W. E. Moerner
Harry S. Mosher Professor of Chemistry and Professor, by courtesy, of Applied Physics
BioX, Biophysics, MIPS, and ChEM-H Programs, Stanford University
Nobel Prize in Chemistry Lecture
Stockholm, Sweden, 8 December 2014
“…we never experiment with just one electron or atom or (small) molecule. In thought-experiments we sometimes assume that we do; this invariably entails ridiculous consequences…In the first place it is fair to state that we are not experimenting with single particles, any more than we can raise Ichthyosaurus in the zoo.

Optical Spectroscopy of Molecules in Solids

Spectrum (absorption vs. wavelength, or color) at room T

860 THz

Frequency=(c/wavelength)

460 THz=460x10^{12} \text{ cps}

Terrylene in p-Terphenyl

Now let's expand the color scale by 25x here, and cool to low T.
Optical Spectroscopy of Molecules in Solids

Spectrum (absorption vs. frequency, or color) at low T (2K)

Frequency = (c/wavelength)

Terrylene in p-Terphenyl

Data: S. Kummer, ... Th. Basché, JChemPhys 1997
Crucial concepts circa 1985 at IBM Research:
• Zero-phonon, purely electronic transitions of fairly flat, rigid molecules in solids at low T become extremely narrow
• An inhomogeneous line profile occurs due to tiny variations in local environments

• To get homogeneous widths: need photon echoes, … or
• Spectral hole-burning: laser-induced changes make a dip or “hole” at chosen laser colors

What are the ultimate limits to “frequency domain” optical storage by spectral hole-burning?

Fundamental Question: Is there a “spectral noise” that results from statistical number fluctuations or the discreteness of individual molecules? – this would define the smallest possible spectral hole that can be detected.
How might statistics appear in spectroscopy?

Suppose you have 10 boxes, and throw 50 balls at these boxes with all boxes being equally likely.

This is the well-known number fluctuation effect, scaling as $\sqrt{N}$, that arises from sampling.

Key Idea: Now think of the horizontal axis as optical frequency (or wavelength). Each box is a bin of width $\Delta v$, the homogeneous width of an optical absorption line. Then the resulting spectrum should have a spectral roughness or fine structure scaling as $\sqrt{N}$, which arises from the discreteness of the individual molecules!
Statistical Fine Structure in an Inhomogeneously Broadened Line

pentacene in p-terphenyl crystal, 1.4K

- Number of molecules per $\Delta v_H$: $N$
- Fluctuations in $N$ should scale as $\sqrt{N}$
- Call this Statistical Fine Structure (SFS)
- Detection achieved with FM spectroscopy since it measures $\Delta \alpha$ on the 100 MHz scale, or $\alpha(\text{upper})-\alpha(\text{lower})$

SFS arises directly from the discreteness of the individual molecules.

The single-molecule limit is within reach!


Detecting Single-Molecule Absorption

Optical Detection and Spectroscopy of Single Molecules in a Solid

W. E. Moerner and L. Kador

IBM Research Division, Almaden Research Center, San Jose, California 95120

(Received 17 March 1989)

- Pentacene in crystalline \( p \)-terphenyl, \( 1.8 \) K, \( 593 \) nm
- Laser FM absorption spectroscopy with Stark (E-field) or ultrasonic (strain field) secondary modulation
- Insensitive to scattering from sample
- Limited by laser shot noise (and out-of-focus molecules from relatively thick cleaved crystal)
- Challenge: focused laser intensity had to be kept low
- Proof-of-principle: single molecules can be optically detected; pentacene/\( p \)-terphenyl is a useful model system


Like FM Radio at 506 THz!
• Used the pentacene/$p$-terphenyl model system

• Detected absorption by measuring emitted fluorescence

• Sensitive to scattering from sample, so careful sample growth required – crystal clear sublimed flakes

• Limited by Rayleigh and Raman scattering background signals, but produced higher SNR for equal bandwidth

PRL 65, 2716 (November 1990)

An application of Laser-Induced Fluorescence (LIF in molecular gas - R.N. Zare, 1968)
Single-Molecule Imaging and Spectroscopy


The width of the peaks along the position axis (FWHM ≈ 5 μm) is a direct measure of the intensity profile of the focused laser beam in the crystal.

Spatial scan: SM measures laser spot size with nm probe!
Freq. scan: Second dimension selects one molecule from many in the same focal volume

Widefield 2D Microscopy

Ultralow intensity: (1.8 mW/cm²)
Lifetime-limited width: 7.6 MHz Wow!

Some of the Surprises from Single Molecules!

