Some Styles of Theory in Biophysics

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Strike! Strike! Strike!

Strike! Strike! Strike!

General models→Often fundamental limits

The most reductionist level→Molecular modeling

Modeling a single process

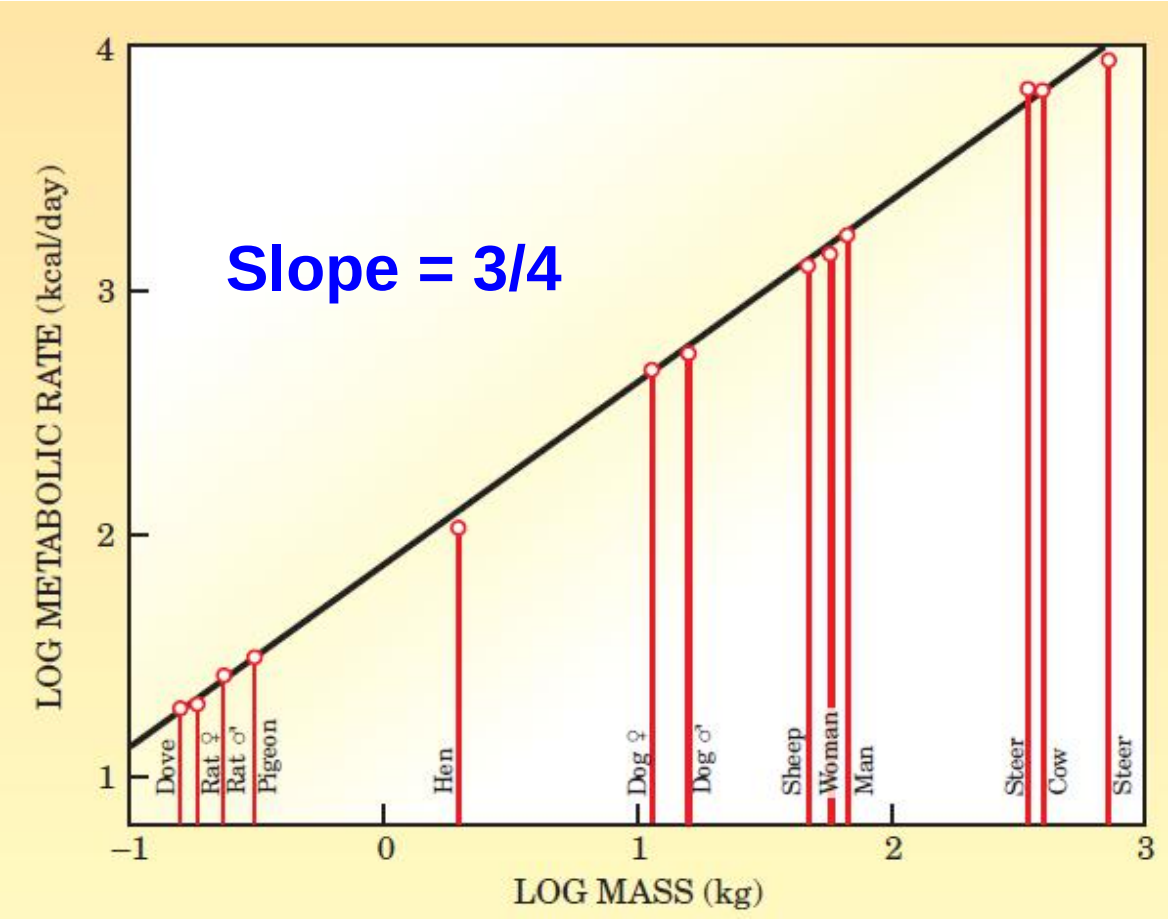
Models of experiments → **Phenomenology**

Models of experiments → **Information limits**

My own work on superresolution

How I got into biophysics

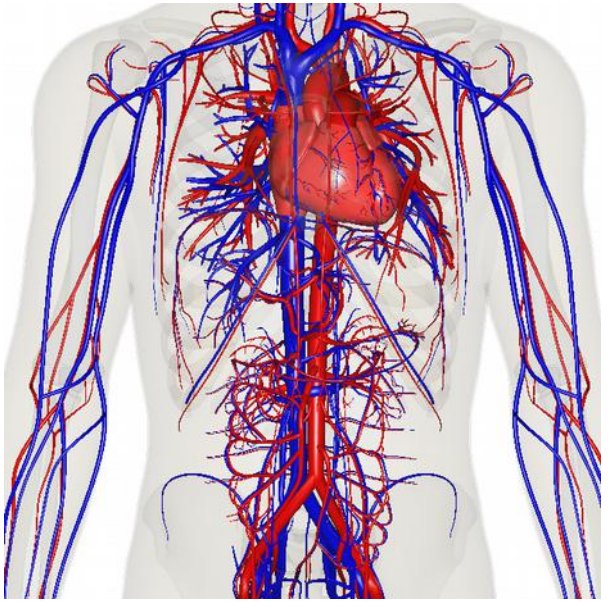
- 1997: West, Brown, Enquist (mostly) explain this:



- But heat loss $\propto$ area $\propto m^{2/3}$???

Model Assumptions

1) Fractal network of blood vessels



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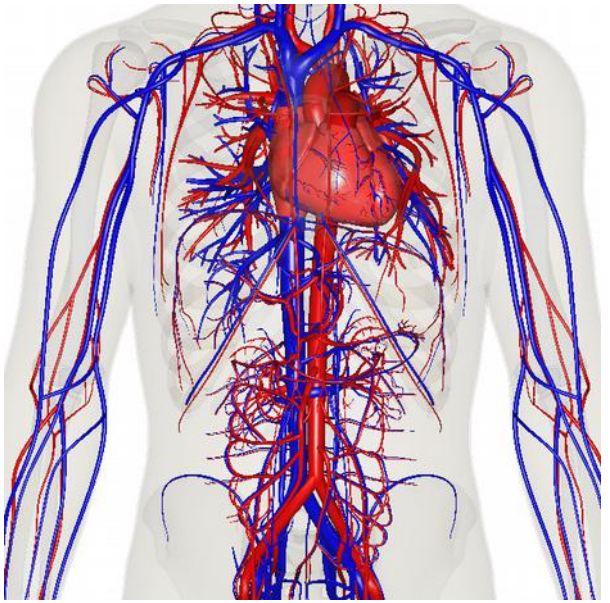

2) Invariant terminal units

Capillaries have to be ~100 μm apart and thick enough for a red blood cell

Model Assumptions

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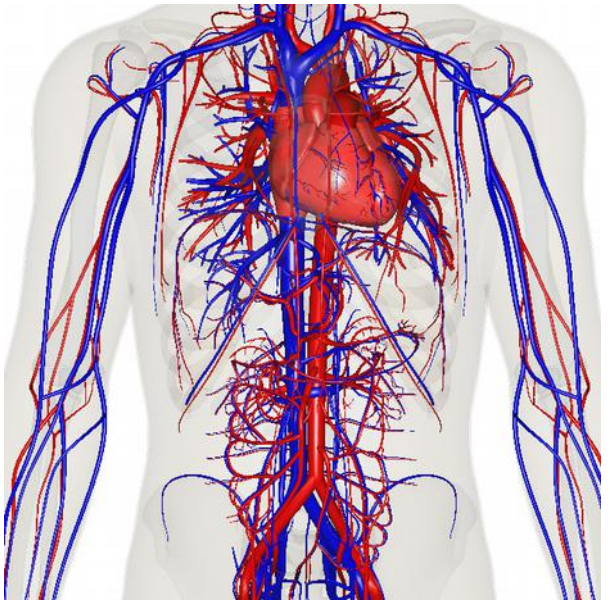
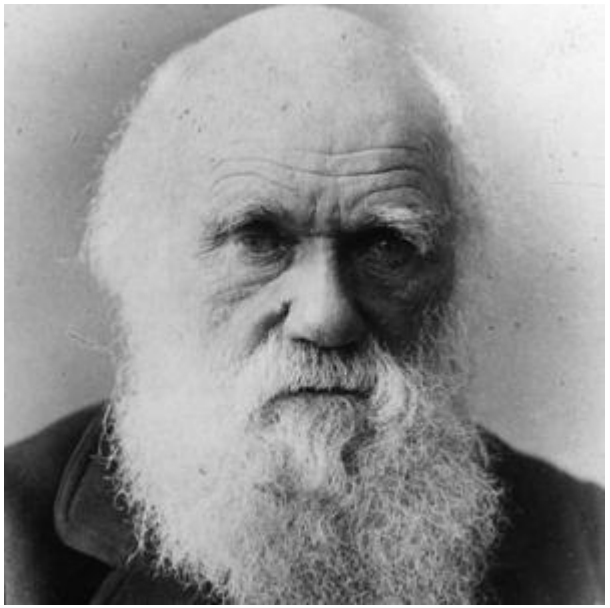


2) Invariant terminal units

Capillaries have to be ~100 μm apart and thick enough for a red blood cell

3) Minimize energy dissipation



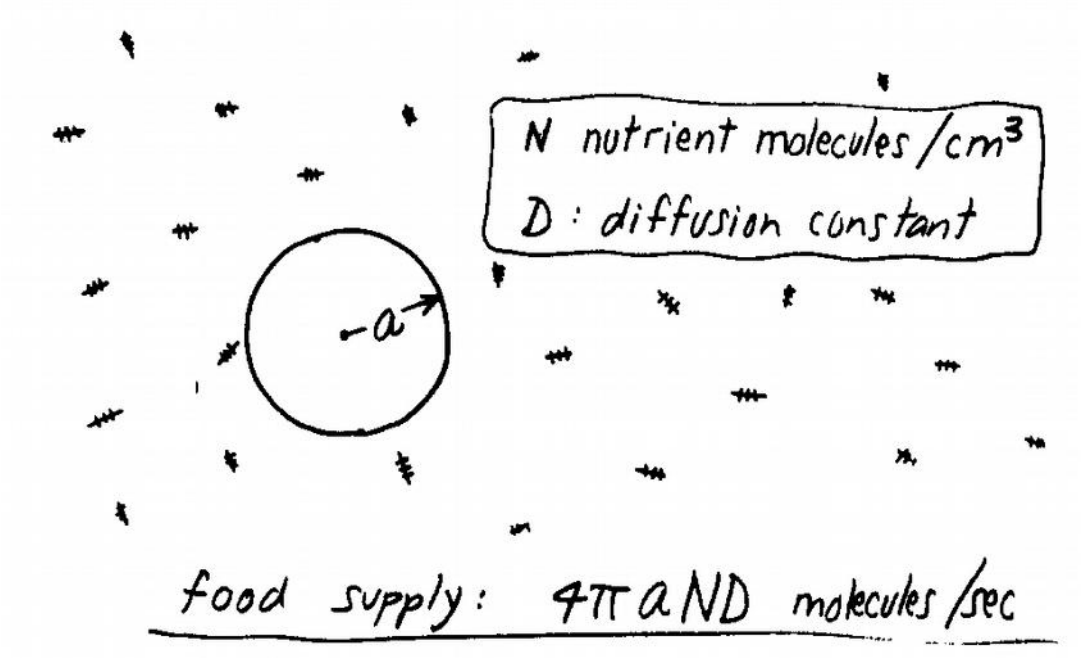
(Caveats)

- The ¾ scaling law is more accurate for large mammals than small ones.
- The fluid dynamics assumptions in WBE are only valid asymptotically.
- WBE errs in wrong directions for small mammals.
- More accurate models fix it.

Biophysics of fundamental questions

Life at low Reynolds number

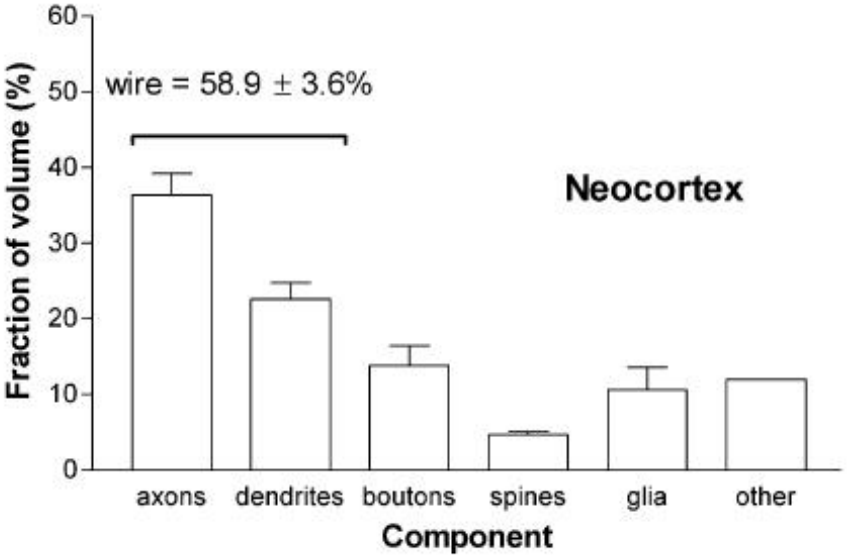
E. M. Purcell
Lyman Laboratory, Harvard University, Cambridge, Massachusetts 02138
American Journal of Physics, Vol. 45, No. 1, January 1977



Biophysics of fundamental questions

Minimize conduction delays
Maximize number of connections
Minimize wire length

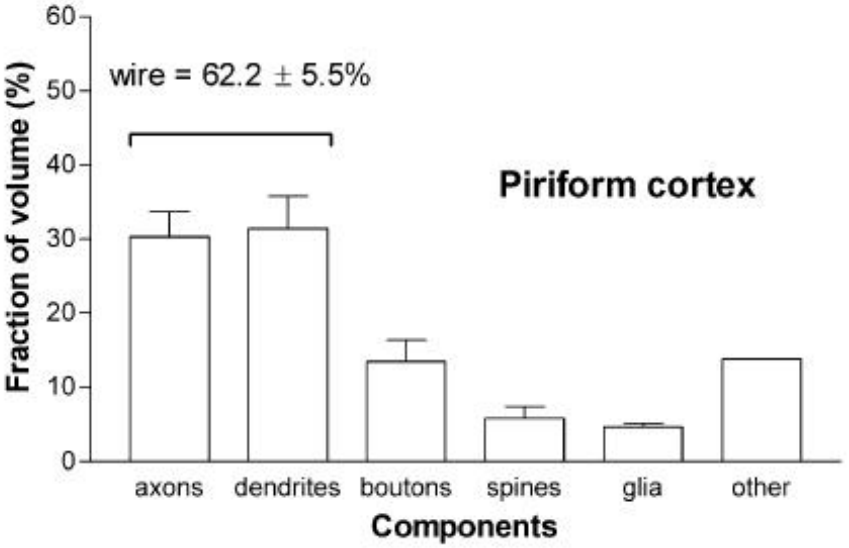
→Brain should be 60% wire



Neuron, Vol. 34, 341–347, April 25, 2002, Copyright ©2002

Wiring Optimization in Cortical Circuits

Dmitri B. Chklovskii,[1] Thomas Schikorski,[2] and Charles F. Stevens[2,3]
[1]Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724
[2] Howard Hughes Medical Institute
The Salk Institute



Other fundamental results

- Optimal ratio of macromolecules to small molecules in bacteria (Vazquez, 2010)
- Morphogen gradients optimized against noise (Saunders, 2009)
- Limits to concentration sensing and chemotaxis (Endres, Levine, Wingreen, etc.)
- Leaf size bounded by diminishing returns to resource investment and flow impedance of tall trees (Jensen and Zwieniecki, PRL, 2013)

And many, many more...

