QLI Lab

UIUC

Quantitative phase imaging
Live cell applications

Mustafa Mir
Quantitative Light Imaging Laboratory
http://light.ece.uiuc.edu

2012 Biophotonics Summer School
May 27th, 2012

64

QLI Lab

Scales in biological imaging

UIUC

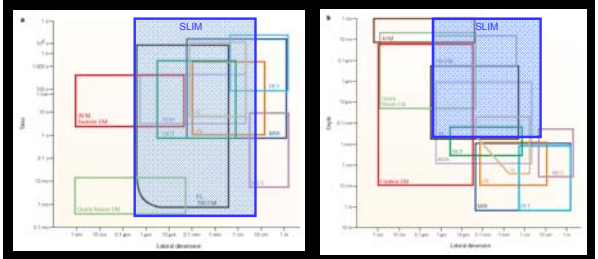
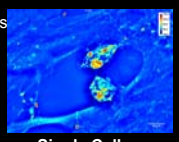


Figure adapted from Tsien RY (2003). "Imagining imaging's future." Nat Rev Mol Cell Biol.

65

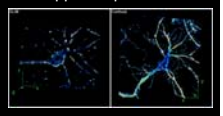
Imaging a *System*

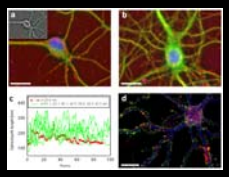
Population Level:
Cell growth over 50 hours



Single Cells:
Microglial cells

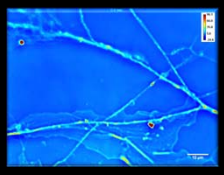
3D Tomography (+4D):
Hippocampal Neuron



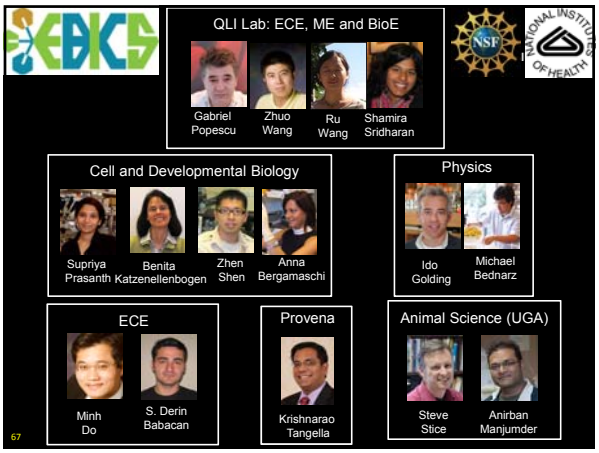


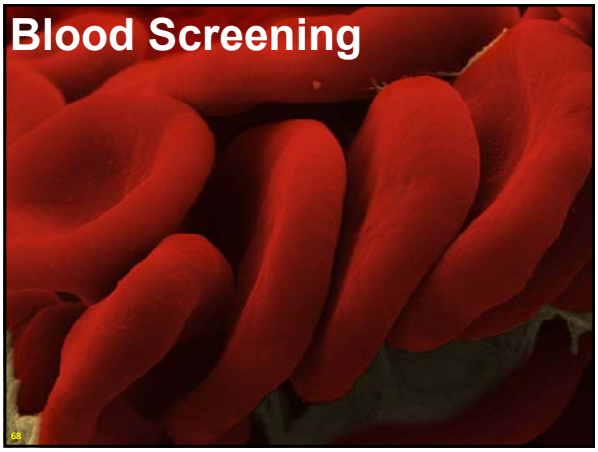
Multimodality:
Fluorescence for identifying synapses

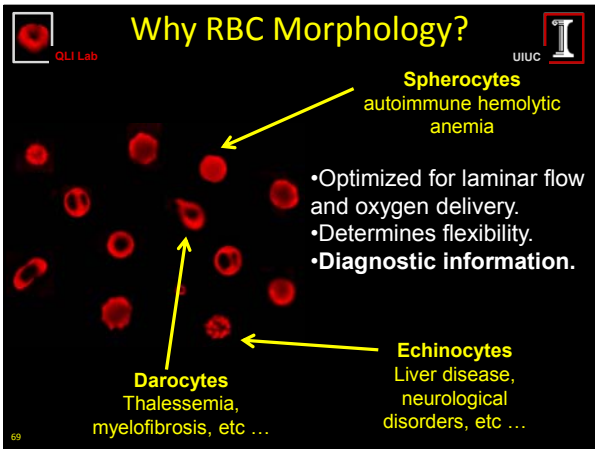
Dynamics And Transport:
Vesicle traffic, membrane fluctuations and microtubules