Fluorescence excitation spectrum during repetitive scanning of cw dye laser over 400 MHz at 506 THz:

Molecule spontaneously jumps in frequency space due to nearby host dynamics!

T-dep: A. Zumbusch, M. Orrit, PRL 70, 3584 (1993)

Th. Basché and WEM, Nature 355, 335 (1992)
Motivations and Impact: Single-Molecule Spectroscopy and Optical Imaging in Complex Systems

Remove Ensemble Averaging
- **Explore heterogeneity**: are the various copies identical in behavior, or are they different?
- **Follow state changes in time**, especially in biological processes and complex materials
- **Test theoretical understanding of stochastic behavior**

Image/Detect nm-Scale Interactions
- Single molecule as a nm-sized reporter and nanometer-sized light source
- Distance rulers by FRET, TJ Ha et al. (1996)
- Probe local fields in nanophotonic structures
- **Super-resolution imaging**

Commercial: Sequence DNA, Imaging
- PacBio sequencing with ZMW’s, …
  …single-photon sources, …
- **Super-resolution Microscopes**
## Room Temperature: Milestones of Single-Molecule Detection and Imaging

| Solution: Correlation functions | Fluorescence Correlation Spectroscopy: Magde, Elson, Webb (1972, 1974); Ehrenberg, Rigler (1974); Pecora (1976); …
| Autocorrelation (FCS) from 1 fluorophore or less in the volume: Rigler, Widengren, BioScience (1990) |
| Single bursts from 1 fluorophore: Shera, Seitzinger, Davis, Keller, Soper, Chem.Phys.Lett. **174**, 553 (1990); Nie, Zare, Science (1994); … |
| Confocal image | Macklin, Trautman, Harris, Brus, Science **272**, 255 (1996); … |
Detecting Single-Molecule Absorption from Emitted Fluorescence at Room T

• Typical organic fluorophore labels are only ~1 nm in size, fluorescent proteins ~3-4 nm
• Light pumps electronic transitions of the molecule
• Signal indirectly reports on local nanoenvironment because only one molecule is pumped and measured, if backgrounds are low and molecule emits light efficiently

- TMR
- Cy3
- GFP, FPs (~3nm x 4 nm)
Immune proteins in membrane of a live CHO cell
Vrljic, Nishimura, McConnell, WEM, Biophys. J. (2002)
How can we use single-molecule labels to surpass Abbé’s optical diffraction limit, a fundamental physical effect in the far-field?

**Single-molecule imaging + 2 key ideas**
Key Idea #1: Super-Localization

cinder cone, bar 120x10^9 nm

Find the position of the emitter by fitting the shape of the single-molecule image.

can easily find peak position to much better precision than the width.

\[ \hat{c} \] center position

\[ \sigma \approx \frac{(Abbe')}{\sqrt{N}} \]

10^2 photons: 20 nm prec.

Summary: WEM, J. Microscopy 246, 213-220 (2012)
## Some Early Examples of Super-Localization in Biological Imaging

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single virus particle on HeLa Cell to 40 nm precision: Seisenberger,…Bräuchle, Science 294, 1929 (2001); …</td>
</tr>
</tbody>
</table>

If all photons come from the same nm-sized object, we can truly extract nanometer-scale position information, and follow the trajectory in time!

Simulation courtesy Lucien Weiss
# Toward Super-Resolution with Single-Molecule Emitters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Use photobleaching of overlapping fluors:</strong> SHRIImP: Gordon, Ha, Selvin, PNAS <strong>101</strong>, 6462 (2004); NALMS: Qu, Wu, Mets, Scherer, PNAS <strong>101</strong>, 11298 (2004)</td>
</tr>
<tr>
<td></td>
<td><strong>Two differently colored probes:</strong> SHREC: Churchman,…,Spudich, PNAS <strong>102</strong>, 1419 (2005)</td>
</tr>
</tbody>
</table>

Acronyms, see WEM, PNAS **104**, 12596 (2007)
Key idea #2: Active control of emitter concentration, sequential imaging

No active control

Active control of concentration

Structural detail beyond DL revealed by sampling

PALM (4/2006)  STORM  F-PALM
E. Betzig/H. Hess  X. Zhuang  S. Hess
(also: PAINT (Hochstrasser), dSTORM (Sauer), YFP reactivation, GSDIM (Hell),
BLINK (Tinnefeld), SPDM (Cremer)…)

Mechanism-Independent: **Single-Molecule Active Control Microscopy: “SMACM”**
1997: Imaging, Blinking, and Photorecovery for Single EYFP
(Enhanced Yellow Fluorescent Protein, a GFP variant)


Imaging of single S65G/S72A/T203Y variants in a gel showed blinking and switching, i.e., thermal AND light-induced recovery from a metastable dark state.