Not all biophysics theory is done at 50,000 feet

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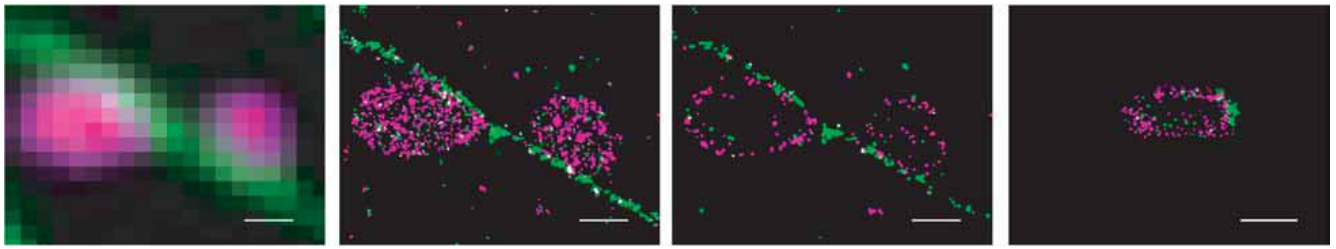
Biophysics of particular systems

- Often borrow approaches from soft matter physics (especially cell membranes, cytoskeleton)



- Cell-cell interactions
- Physiology
- Muscles
- Fluid mechanics
- Nervous system

Membranes & cytoskeleton

Molecular crowding & Interactions

Molecular modeling

e^- transfer

0.1 nm | 1 nm | 10 nm | 100 nm | 1 μm | 10 μm

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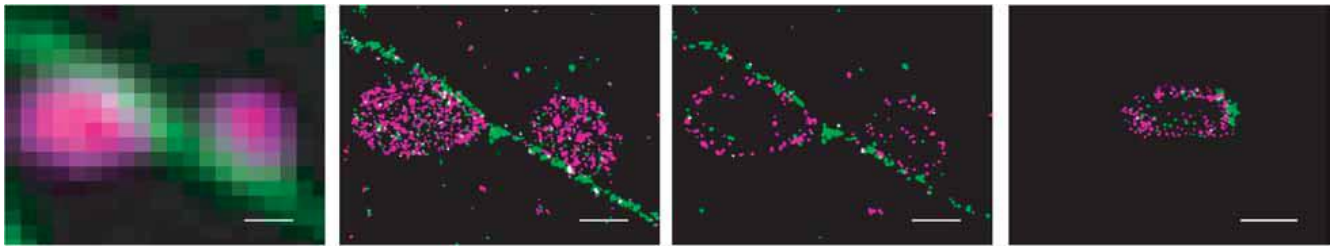

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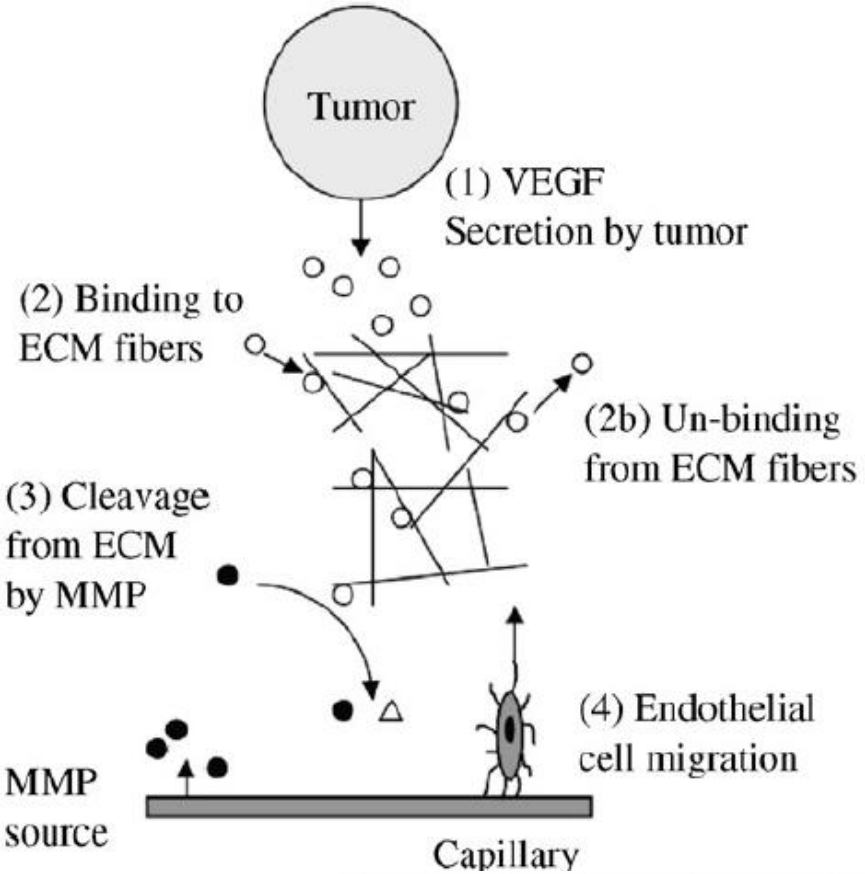
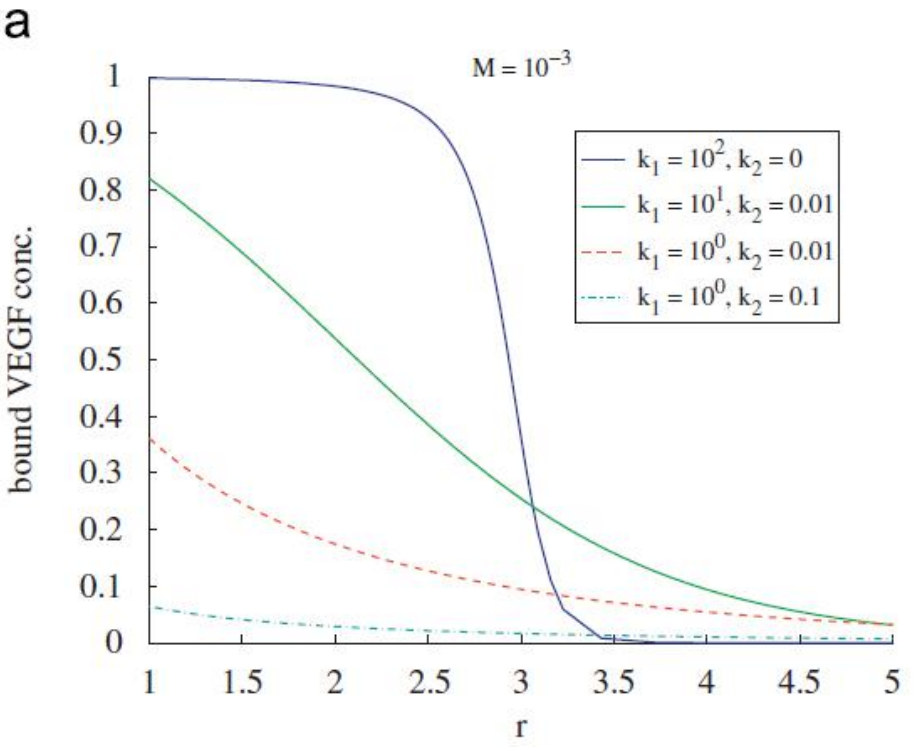
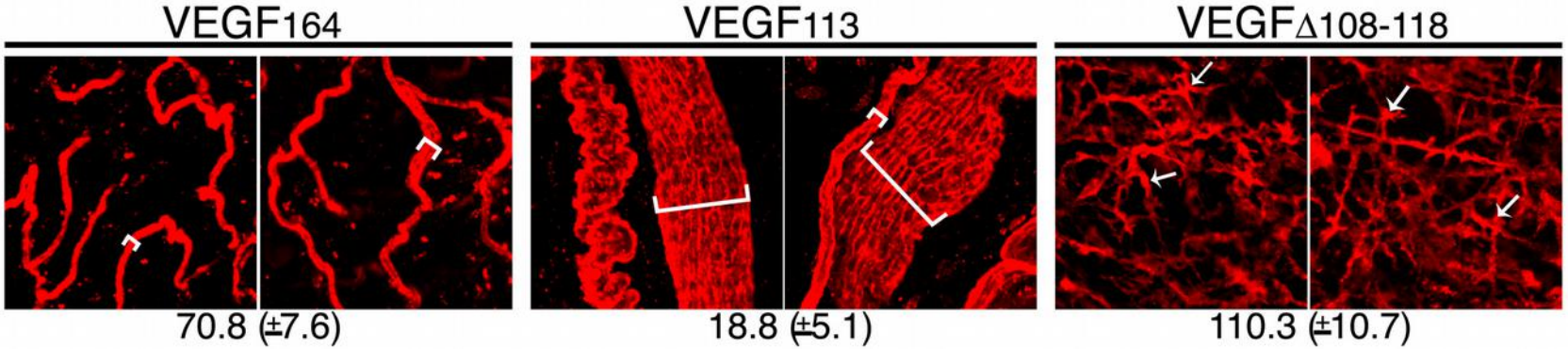
0.1 nm | 1 nm | 10 nm | 100 nm | 1 μm | 10 μm

Reaction-Diffusion Models
Solvable for undergrads w/ software

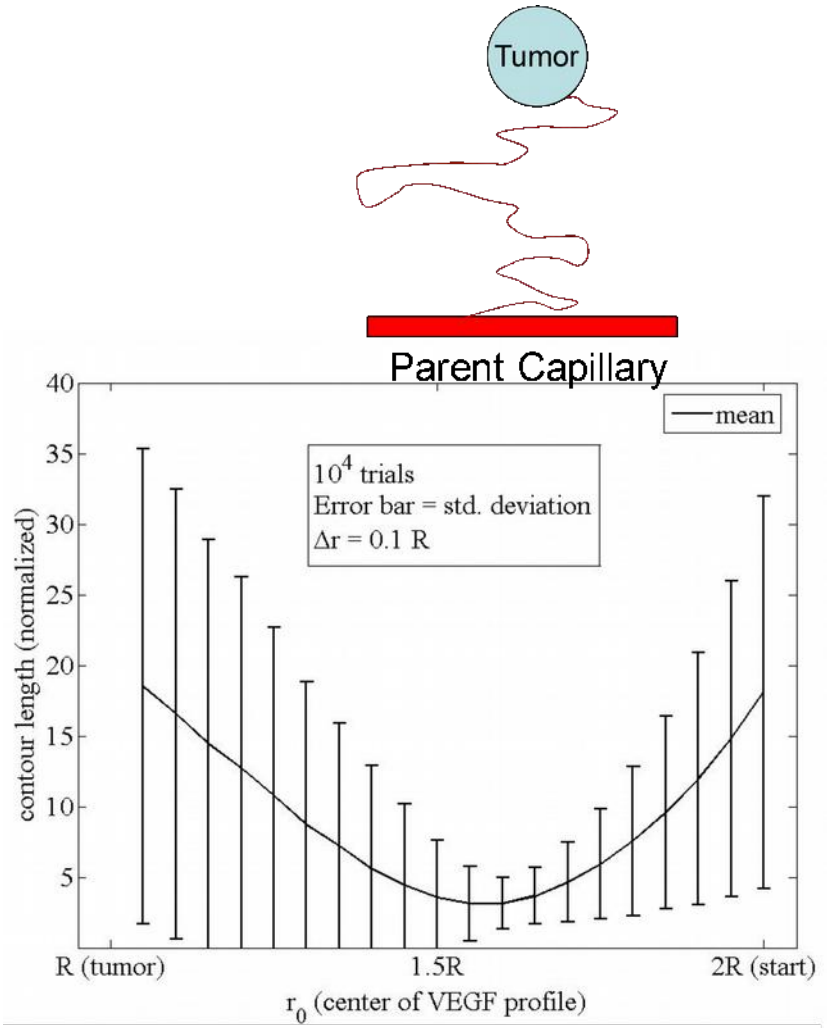
Example: Tumor Angiogenesis

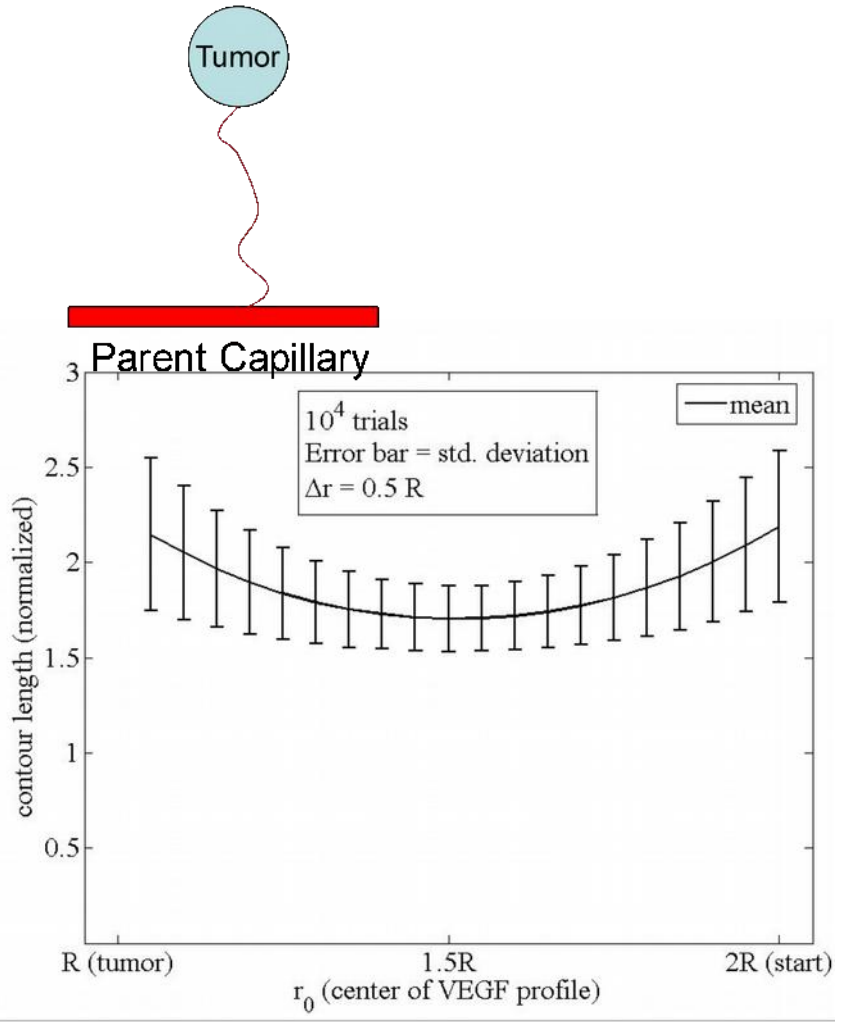






Relation to vessel morphology





- Step-like gradients lead to longer migration times
- More tortuous morphology, fewer vessels reach tumor
- Consistent with differential potencies of $VEGF_{189}$ vs. $VEGF_{165}$

Who does what

- Fundamental limits: Primarily physicists
- Membranes, cytoskeletons, ordering, interactions: Primarily physicists
- Reaction-diffusion models: Physicists and mathematicians
- Cascades of reactions: Primarily mathematicians
- Developmental biology: Mostly mathematicians, and also Bill Bialek

Molecular simulation

- Mostly done by chemists
- A few physicists→Note on history of the field
- Enough canned and standardized code that you can get undergrads involved on some level.

- Now for some slides from Paul Nerenberg (formerly of Claremont Colleges, about to start at CSU LA)

What are molecular dynamics (MD) simulations?