66


QLI Lab: ECE, ME and BioE
Gabriel Popescu
Zhuo Wang
Ru Wang
Shamira Sridharan
Cell and Developmental Biology
Supriya Prasanth
Benita Katzenellenbogen
Zhen Shen
Anna Bergamaschi
Physics
Ido Golding
Michael Bednarz
ECE
Minh Do
S. Derin Babacan
Provena
Krishnarao Tangella
Animal Science (UGA)
Steve Stice
Anirban Manjumder
67


Blood Screening
68


Why RBC Morphology?
QLI Lab
UIUC
Spherocytes
autoimmune hemolytic anemia
•Optimized for laminar flow and oxygen delivery.
•Determines flexibility.
•Diagnostic information.
Echinocytes
Liver disease, neurological disorders, etc …
Darocytes
Thalessemia, myelofibrosis, etc …
69

Current Technology

QLI Lab

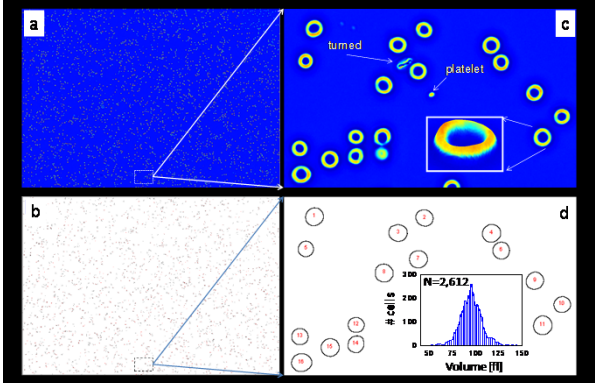
UIUC

- ~ 50 years old
- Population level statistics
- Expensive
- Bulky
- Costly
- **No Morphology**

70

Cell Detection

High throughput



Blood Cytometry

Refractive Index

$$n_c = n_s + \beta * C_{Hb}$$

Height

$$h(x,y) = \frac{\lambda}{2\pi\Delta n}\phi(x,y)$$

Volume

$$V = \iint h(x,y)dxdy$$

Surface Area

$$dA = dxdy\sqrt{1 + \partial_x^2 + \partial_y^2}$$

Sphericity

$$\psi = 4.84\frac{V^{2/3}}{SA}$$

Minimum Cylindrical Diameter

$$V = SA * MCD - \frac{\pi MCD^3}{12}$$

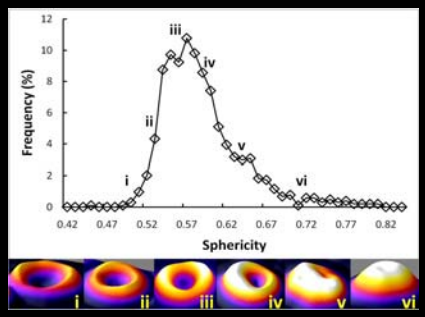
Clinical Trial

- 32 patients
- Compared measurements with hematology analyzer (Beckman Coulter).
- Determine capabilities in a clinical setting.
- Agreement with current instruments.
- Explore translational pathways.
- *Reduced need for an expert?*

PROVENA Health

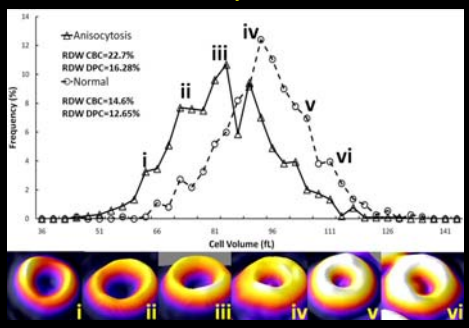
M.Mir, H.Ding, Z.Wang, J. Reedy, K. Tangella and G. Popescu, Blood screening using diffraction phase cytometry, J. Biomed. Opt., 15 (2), (2010).

Sphericity



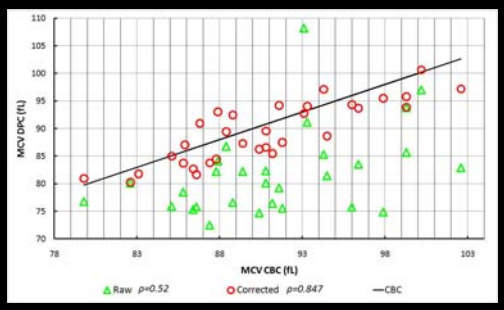
M.Mir, H.Ding, Z.Wang, J. Reedy, K. Tangella and G. Popescu, *Blood screening using diffraction phase cytometry*, J. Biomed. Opt., 15 (2), (2010).

Anisocytosis



M.Mir, H.Ding, Z.Wang, J. Reedy, K. Tangella and G. Popescu, *Blood screening using diffraction phase cytometry*, J. Biomed. Opt., 15 (2), (2010).