Blinking (488 nm, 100 ms)

Switching: 405 nm causes recovery of yellow emission for one single molecule

Further development of switchable FPs:
PA-GFP: Patterson, Lippincott-Schwartz (2002)

EYFP immobilized in polyacrylamide gels
Likely photoinduced $E,Z$-isomerization of the GFP chromophore
What the data look like….

eYFP fusions to a bacterial protein blinking in *Caulobacter crescentus* cells

5μm Scale bar, 10ms per frame, movie slowed down 3X

eYFP blinking and photocontrol:
Epi-fluorescence images, of 3 different eYFP fusions in *Caulobacter crescentus*

MreB    ParA    HU

Super-Resolution (40 nm loc. precision) reveals multiple localization patterns!

Lee, Thompson, et al. BPJ Lett 2011

(Collaboration with Lucy Shapiro lab, Stanford)
Neuritic Spine-Like Structures: Na\textsubscript{V} Channels in a Differentiated PC12 Cell

Super-Resolution Reconstruction, 500 ms sliding window

New Fluorophores, New 3D Imaging Methods

Marissa Lee, P. Rai, J. Williams, R. J. Twieg, WEM

Optimized rhodamine spirolactams photoswitch with blue light

Object depth is encoded into PSF rotation for the DH-PSF

Collaboration: R. Piestun, Univ. Colorado

Labeling free amines on live bacteria surfaces highlights sub-diffraction-sized stalks
Impact of Single-Molecule Spectroscopy/Imaging

**Chemistry:**
- spectral diffusion,
- intersystem crossing,
- molecular distortions,
- nanoantennas, structures of materials, photocontrol, catalysis,…

**Physics:**
- nanoenvironments, magnetic interactions, diffusion, structure, EM enhancements quantum optics,…

**Biology:**
- fluctuations, enzymatic states/mechanisms, cell biology, folding, membrane behavior, cellular structures beyond the diffraction limit …

Working at the ultimate single-molecule limit has attracted the attention of many talented scientists around the world, who continue to make seminal contributions to this field!

FRET: TJ Ha, S Weiss …
Enzymes: XS Xie, H Yang, …
RNA folding, actin bands: X Zhuang, …
Motors, DNA processing, DNA dynamics, gene expression, nuclear pores, RNA/proteins in cells, chaperonins, viral entry, quantum optics, new labels, 3D, …
Thanks to Moerner Lab Alumni!

**IBM Almaden Research Ctr., San Jose:**
- Dr. Alan Huston
- Dr. Howard Lee
- Dr. Thomas Carter
- Dr. Lothar Kador
- Dr. W. Pat Ambrose
- Prof. Dr. Thomas Basché
- Prof. Anne Myers
- Dr. Paul Tchenio
- Dr. Jürgen Köhler
- Prof. Stephen Ducharme
- Dr. Peggy Walsh
- Dr. John Stankus
- Dr. Scott Silence
- Dr. Constantina Poga
- Dr. Yiwei Jia

**Stanford University:**
- Dr. Erwin J. G. Peterman
- Dr. Arosha Goonesekera
- Dr. Sophie Brasselet
- Dr. Brahim Lounis
- Mr. Andre Leopold
- Mr. Erik Bjerneld
- Mr. Shaumo Sudhukhan
- Ms. Yeonsuk Roh
- Dr. Ueli Gubler
- Dr. Dan Wright
- Dr. Matt Paige
- Dr. Oksana Ostroverkhova
- Dr. Stephan Hess
- Dr. Marija Vrljic
- Dr. Jason Deich
- Mr. Johann Schleier-Smith
- Dr. Kallie Willets
- Dr. Hans-Philipp Lerch
- Dr. Stefanie Nishimura
- Dr. David P. Fromm
- Dr. P. James Schuck
- Ms. Jennifer Alyono
- Dr. Jaesuk Hwang
- Mr. Kit Werley
- Dr. Hanshin Hwang
- Mr. Naveen Sinha
- Dr. Adam E. Cohen
- Dr. Laurent Coolen

**University of California, San Diego:**
- Ms. Courtney Thompson
- Dr. David J. Norris,
- Dr. Anders Grunnet-Jepsen
- Dr. Susanne Kummer
- Dr. Rob Dickson
- Dr. Maria Diaz-Garcia
- Mr. James Frazier
- Mr. Tim Marsh
- Ms. Julie Casperson
- Ms. Laura Neurauter
- Mr. Barry Smith