- Basic idea: simulate molecules in time by calculating forces at each instant and applying Newton's second law
- Forces are derived from a potential energy function or *force field*
- A fundamental trade-off: accuracy of the potential energy function vs. sampling

The various flavors of MD

Simulation type	**Resolution**	**Number of atoms**	**Time scale**
ab initio MD	Atomic+	10^2	200 ps
All-atom polarizable MD	Atomic	10^2-10^4	50 ns
All-atom fixed-charge MD	Atomic	10^3-10^5 (max: 10^7)	1 µs (max: 1 ms)
Coarse-grained MD	~Residue	10^3-10^5	100 µs

The MARTINI force field

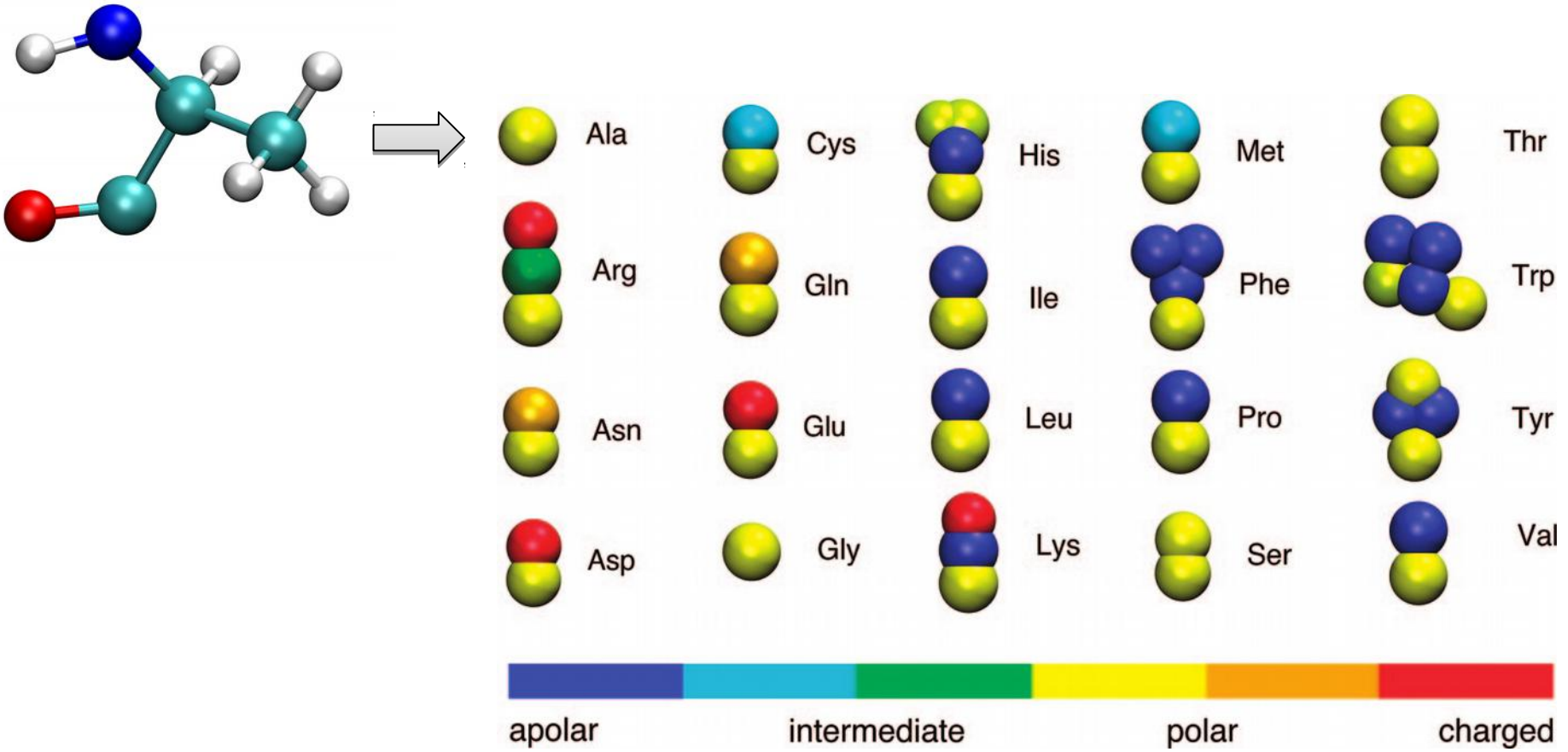


Figure 1. Coarse-grained representation of all amino acids. Different colors represent different particle types.

L Monticelli et al., *J Chem Theory Comput* (2008).

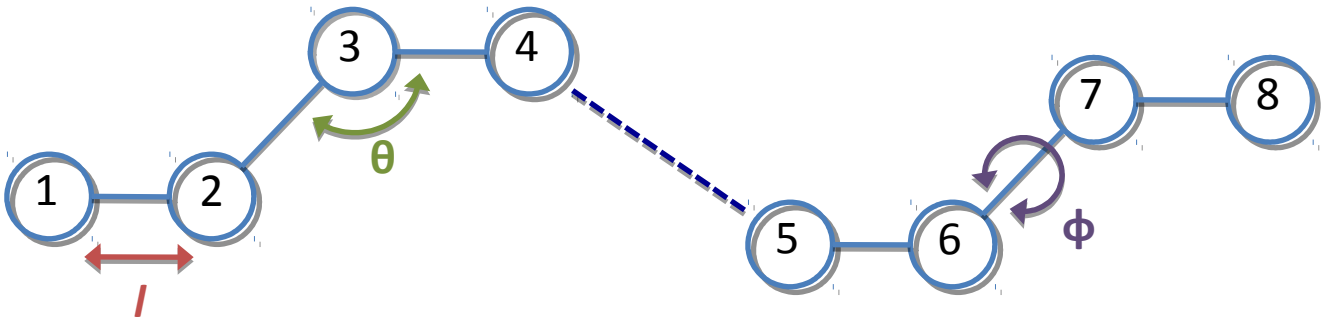
CG MD in a nutshell

- Quasi-atoms are spheres of mass m_i, charge q_i, and van der Waals size σ_i
- Potential energy function (force field):

$$U = U_{bonded} + U_{non-bonded}$$

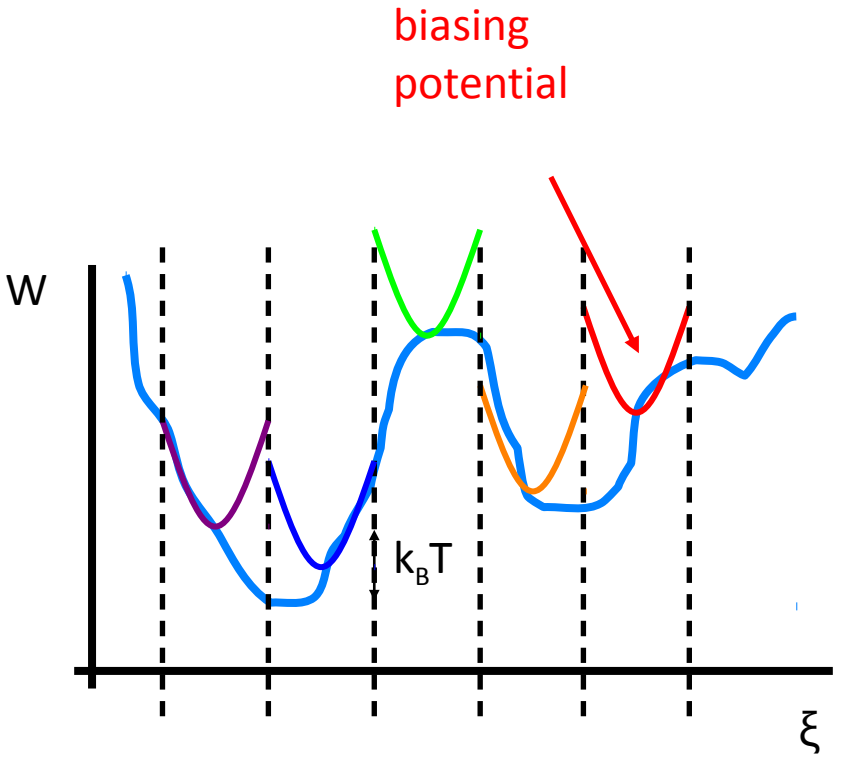
$$= \sum_{bonds} \frac{1}{2} k_b \left(l_{ij} - l_{ij}^{eq} \right)^2 + \sum_{angles} \frac{1}{2} k_a \left(\cos\theta_{ijk} - \cos\theta_{ijk}^{eq} \right)^2 + \sum_{dihedrals} k_d \left(1 + \cos\left(n\phi_{ijkl} - \phi_{ijkl}^{eq} \right) \right)$$

$$+ \sum_{nonbonded} \left[\frac{k_e q_i q_j}{\epsilon r_{ij}} + 4\varepsilon_{ij} \left(\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right) \right]$$

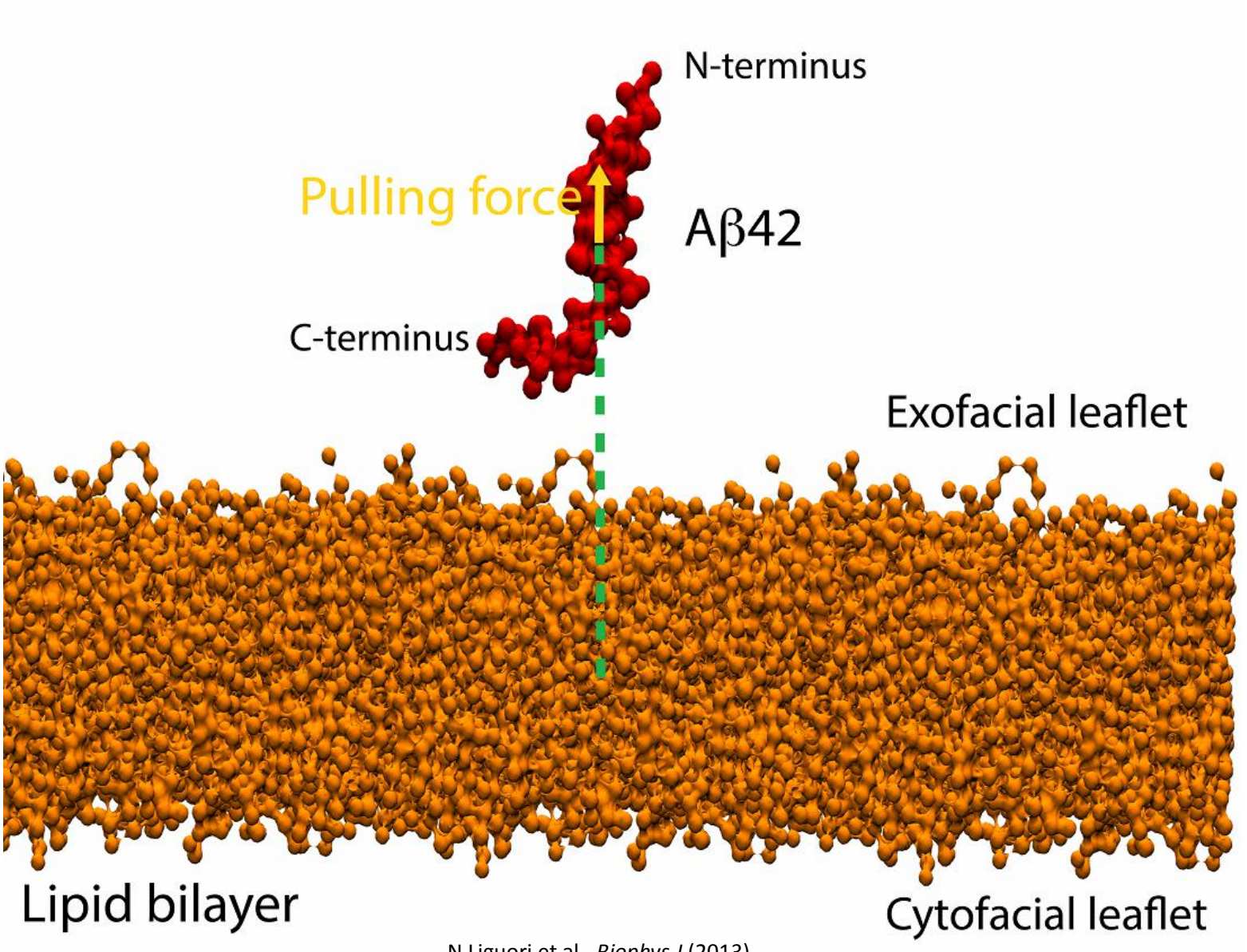


Umbrella sampling

- Enhanced conformational sampling method along reaction coordinate: can traverse large energy barriers between states
- Create a biasing potential to sample small regions of the reaction coordinate
- Sample many windows to cover full range of reaction coordinate
- Unbias data at the end to recover underlying energy landscape (WHAM)



Typical graphics



N Liguori et al., *Biophys J* (2013).

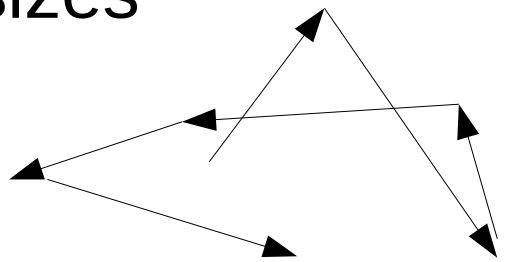
Phenomenology and Information Limits

- Biological systems are messy
- Predicting the sorts of signals that we'll see from an underlying model/mechanism is hard
- Examples: Protein folding, random walks, imaging

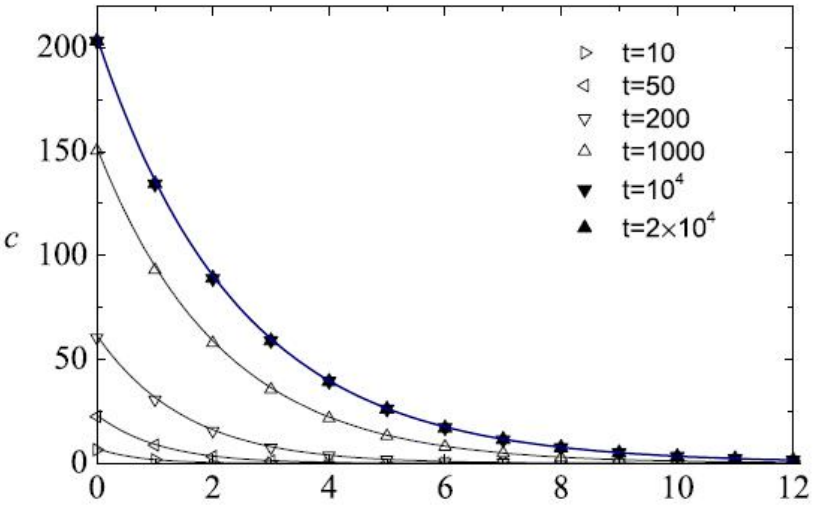
Random Walks

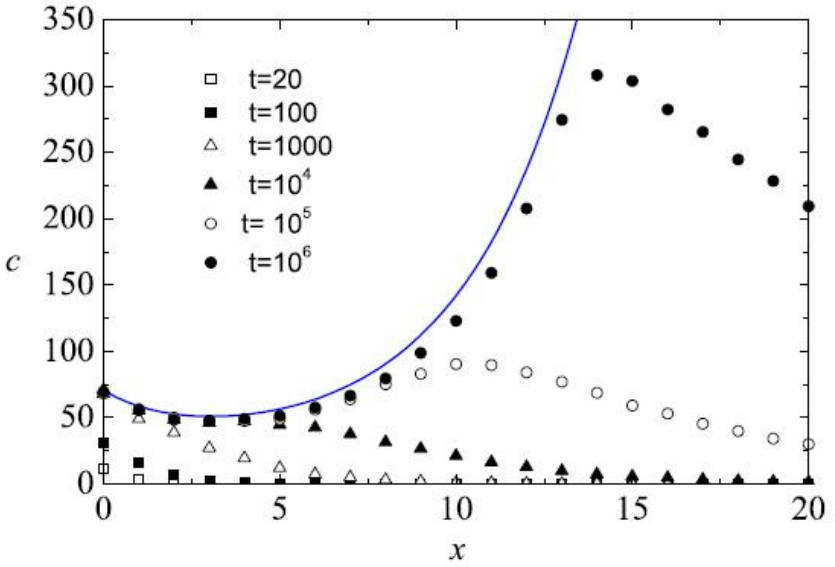
- Simple diffusion is simple
- Anomalous diffusion: Often modeled with Continuous Time Random Walk

  $\rightarrow$Interesting for power-law distribution of wait times and/or step sizes