Hemoglobin Concentration

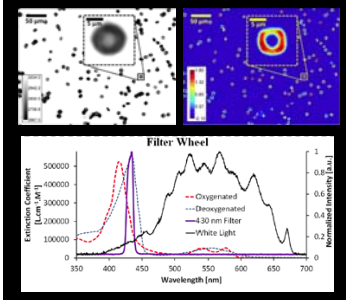


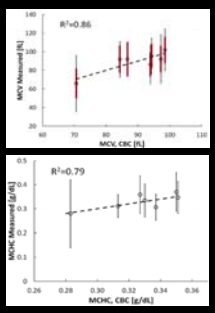
M.Mir, H.Ding, Z.Wang, J. Reedy, K. Tangella and G. Popescu, Blood screening using diffraction phase cytometry, J. Biomed. Opt., 15 (2), (2010).

Soret band Absorption

QLI Lab

UIUC



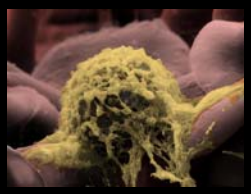


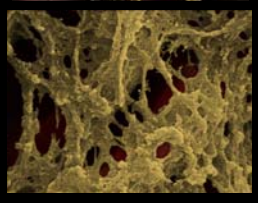
77

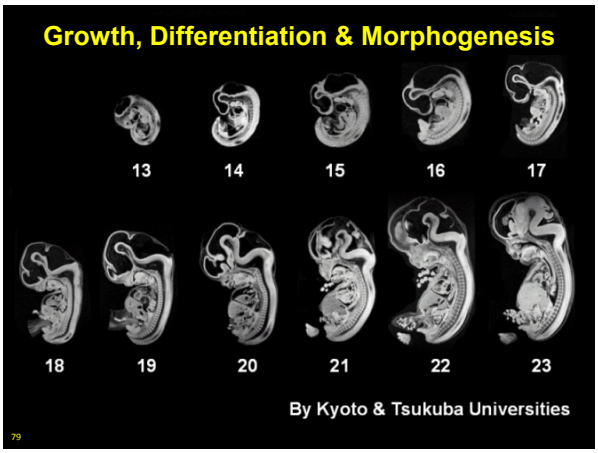
(M.Mir, et. al., Opt. Exp 2011)

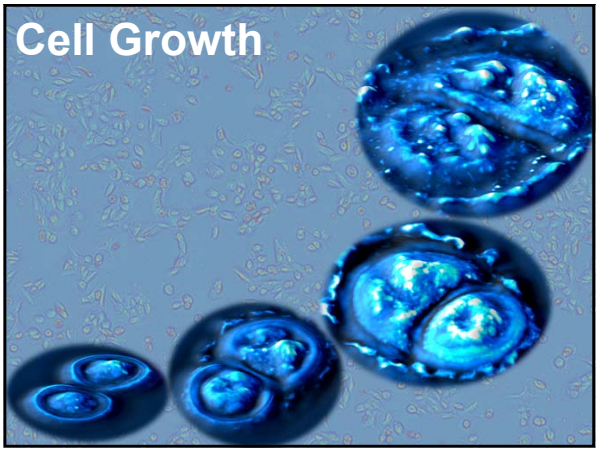
Conclusions

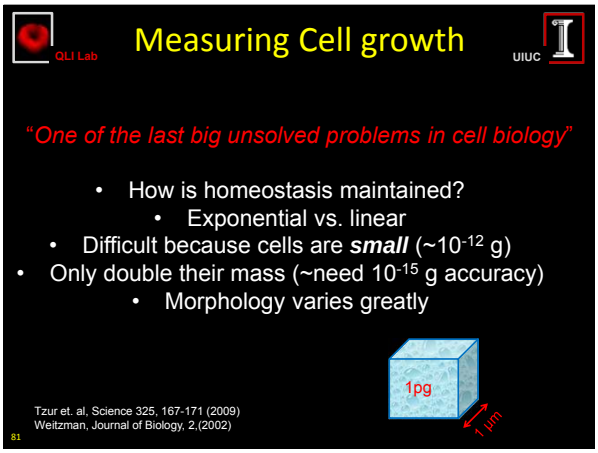
- QPI techniques provide powerful tools for blood screening.
- Simplicity and versatility is essential for a successful instrument.
- Clinical trials need to be conducted to determine where the greatest impact may be made.