- Dr. Marcelle Koenig
- Dr. Andrea Kurtz
- Dr. So Yeon Kim
- Dr. Frank Jaeckel
- Ms. Nicole Tselentis
- Dr. Magnus Hsu
- Dr. Nick Conley
- Dr. Julie Biteen
- Dr. Sam Lord
- Dr. Shigeki Iwanaga
- Dr. Anika Kinkhabwala
- Dr. Alexandre Fuerstenberg
- Mr. Andrey Andreev
- Dr. Jianwei Liu
- Dr. Steven F. Lee
- Dr. Majid Badeirostami
- Dr. Randall Goldsmith
- Dr. Michael Thompson
- Dr. Hsiao-Iu Denise Lee
- Ms. Yao Yue
- Dr. Whitney Duim
- Dr. Yan Jiang
- Ms. Katie Evans
- Dr. Lana Lau
- Dr. Sam Bockenhauer
- Dr. Andreas Gahlmann
- Dr. Steffen Sahl
- Dr. Gabriela Schlau-Cohen
- Dr. Matthew Lew
- Prof. Michael Börsch
More Thanks:
The Current Guacamole Team!

Dr. Yoav Shechtman
Dr. Saumya Saurabh
Dr. Quan Wang
Dr. Allison Squires
Marissa Lee
Mikael Backlund
Lucien Weiss
Adam Backer
Alex Diezmann
Hsiang-yu Yang
Colin Comerci
Camille Bayas
Josh Yoon
Maurice Lee
Petar Petrov
Jingying Yue (rotator)

one molecule = one guacamole
(i.e., 1 over Avocado’s Number of moles, 1/N_A moles)
(with apologies to the memory of Amadeo Avogadro)
Thanks to My Collaborators/Colleagues!

Washington University:
• Jan Brown, Harry Ringermacher, Marjorie Yuhas, …

Cornell University
• Yves Chabal, Aland Chin, Andy Chraplyvy, Fred Pinkerton, Eric Schiff, Don Trotter, …

IBM Research:
• Gary C. Bjorklund, Christoph Bräuchle (TU Munich), Don Burland, Bryan Kohler (Wesleyan), Bill Lenth, Marc Levenson, Roger MacFarlane, Chris Moylan, Michel Orrit (CNRS), Jan Schmidt (Leiden), Robert Shelby, Campbell Scott, Robert Twieg, …

ETH Zürich:
• Bert Hecht, Thomas Irngartinger, Viktor Palm, Taras Plakhotnik, Dieter Pohl (IBM), Aleks Rebane, Urs P. Wild, …

UCSD:
• Larry Goldstein, Jay Siegel, Susan Taylor, Mark Thiemens, Roger Tsien, Bruno Zimm, …

Stanford:
• Thijs Aartsma (Leiden), Steve Boxer, Chris Calderon (Numerica), Gerard Canters (Leiden), Wah Chiu (BCM), Justin DuBois, Shanhui Fan, Gordon Kino, Eric Kool, Harden McConnell, Rafael Peistun (CU), Matthew Scott, Lucy Shapiro, Andy Spakowitz, Tim Stearns, Bob Waymouth, Karsten Weis (UCB), Paul Wender, and many more
Thanks to My Mentors, Homes, Funding Sources

Mentors:
- High School (Thomas Jefferson): Mrs. Blanche Rodriguez, Dr. Richard G. Domey (Bioengineering, UTMSSA)
- Undergrad (Wash U): James G. Miller
- Graduate (Cornell): Albert J. Sievers III
- Professional:
  - IBM: Gary C. Bjorklund, Dan Auerbach, Jerry Swalen, George Castro, Grant Willson
  - UCSD: Kent Wilson, Katja Lindenberg
  - Stanford: Harden McConnell, Dick Zare, Michael Fayer

Funding: U. S. Agencies: ONR, NSF, NIH-NIGMS, NIH-NEI, DOE-BES

Institutions post PhD:
- IBM Research, San Jose and Almaden Research Centers
- ETH Zurich (Guest Professor of Urs P. Wild)
- The University of California, San Diego, Dept. Chemistry and Biochemistry
- Stanford University, Department of Chemistry
- Administrators and Staff, Administrative Assistants Kathi Robbins, Ann Olive
Thanks to My Family and Friends

Friends: Burr Stewart, Ed Snyder, Dave Palmer, and many, many more
In-Laws: Ruth and Michel Stein
Parents: William A. and Frances R. Moerner; Stepmother: Maria Esther Moerner

Wife and Son: Sharon S. Moerner and Daniel E. Moerner and my entire family!