  $\rightarrow$Even more interesting for spatially varying reaction rates





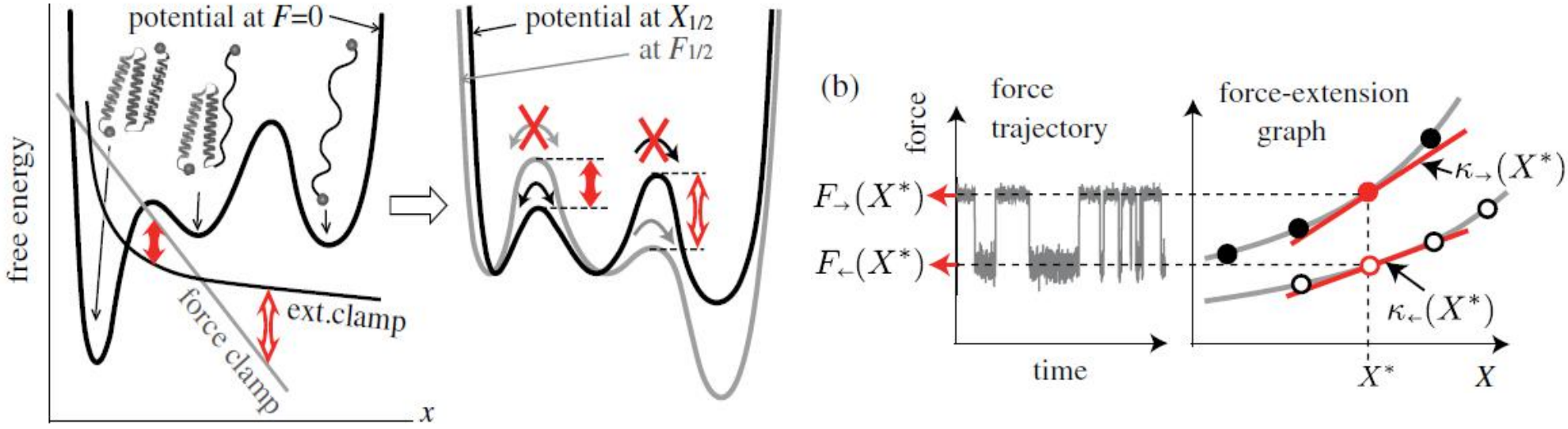
Yuste, Abad, Lindenberg, PRE, 2010

Single molecule experiments

PRL **110,** 158105 (2013) PHYSICAL REVIEW LETTERS week ending 12 APRIL 2013

Single Molecules in an Extension Clamp: Extracting Rates and Activation Barriers

Yohichi Suzuki and Olga K. Dudko
Department of Physics and Center for Theoretical Biological Physics, University of California at San Diego, La Jolla, California 92093, USA
(Received 4 December 2012; published 12 April 2013)

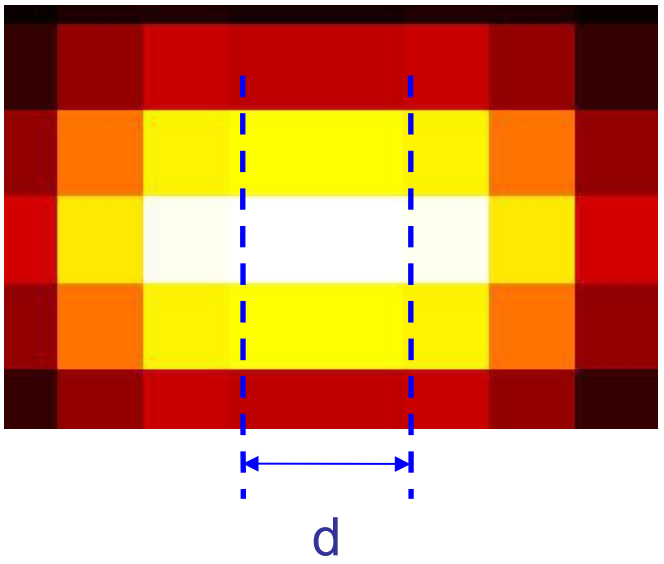


Theory of superresolution

- Every new technique requires a theory to predict the maximum information obtainable
- That's what I've been doing

Single-Molecule Localization

•2 fluorescent molecules close together would look like this under microscope



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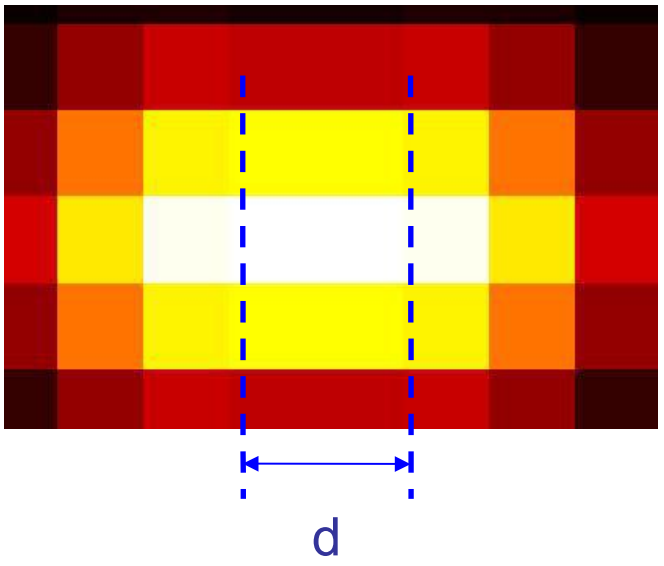
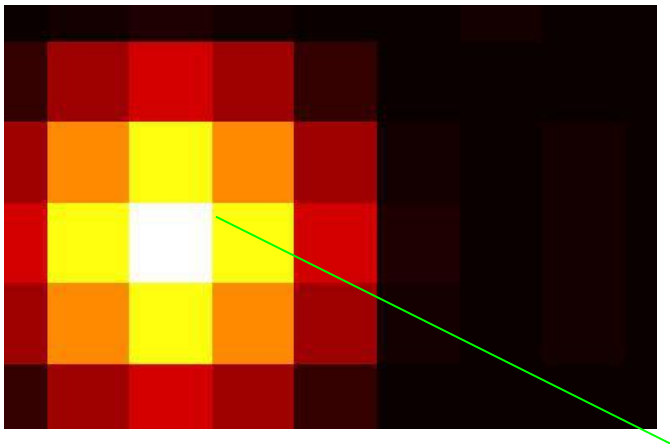
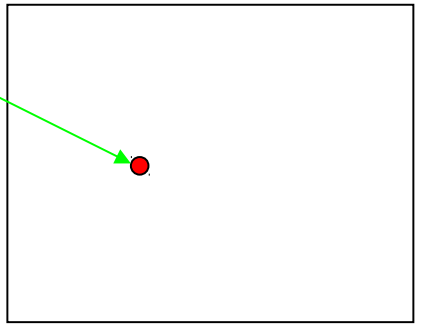


What if only one at a time is shining?



Find centers and infer molecule locations



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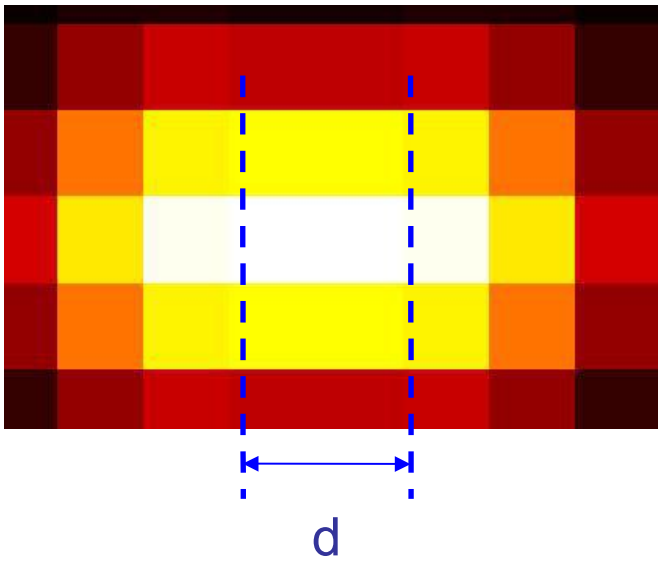

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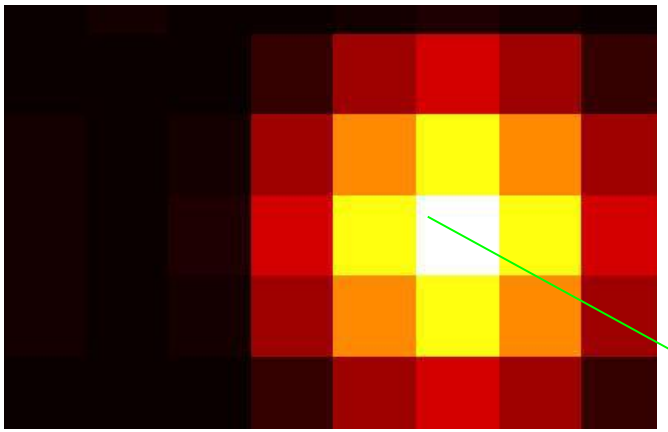

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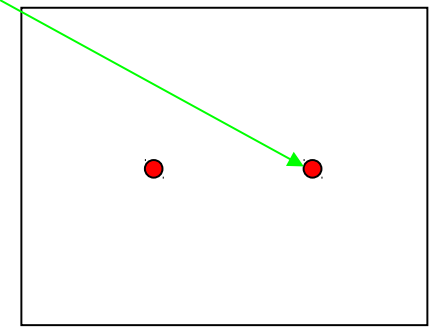

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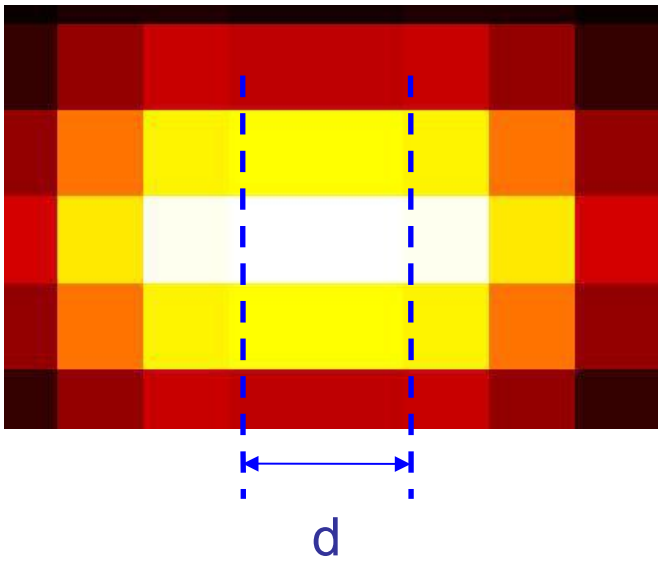

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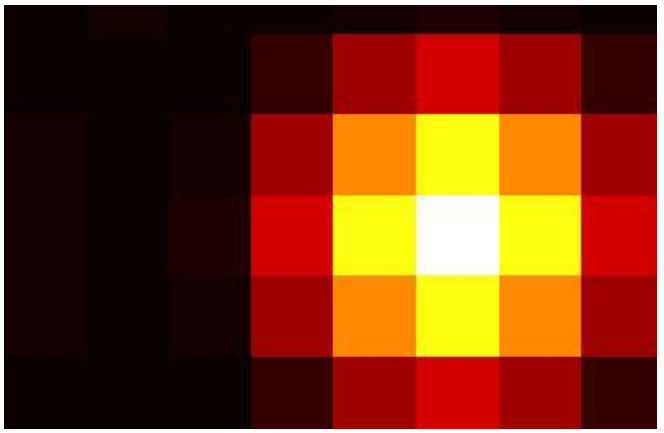

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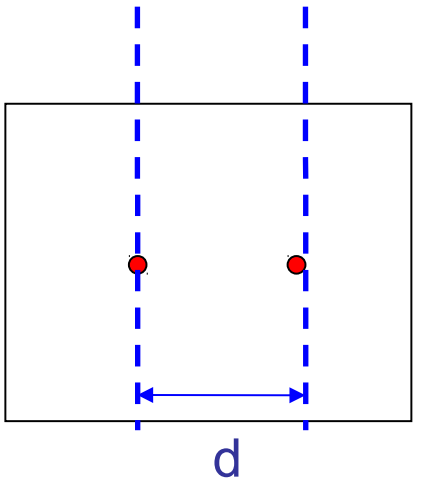
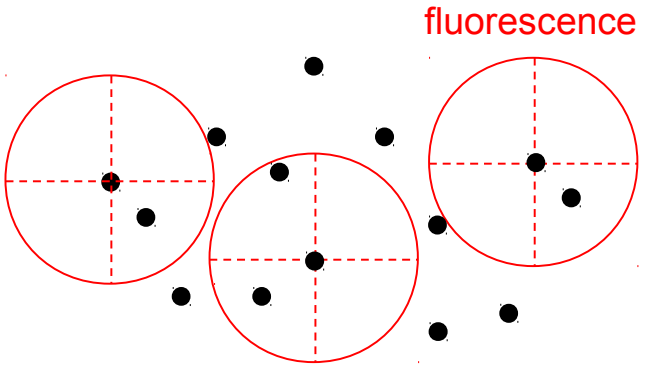


Do this enough times, and eventually you know where every molecule is!

Sequential Localization

Not all the molecules in a crowded image are “on” at once.

Localize those that are on



Time = 1

(no overlaps)

Sequential Localization

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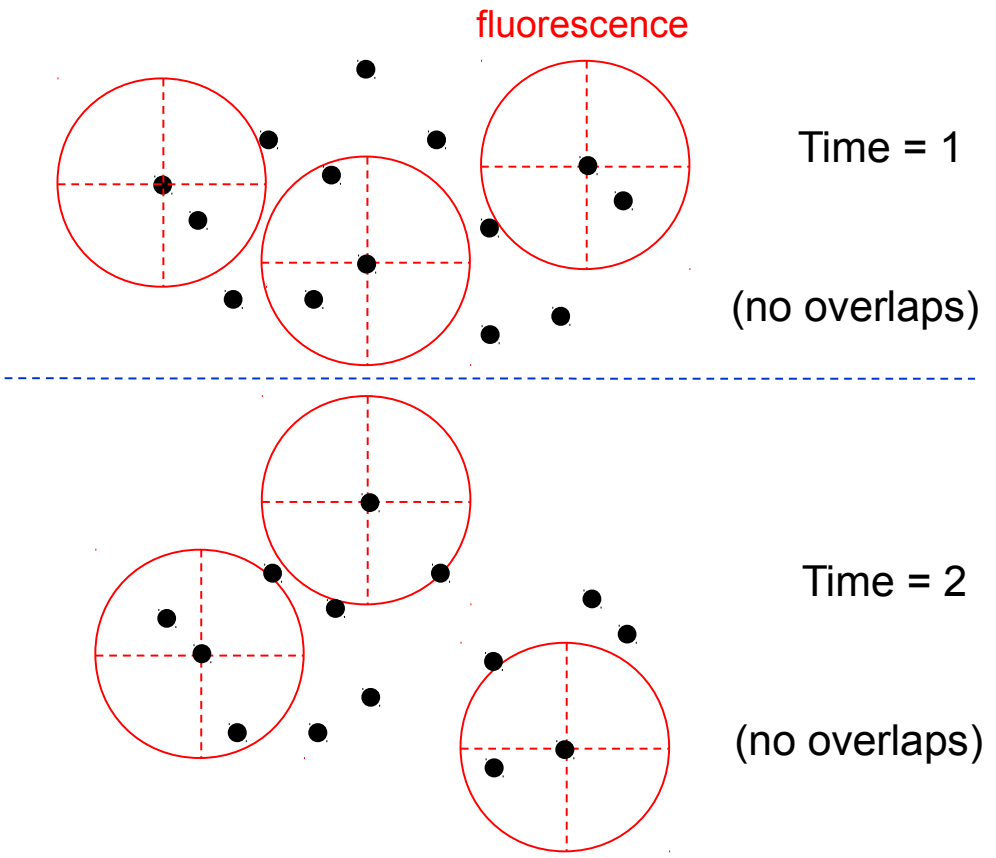
Repeat for different set of on molecules