Growth, Differentiation & Morphogenesis
13
14
15
16
17
18
19
20
21
22
23
By Kyoto & Tsukuba Universities
79


Cell Growth

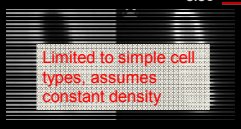

QLI Lab
Measuring Cell growth
UIUC
"One of the last big unsolved problems in cell biology"
• How is homeostasis maintained?
• Exponential vs. linear
• Difficult because cells are ***small*** (~10^{-12} g)
• Only double their mass (~need 10^{-15} g accuracy)
• Morphology varies greatly
1pg
1 µm
Tzur et. al, Science 325, 167-171 (2009)
Weitzman, Journal of Biology, 2,(2002)
81

Current Methods

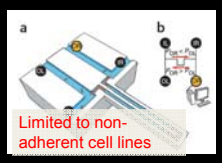
QLI Lab

UIUC

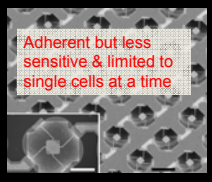
Traditional instrument: Beckman Coulter Counter Recently by Tzur et. al, Science 2008



Phase Contrast and fluorescence-eg. from Reshes et al., Biophys. J., 2008



Suspended microchannel Resonator (SMR), Godin et al Nat. Meth. 2010



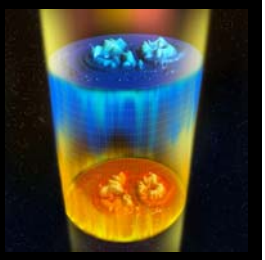
MEMS sensors, Park et al, PNAS 2010

Ideal Method (Optical)

QLI Lab

UIUC

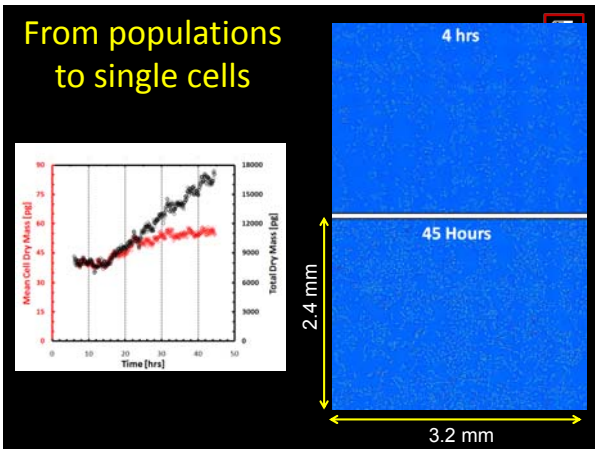
- Quantitative and highly sensitive
- Non-invasive
- Stable for several cell cycles
- Follow single cells
- Compatible with many cell types
- Differentiate between cells in close proximity
- Fully biocompatible
- Multimodal

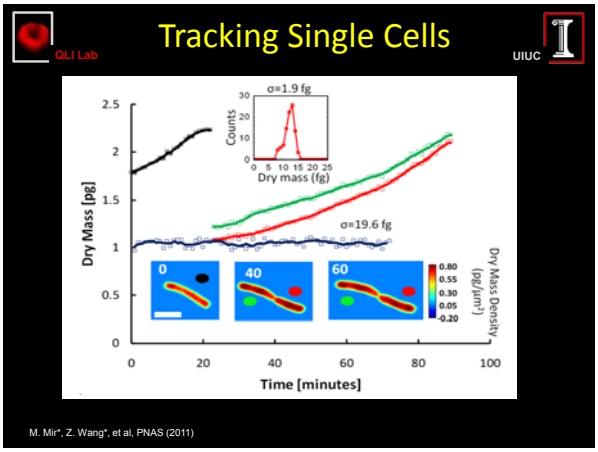


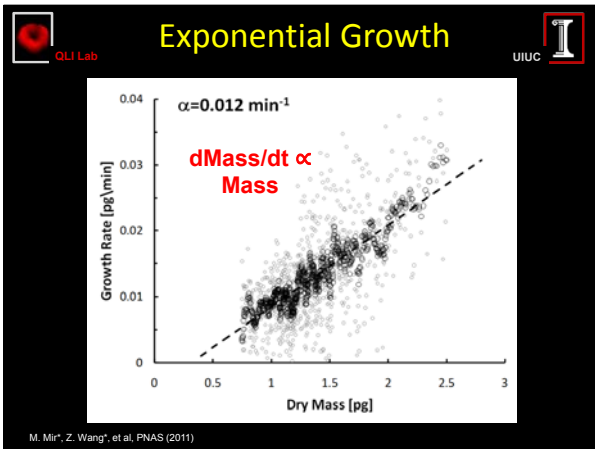
<u>SLIM Sensitivity to Dry Mass</u>
Spatial:1.5 fg/μm²
Temporal: 0.15 fg/μm²

Environmental Control

- Perfusion Chamber
- Temperature Control
- Humidity
- CO2


From populations to single cells
Mean Cell Dry Mass [pg]
Total Dry Mass [pg]
Time [hrs]
4 hrs
45 Hours
2.4 mm
3.2 mm

