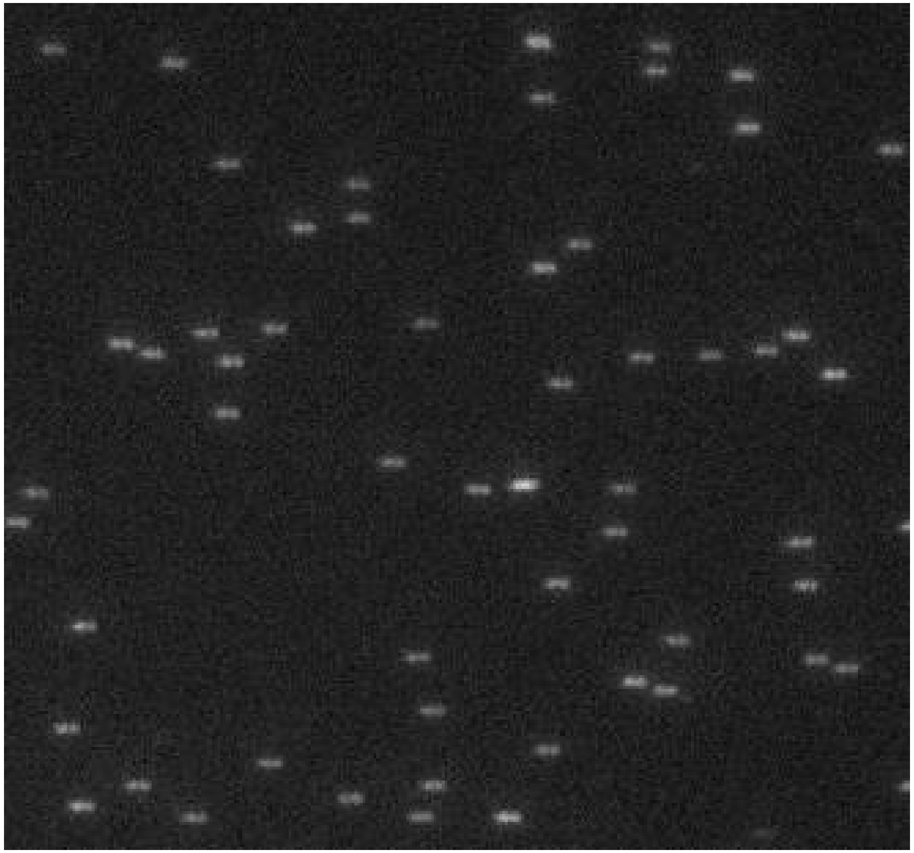
4) Confidence measure

Accurate object detection leads to reliable confidence metric

A confidence measure ~ residual light after light of all found objects is subtracted from the light in the image



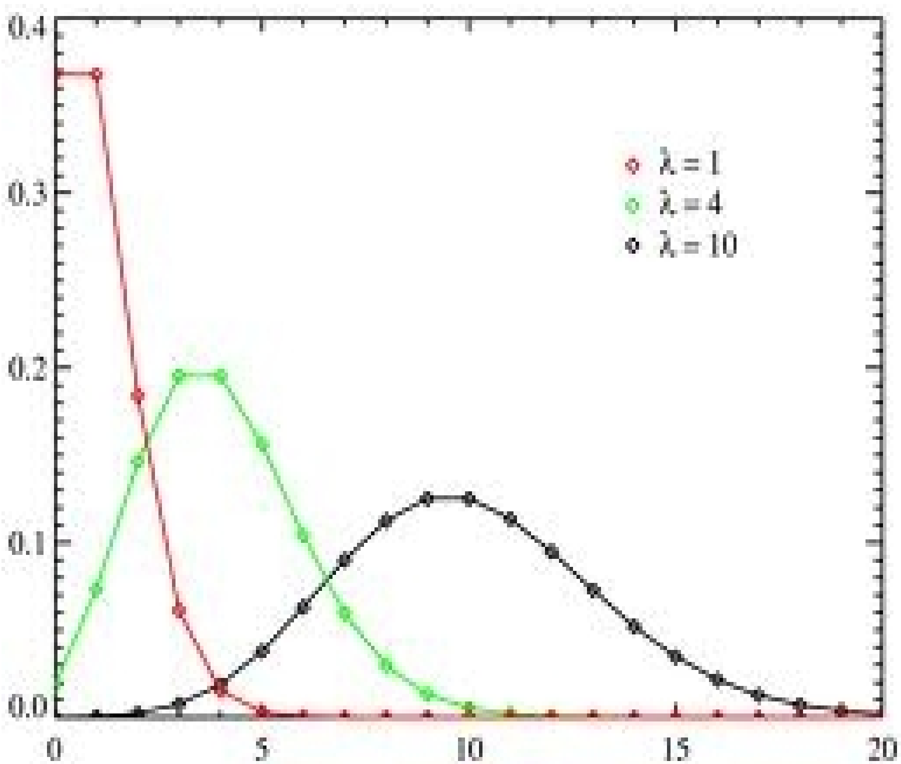
5) Object detection based instrument imaging quality measures (and diagnostics for the instruments optical system)



Example: stage moved,
all objects are seen as doubles.

While a-state-of-the-art object detection would not even see the objects as doubles

6) Object detection based object distribution analysis can be used for chem-bio trouble shooting – accurate density and state of the reagents measures



These curves describe the probability of finding the amount of X (x-axis) objects within a unit area (or volume) fora true Poisson distribution of density λ. Accurate Object detection enables object distribution analysis. If it shows deviations from the distribution expected it suggests a deviation of chemical density of reagents. The distribution analysis can recommend a specific quantitative change to achieve an optimal density for the instrument.

If accurate Object detection reports and object distribution analysis show deviations from the distribution expected it suggests a deviation of chemical density of reagents. The distribution analysis can pinpoint excess of insufficient density compared to optimal density range for the instrument.