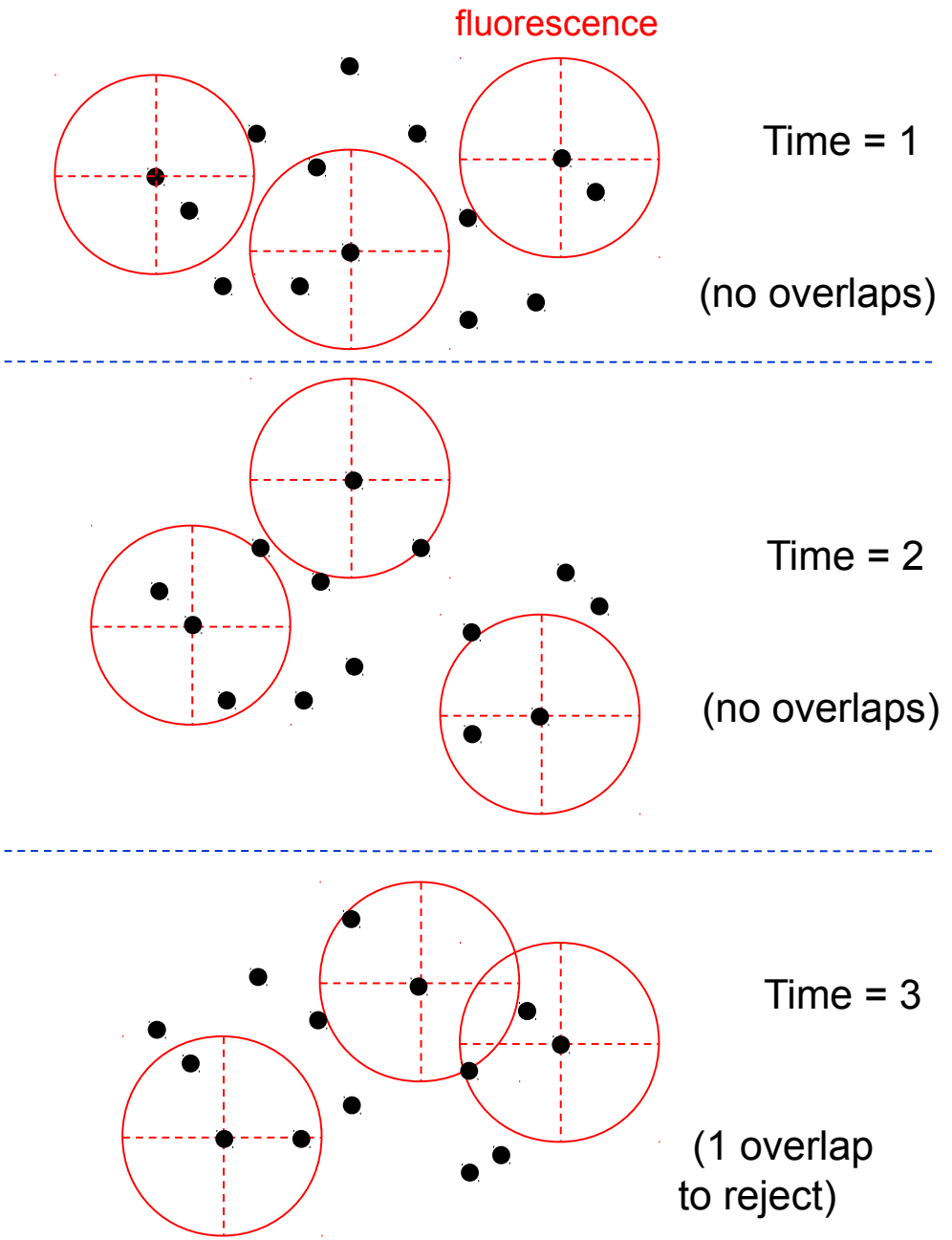
Sequential Localization

Not all the molecules in a crowded image are “on” at once.

Localize those that are on

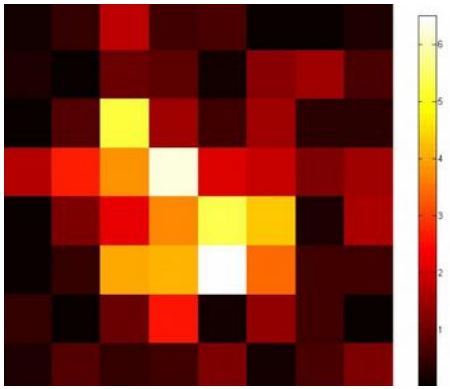
Repeat for different set of on molecules

2 close molecules are “on” at the same time: Need to discard that image.



Noise and Single-Molecule Resolution

- No image is perfect
- We get photons one at a time, in an unpredictable sequence.



50 photons

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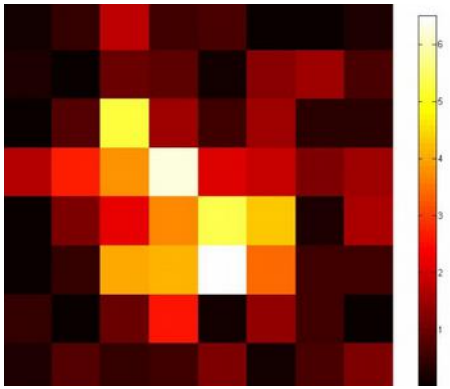
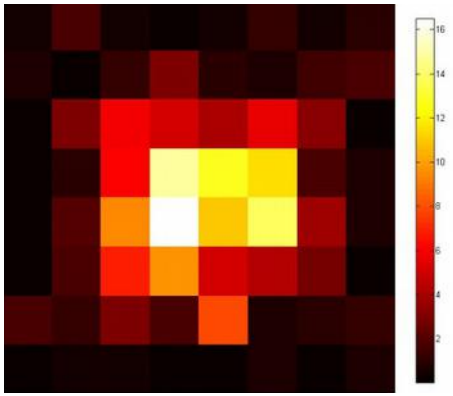


50 photons



200 photons

Noise and Single Molecule Resolution

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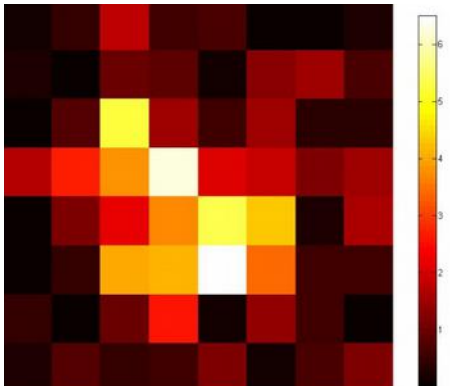
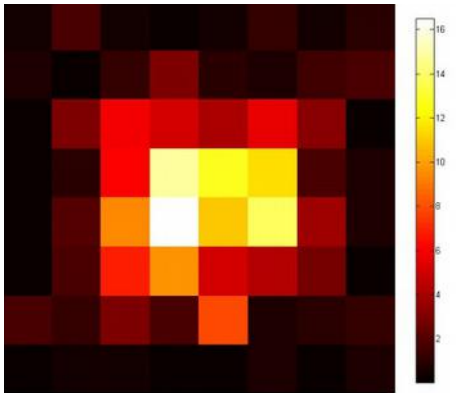
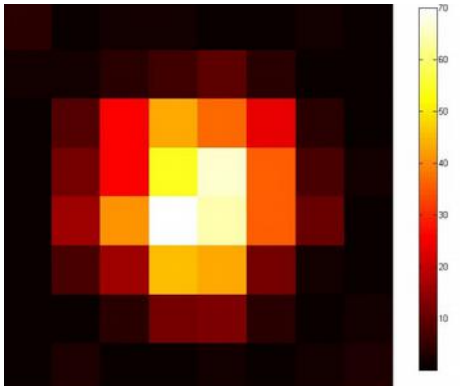


50 photons



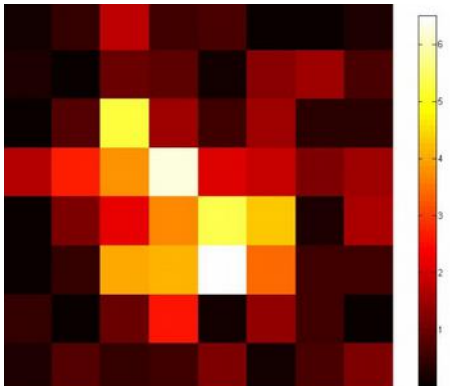
200 photons



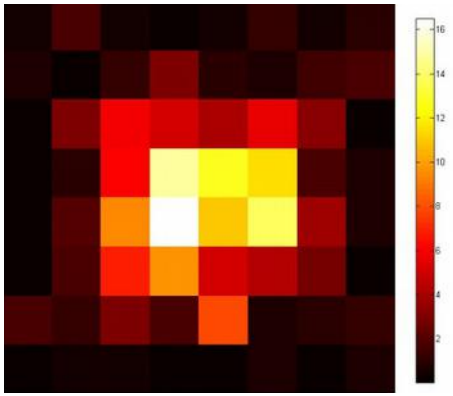
800 photons

Noise and Single Molecule Resolution

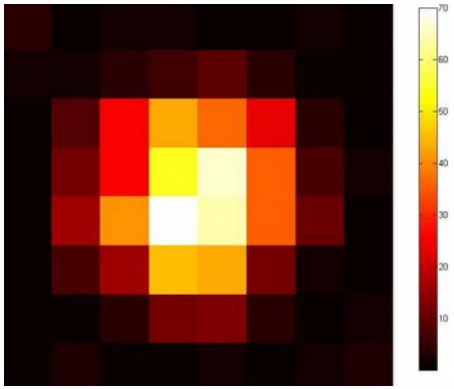
- No image is perfect
- We get photons one at a time, in an unpredictable sequence.



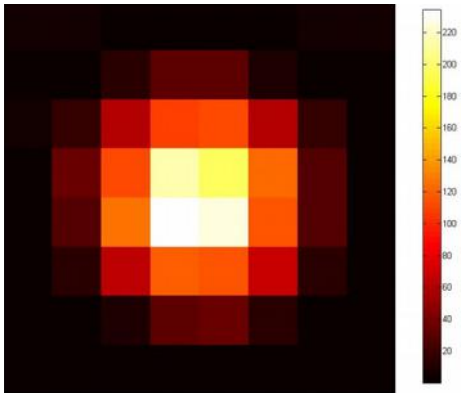
50 photons



200 photons



800 photons



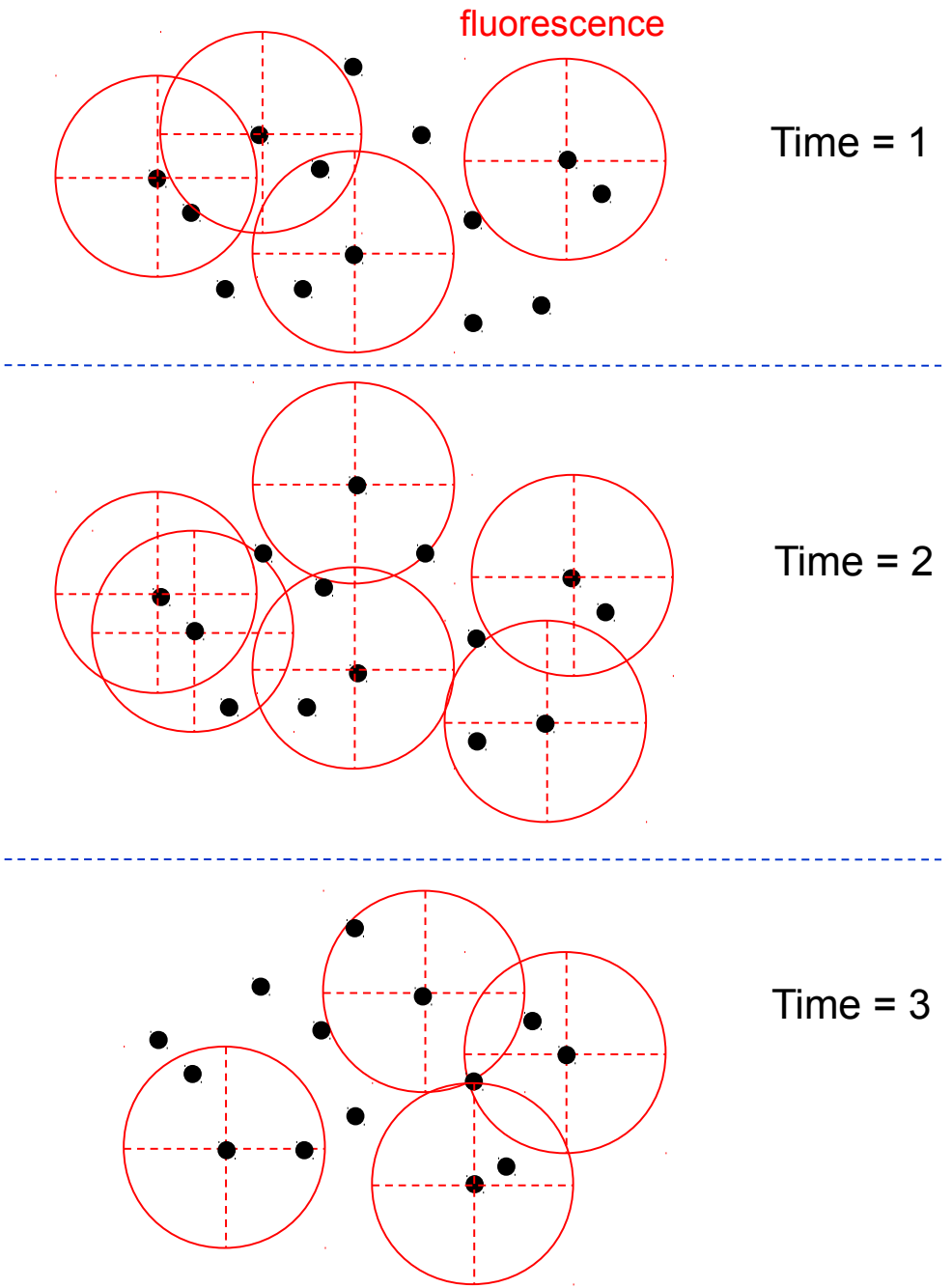
2500 photons

Best possible precision in estimate of molecular position:

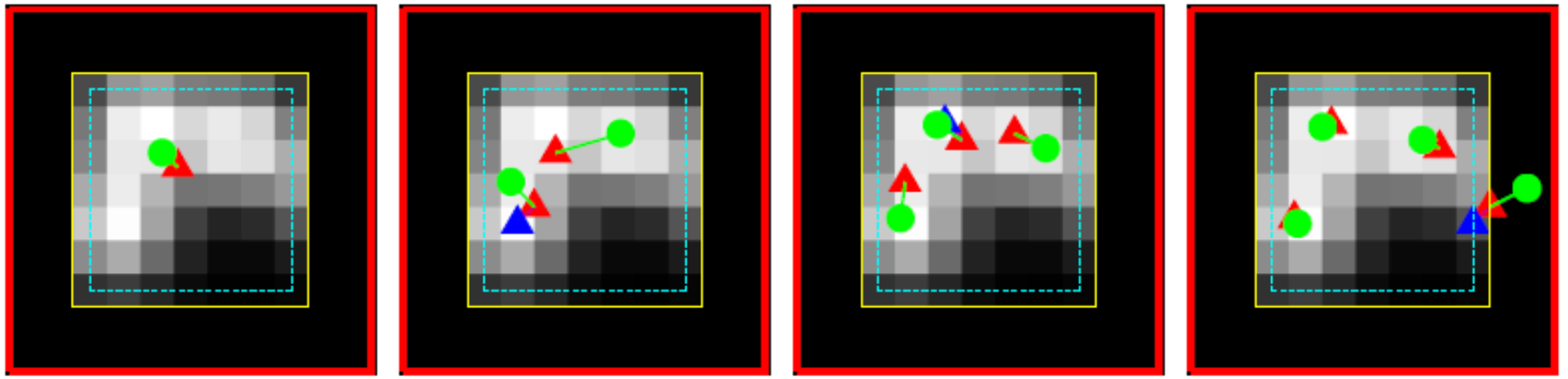
$$\approx \frac{\lambda}{\sqrt{\#\text{ of photons collected}}}$$

Simultaneous Localization

Use software to estimate many positions at once, from overlapping images.