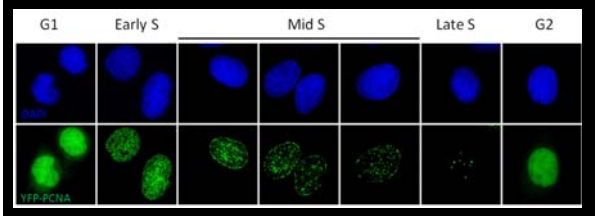

QLI Lab
Tracking Single Cells
UIUC
σ=1.9 fg
Counts
Dry mass (fg)
σ=19.6 fg
Dry Mass [pg]
Time [minutes]
Dry Mass Density (pg/μm²)
M. Mir*, Z. Wang*, et al, PNAS (2011)


QLI Lab
Exponential Growth
UIUC
α=0.012 min⁻¹
dMass/dt ∝ Mass
Growth Rate [pg\min]
Dry Mass [pg]
M. Mir*, Z. Wang*, et al, PNAS (2011)

Cell Cycle Markers

QLI Lab

UIUC



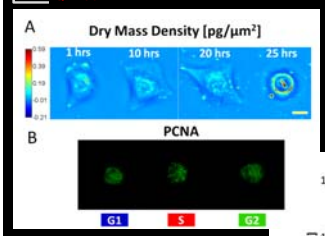
Proliferating Cell Nuclear Antigen (PCNA) acts as a processivity factor for DNA polymerase.

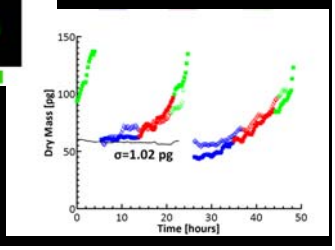
M. Mir*, Z. Wang*, et al, PNAS (2011)

Cycle Dependent Growth

QLI Lab

UIUC



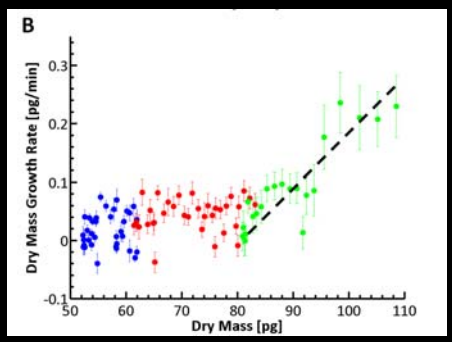


M. Mir*, Z. Wang*, et al, PNAS (2011)

Growth Patterns

QLI Lab

UIUC

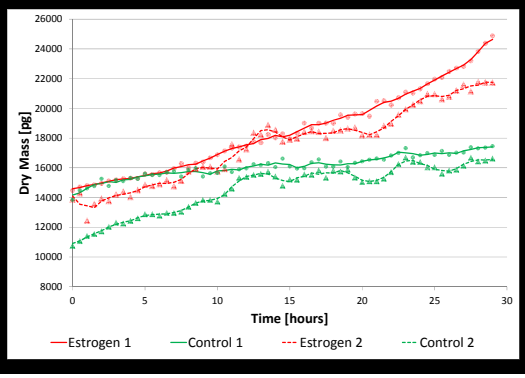


90

Modulating Cancer growth

QLI Lab

UIUC



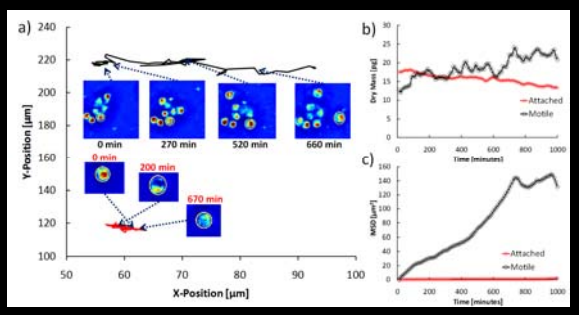
91

Cellular Motility

Measuring Motility

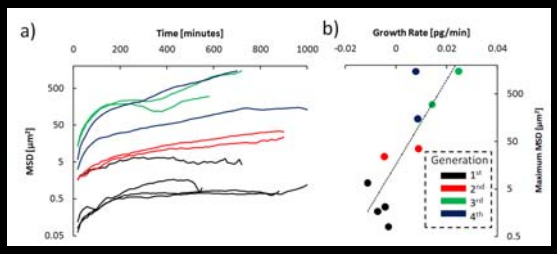
QLI Lab

UIUC



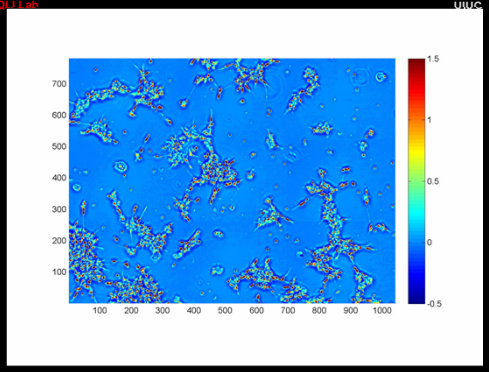
93

Effects of motility on growth



94

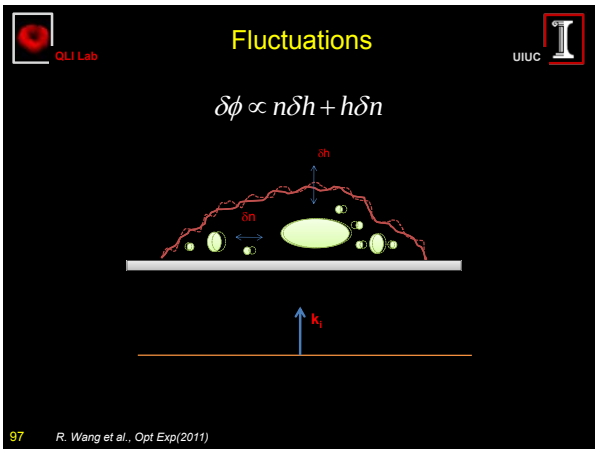
Establishment of Neural Network

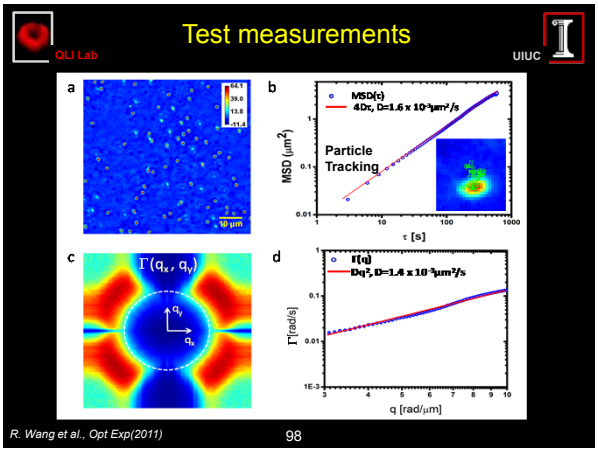


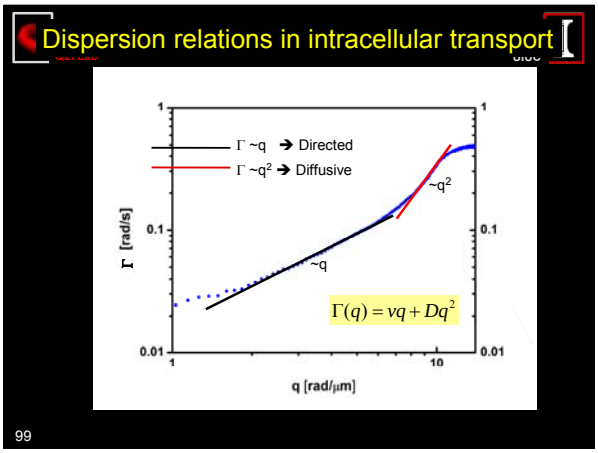
95

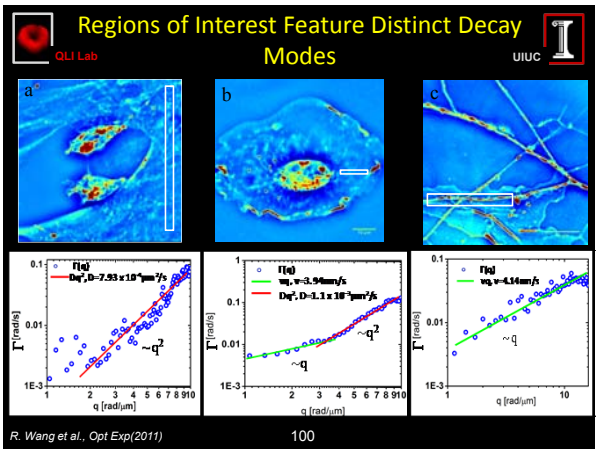
3x3 Mosaic of 10X Images, (0.750 mm x 1 mm), every 15 minutes