Multifluorophore Localization



(b) Single emitter fitting (c) Two emitter fitting (d) Three emitter fitting (e) Four emitter fitting

Huang, Lidke, et. al. 2011

- Select model that best matches data

Caution: You can always add more fluorophores with low intensity to “mop up” residuals

Motive: Speed

Max Fluorophores per Frame	Normalized Min. # frames needed	Speed improvement
1	1	1
2	0.44	2.27
3	0.26	3.85
4	0.19	5.26
5	0.14	7.14
10	0.023	43.5

Small, Biophysical Journal, 2009

What about resolution?

- MLE software can localize multi-fluorophore images w/ theoretical limit to precision.
- Most approaches consider images of N~5 fluorophores at once.
- Densities up to 8-10 fluorophores/μm^2.

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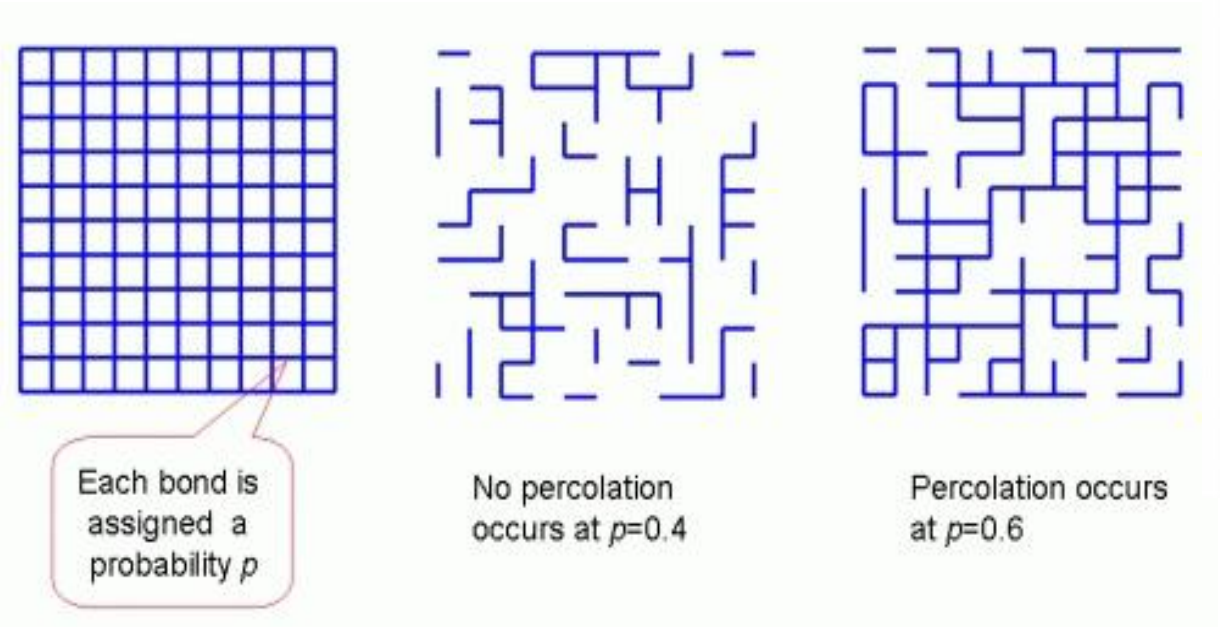
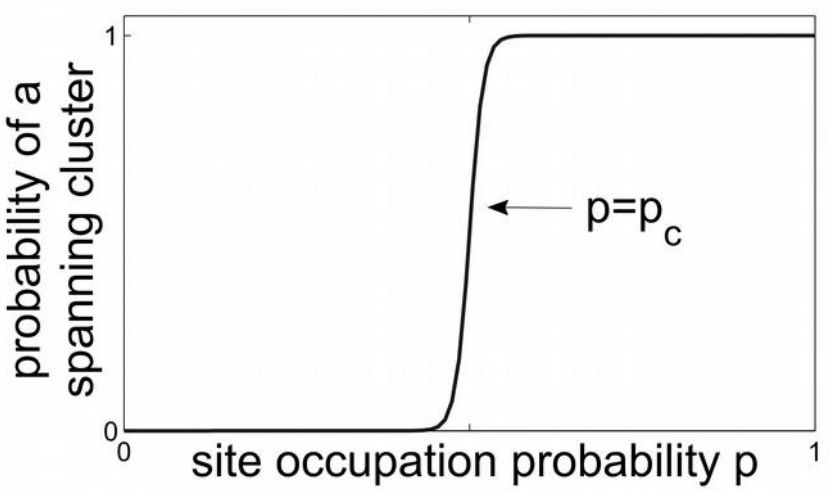
- Densities up to 8-10 fluorophores/μm^2.

Is this the limit?

Percolation Theory

- In a system with randomly-placed components, how many do you need to be assured a path from side to side?

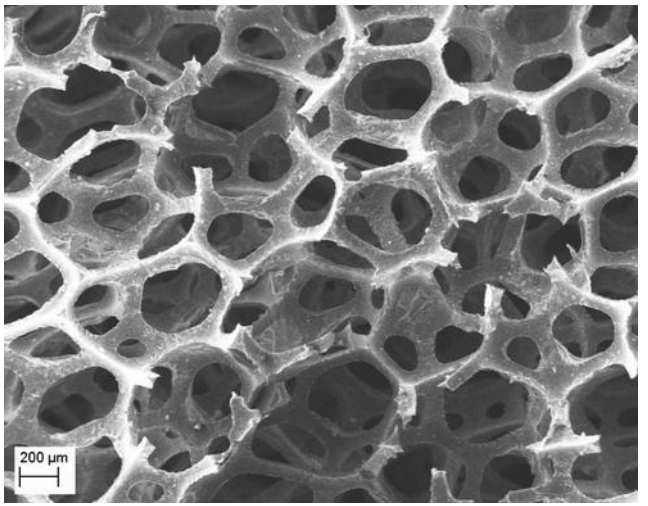




http://mysite.du.edu/~yyi2/percolation.html

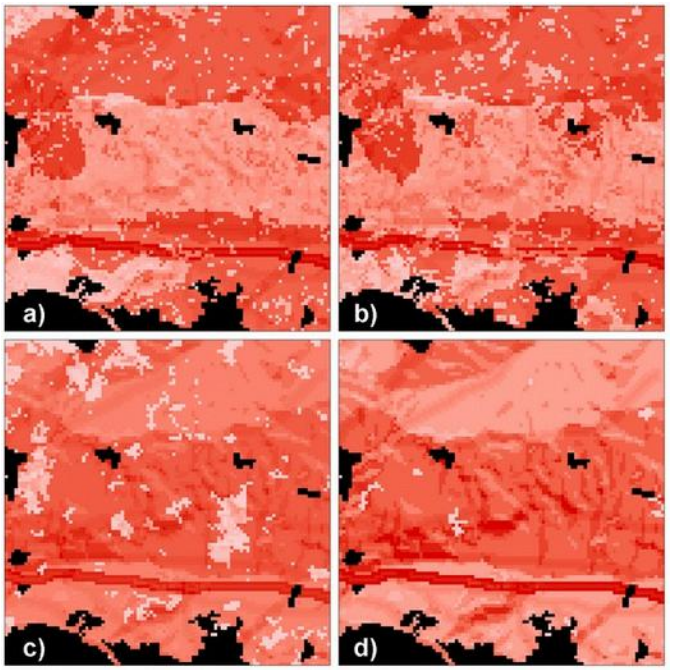
Why is there interest in percolation?

- Porous flow
- Electrical conduction
- Disease
- Forest fires



Bibikov and Prokof'ev, *Composite Materials for Some Radiophysics Applications*

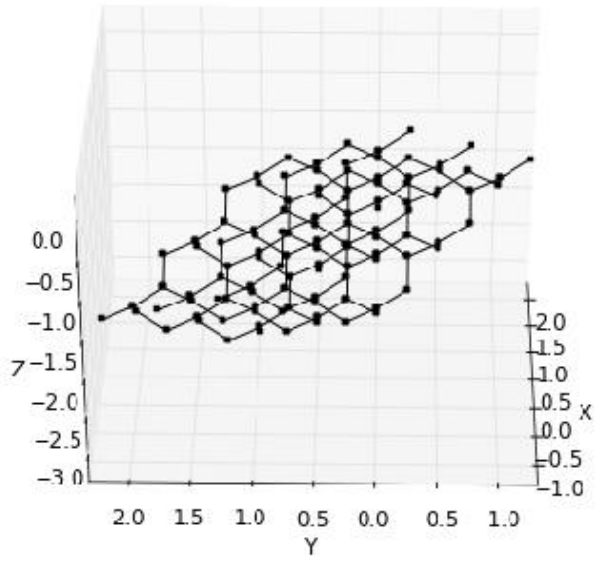
Aerial photo of rural Spain

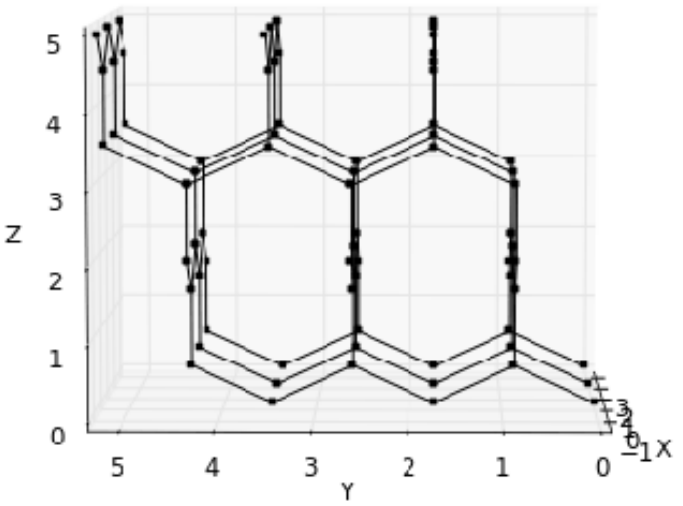


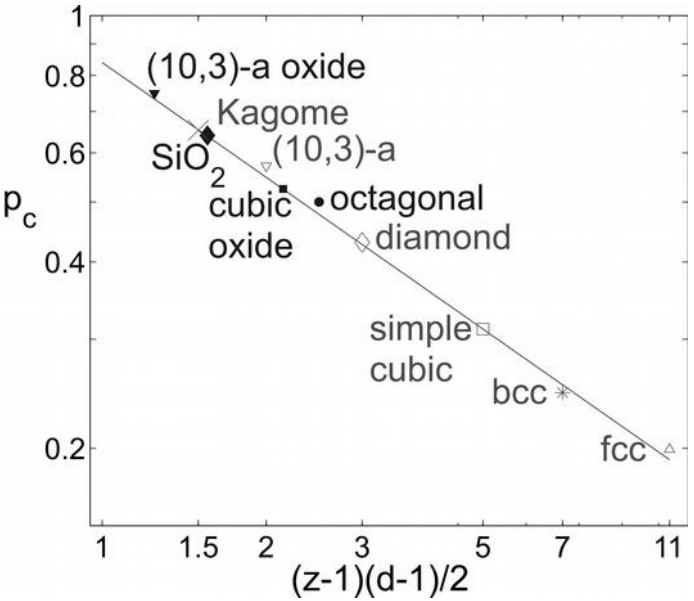
Computer simulations of wildfire risk in different land use scenarios.

Why did I get into percolation?

- (10,3)-a
- (10,3)-b





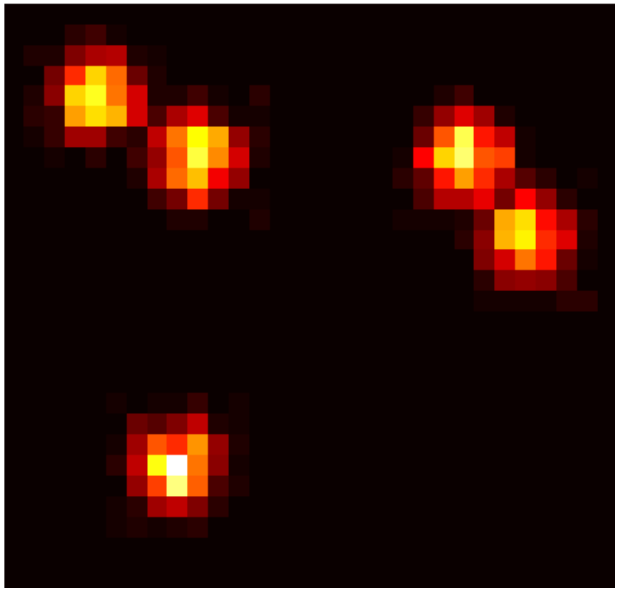


Low-coordinated lattices: Side project for my undergrads

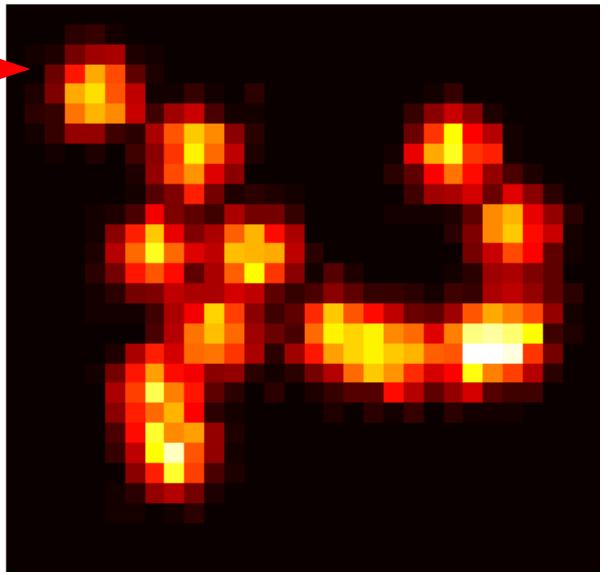
Tran, JStat, 2013
Yoo, Jstat, 2014

Why should microscopists care about percolation?

- Low density
- Small clusters are independent

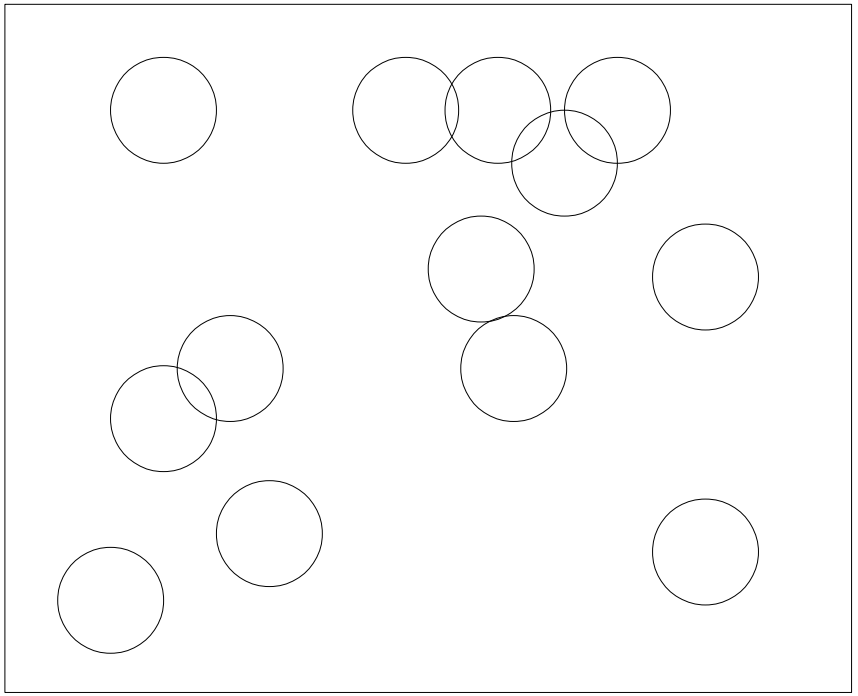


- **High density (percolation)**
- Many molecules are coupled
- Position estimates have higher variance.