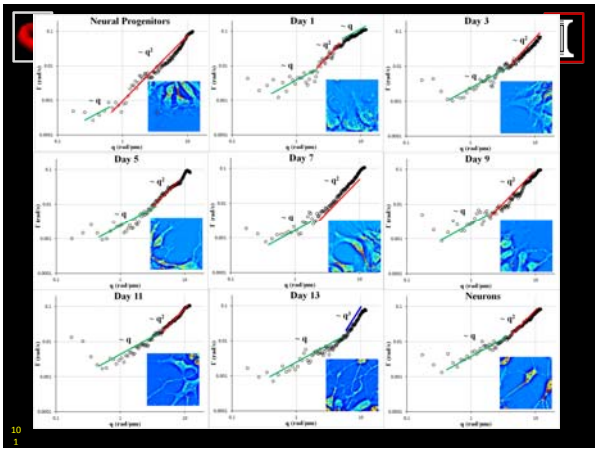
Differentiation

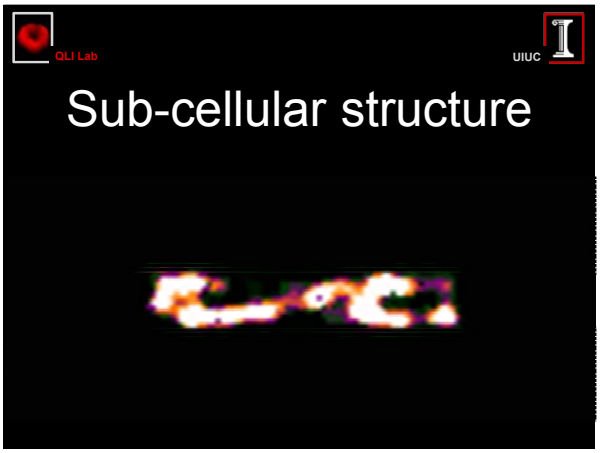

QLI Lab
Fluctuations
UIUC
$\delta\phi \propto n\delta h + h\delta n$
δh
δn
k_i
97
R. Wang et al., Opt Exp(2011)

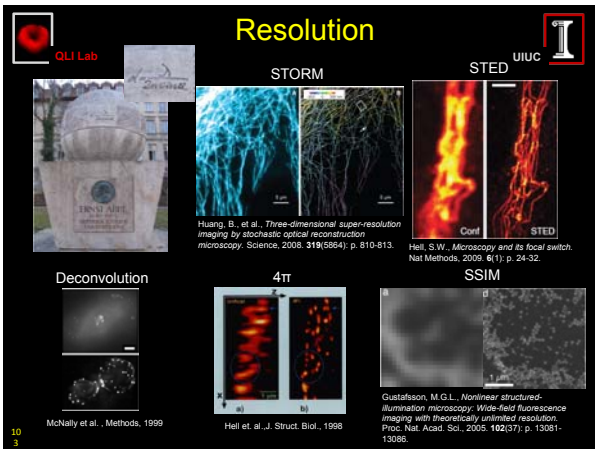

QLI Lab
Test measurements
UIUC
a
b
MSD(τ)
4Dτ, D=1.6 x 10⁻³μm²/s
Particle Tracking
MSD (μm²)
τ [s]
c
Γ(qx, qy)
d
Γ(q)
Dq², D=1.4 x 10⁻³μm²/s
Γ[rad/s]
q [rad/μm]
R. Wang et al., Opt Exp(2011)
98

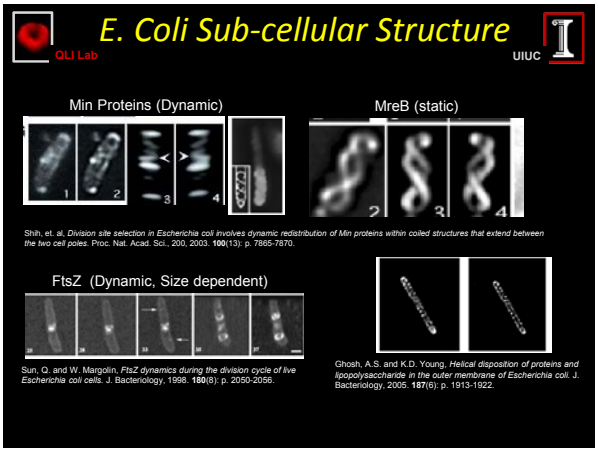

Dispersion relations in intracellular transport
Γ ~q → Directed
Γ ~q² → Diffusive
~q²
~q
$\Gamma(q) = vq + Dq^2$
Γ [rad/s]
q [rad/μm]
99

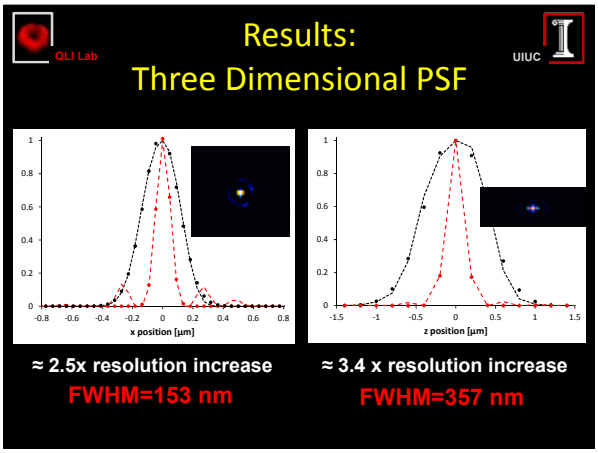

Regions of Interest Feature Distinct Decay Modes
QLI Lab
UIUC
a
b
c
Γ [rad/s]
q [rad/μm]
~q²
~q
R. Wang et al., Opt Exp(2011)
100