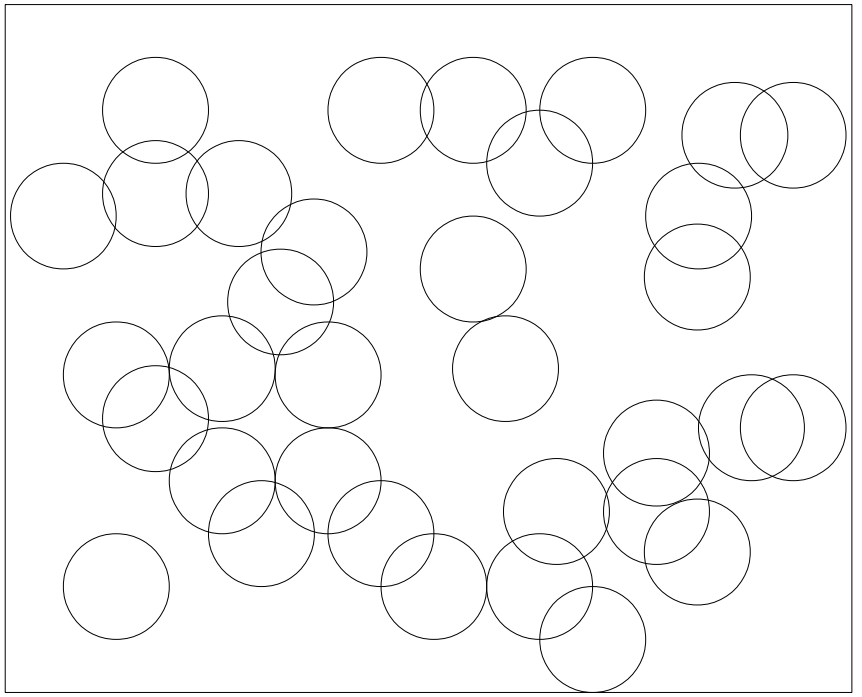


Continuum Percolation

- We don't need to do this on graph paper.



Low coverage

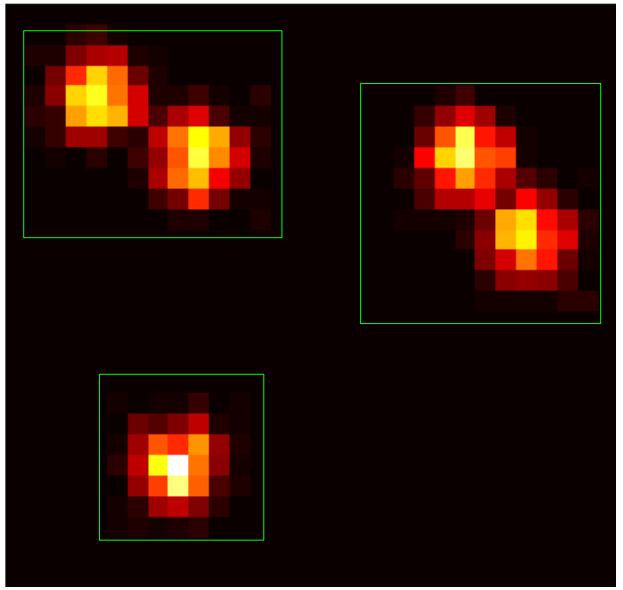


High coverage (**≥67.6%**)

Boundary Issues

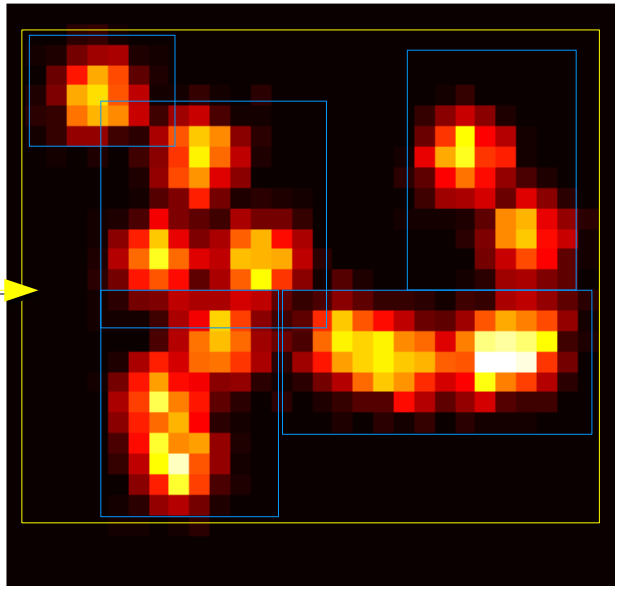
Percolation→Spanning Cluster→Images that can't be contained in small windows

Tractable estimation problems.



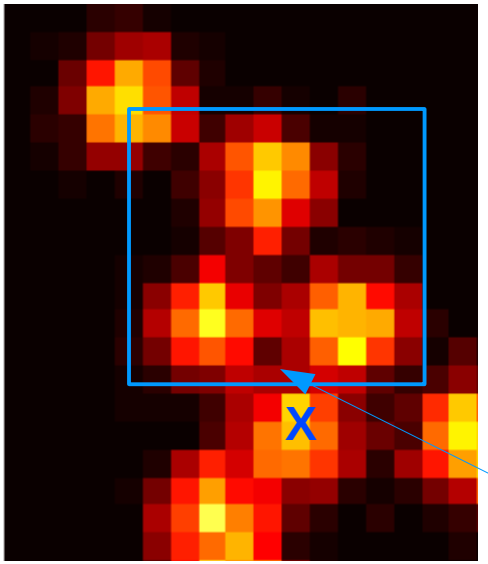
Regions with “spill-over”

Intractable →



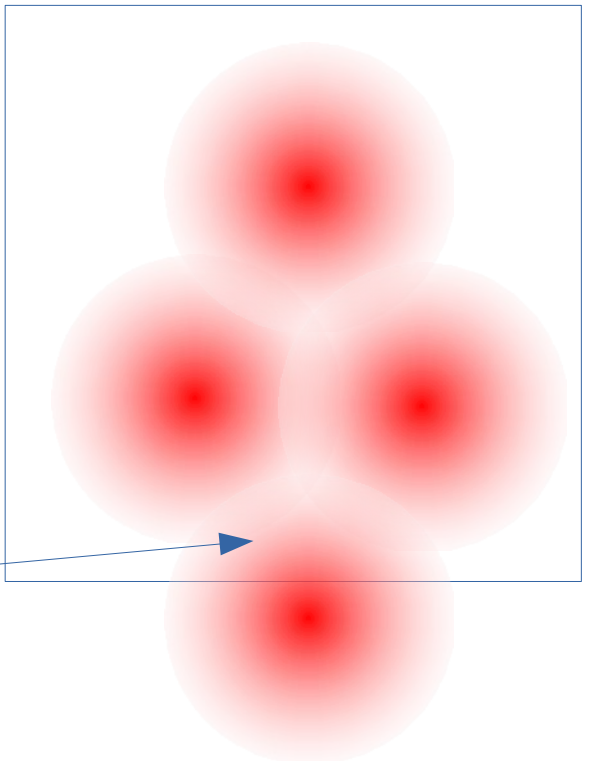
Boundary Issues (2)

- Fluorophore outside the window cannot be estimated well
- But it still biases other estimates



Spill-over photons from molecule X outside ROI (blue box)

Schematic



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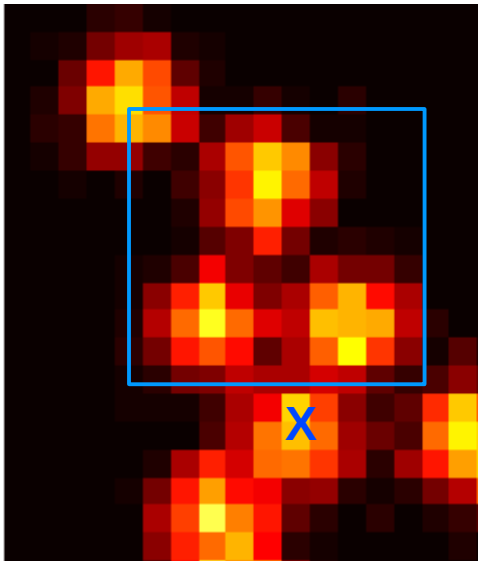
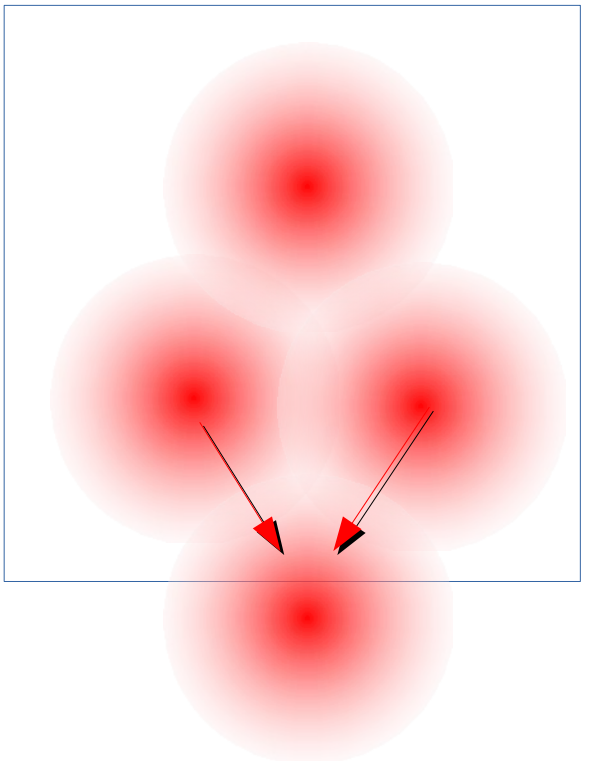


Bias in position estimates due to spill-over photons.

Schematic

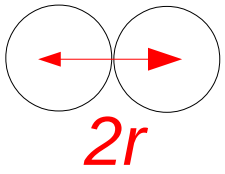


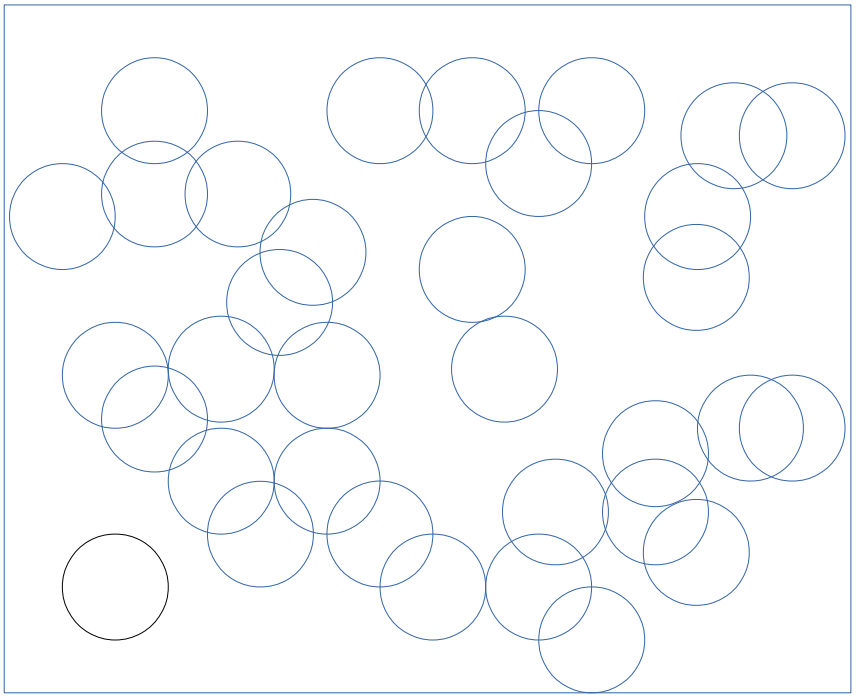
When is overlap inevitable?

- Easy answer: Above continuum percolation threshold (67.6% coverage).
- σ=density of circles

  $=1.13/\pi r^2$

  $=4.52$ neighbors$/\pi(2r)^2$





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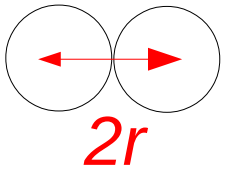
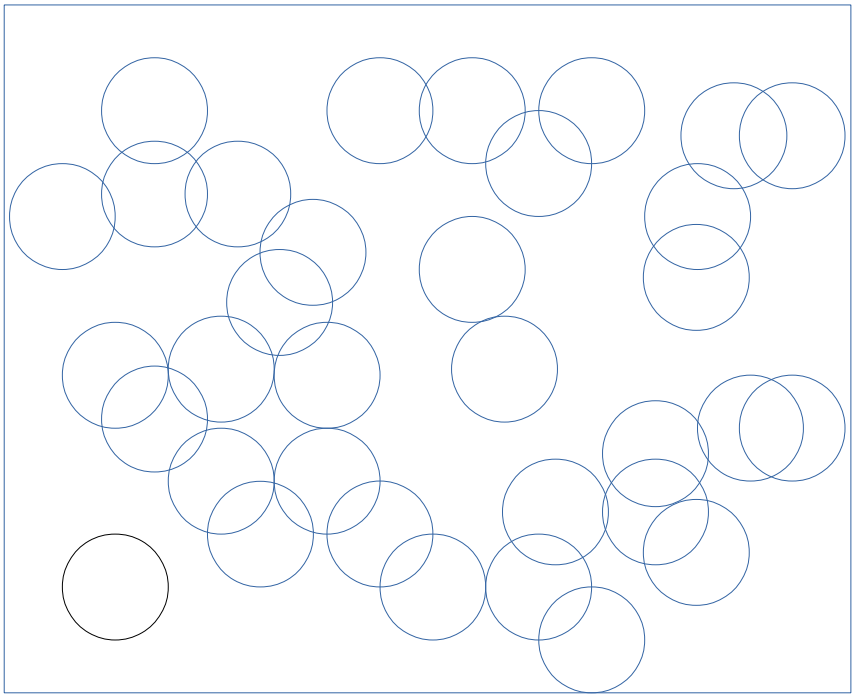
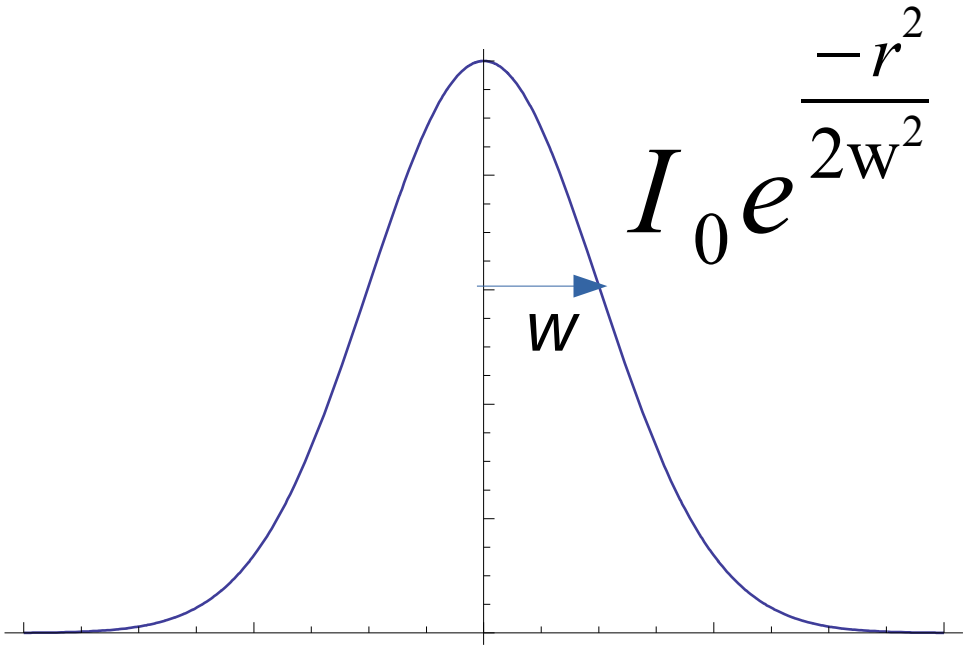




What is *r*?

Radius for overlaps

- *w*? 2*w*?



- Useful answer: “Close enough to matter”

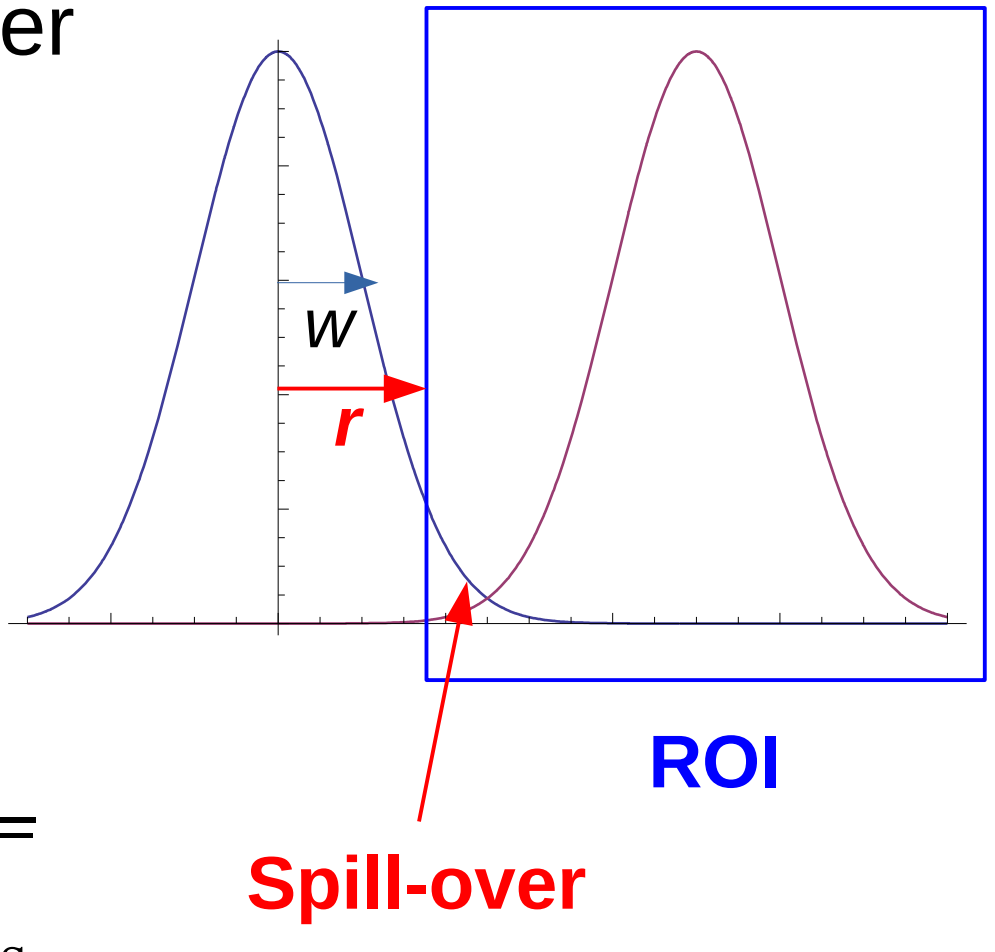
Bias = std. dev. position estimate

Bias and Uncertainty

- Cut off a Gaussian PSF at *r* and overlap with another PSF

$$\text{bias} = \frac{w\, e^{-r^2/2w^2}}{\sqrt{2\pi}}$$

$$\text{std. dev.} = \frac{w}{\sqrt{N_{\text{photons}}}}$$



r depends on error tolerance

bias ≤ α*std. dev.

$$r = w\sqrt{\log \frac{N_{\text{photons}}}{2\pi\alpha^2}}$$

Prediction

$$\sigma = \text{Density} \leq \frac{\eta}{\pi r^2} = \frac{8\pi\beta\eta\,\mathrm{NA}^2}{\lambda^2 \log \frac{N_{\text{photons}}}{2\pi\alpha^2}}$$

Prediction

Depends on percolation model, 1.13 for circles

Depends on PSF, 0.28 if approximating Airy w/ Gaussian

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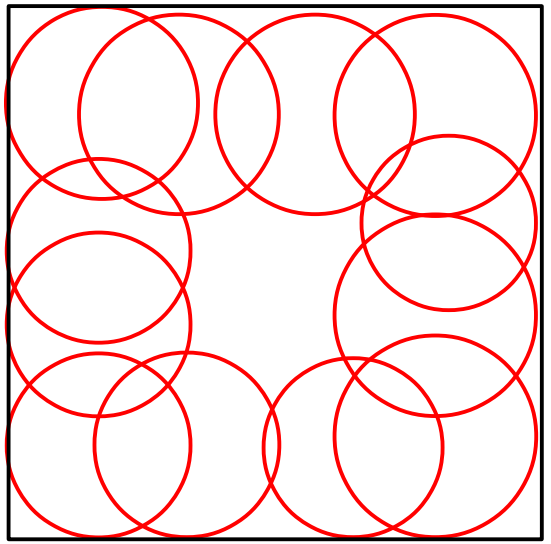
#'s: 647 nm light **(Alexa)**, 1.45 NA objective, ~750 photons, α=1

σ=8.35 fluorophores/μm^2 before edge effects matter

→Near-exact match to Huang & Lidke 2011

Interpretation:

- Higher density: molecules *not* in middle of ROI are mis-localized.



- For <20 fluorophores/ROI (typical)

  and

  density > p_c

  majority of fluorophores contaminated with spill-over

Consequence: Speed

Max Fluorophores per Frame	Normalized Min. # frames needed	Speed improvement
1	1	1
2	0.44	2.27
3	0.26	3.85
4	0.19	5.26
5	**0.14**	**7.14**
10	0.023	43.5

Small, Biophysical Journal, 2009

Localization precision

- Fundamental Limit: Cramer-Rao Lower Bound (CRLB)
- CRLB calcs can be done for multi-flourophore fits (e.g. Yi Sun, JBO, 2013)

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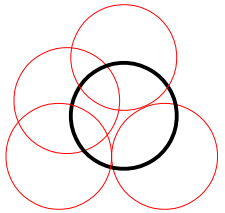

- Percolation: ~4-5 neighbors per fluorophore
- Can a “mean-field” model get close to more precise calculations?

Thompson, Larson, Webb 2002

- Formula not exact, but often useful.
- For spill-over background and pure shot noise, formula simplifies to:

$$\mathrm{Var}(x) = \frac{w^2}{N}\left(1 + \frac{4\eta w\, a_{\mathrm{pixel}}}{\sqrt{\pi}\, r^2}\right) + \frac{a_{\mathrm{pixel}}^2}{N}$$

Typical #'s: w~0.5r, a~0.4r

std. dev.(multi-molecule) ~ 1.3 std. dev. (single molecule)

Thompson, Larson, Webb 2002

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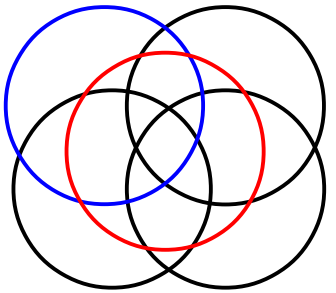
Typical #'s: w~0.5r, a~0.4r

std. dev.(multi-molecule) ~ 1.3 std. dev. (single molecule)

→Off from Sun by ~2

Simplest cluster model

Explicitly construct Fisher information matrix



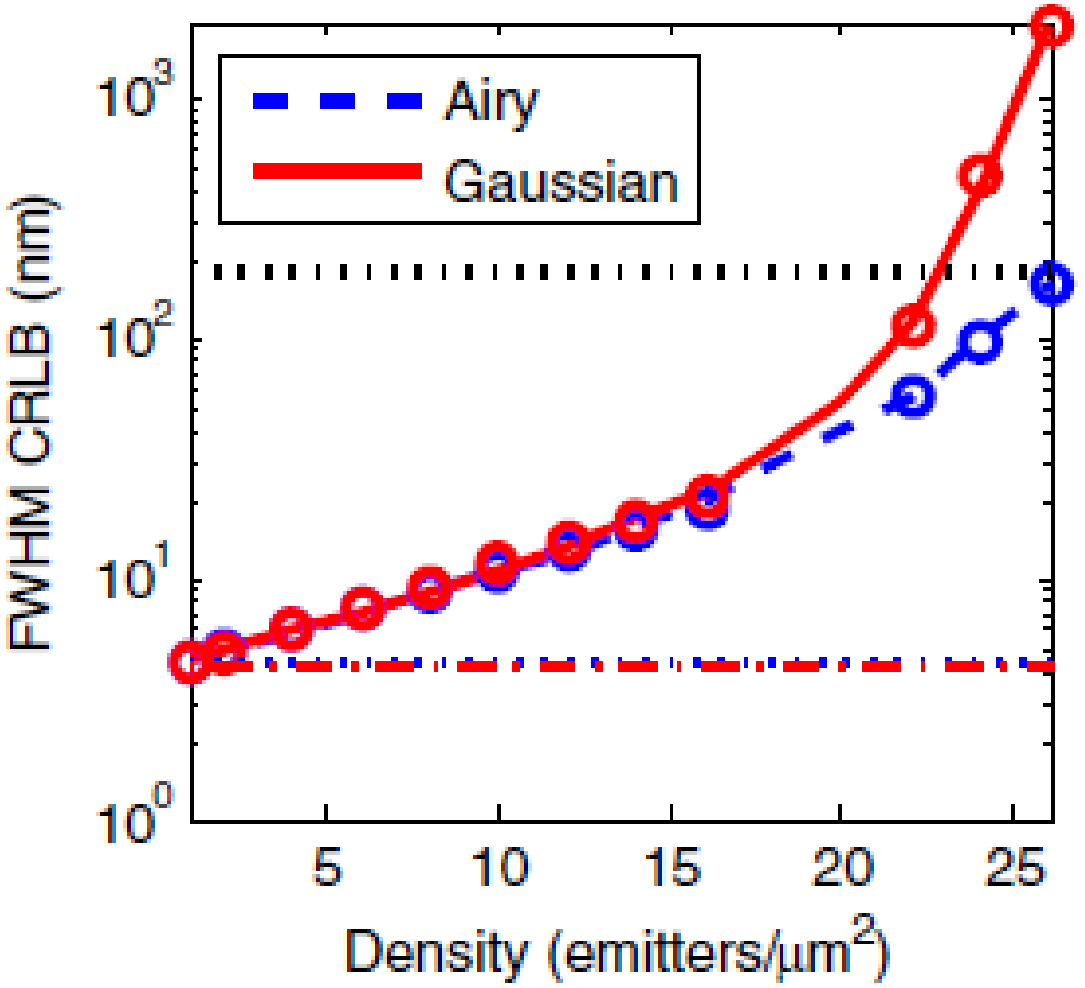
Central: 4 equidistant neighbors @ 2w

→Off-diagonals dominated by r=w,
→diagonal/$e^{0.5}$

Corners: 1 neighbor @ w (diagonal/$e^{0.5}$)
2 neighbors @ $\sqrt{2}$w (diagonal/e)
1 neighbor @ 2w (diagonal/e^{2})

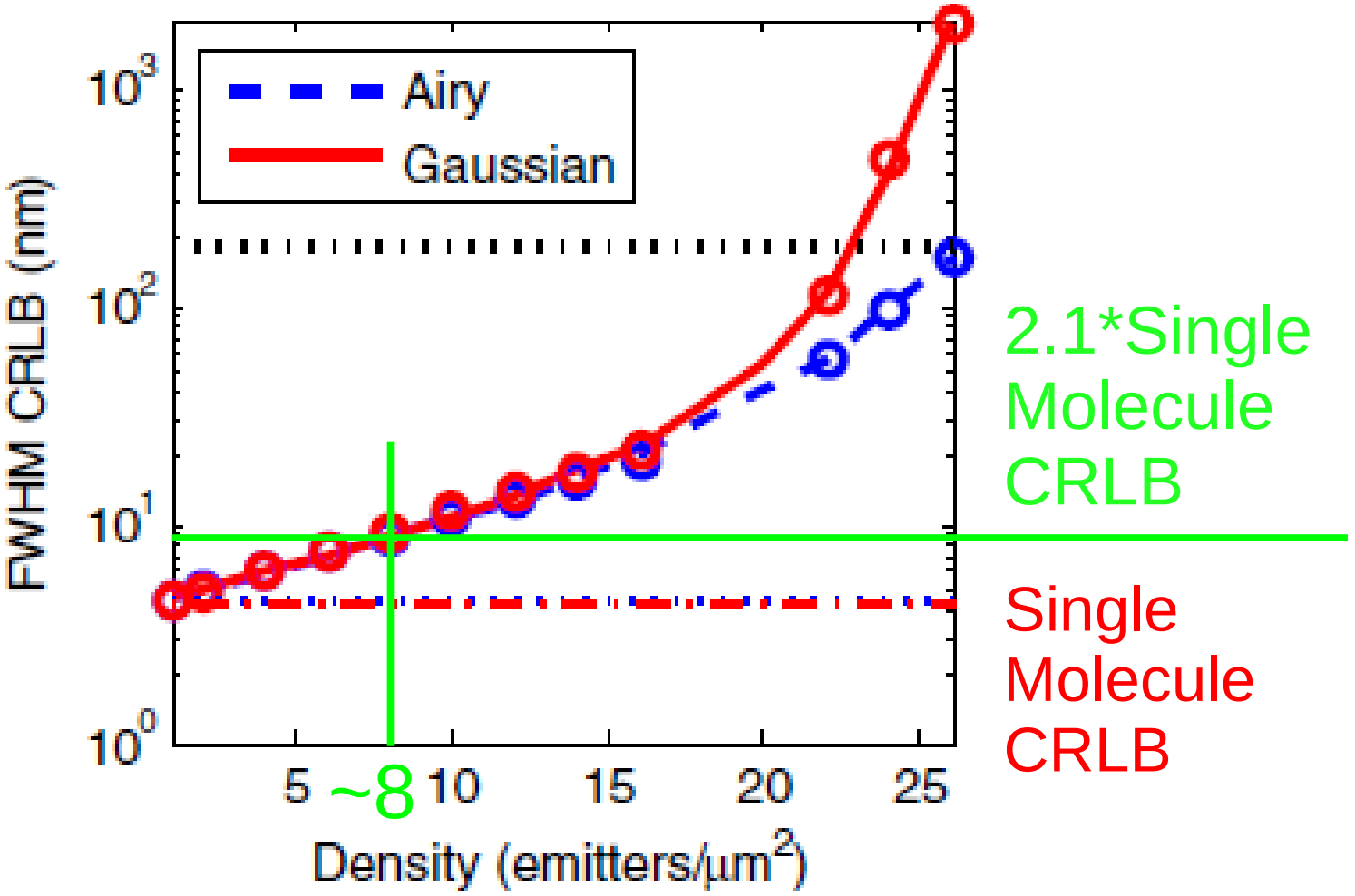
Std. Dev. Of center = 2.1*Single-Molecule Case!

From Sun, 2013



Single Molecule CRLB

From Sun, 2013



Conclusions

- Multi-fluorophore localization performance degrades due to percolation effects
- Localization precision: fluorophore has an average environment of 4 neighbors
- Speed improvements beyond ~7 unlikely w/o accuracy trade-offs and/or large ROIs

Acknowledgments

- Some of this work started during stay at LFD (Enrico Gratton, UCI)
- Discussions of percolation w/ Suketu Bhavsar (CPP)
- Percolation theory students:
  - Shane Stahlheber
  - Jon Tran
  - Ted Yoo
  - Carina Kaainoa
  - Kevin Djepang
  - Brianna Thierjung