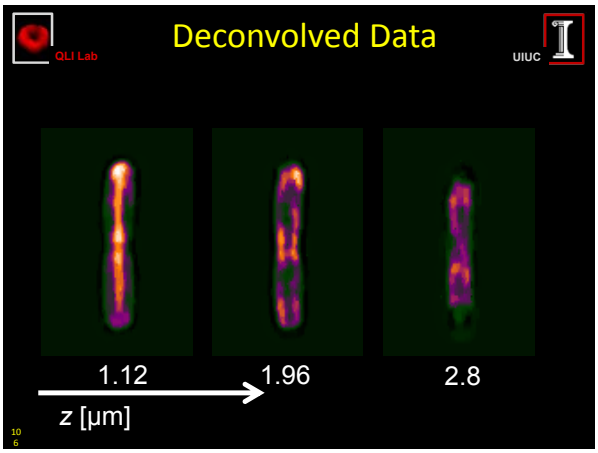

Neural Progenitors
Day 1
Day 3
Day 5
Day 7
Day 9
Day 11
Day 13
Neurons
101

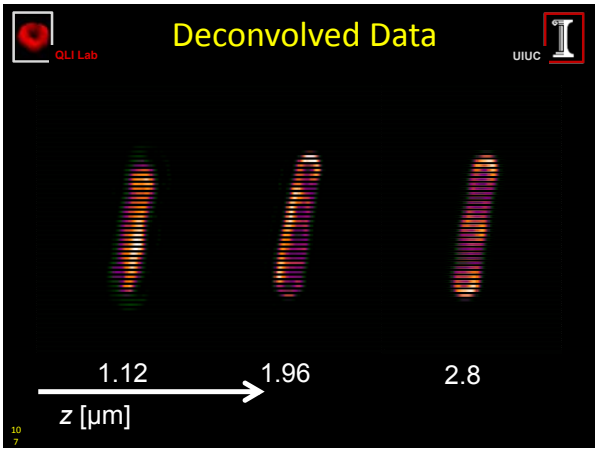

QLI Lab
UIUC
Sub-cellular structure

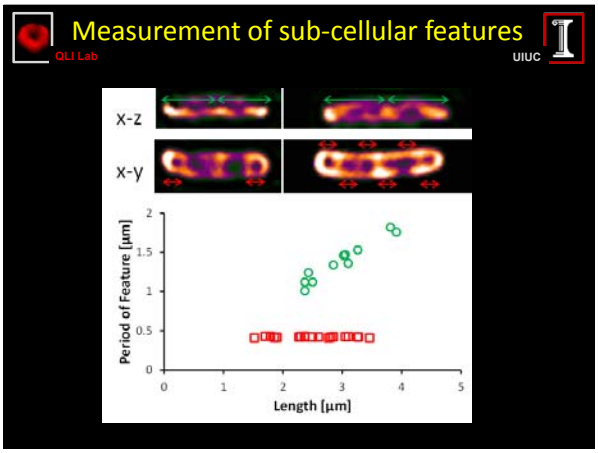

QLI Lab
Resolution
UIUC
STORM
STED
Huang, B., et al., Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy. Science, 2008. 319(5864): p. 810-813.
Hell, S.W., Microscopy and its focal switch. Nat Methods, 2009. 6(1): p. 24-32.
Deconvolution
4π
SSIM
McNally et al. , Methods, 1999
Hell et. al.,J. Struct. Biol., 1998
Gustafsson, M.G.L., Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution. Proc. Nat. Acad. Sci., 2005. 102(37): p. 13081-13086.
103


QLI Lab
E. Coli Sub-cellular Structure
UIUC
Min Proteins (Dynamic)
MreB (static)
Shih, et. al, Division site selection in Escherichia coli involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. Proc. Nat. Acad. Sci., 200, 2003. 100(13): p. 7865-7870.
FtsZ (Dynamic, Size dependent)
Sun, Q. and W. Margolin, FtsZ dynamics during the division cycle of live Escherichia coli cells. J. Bacteriology, 1998. 180(8): p. 2050-2056.
Ghosh, A.S. and K.D. Young, Helical disposition of proteins and lipopolysaccharide in the outer membrane of Escherichia coli. J. Bacteriology, 2005. 187(6): p. 1913-1922.


QLI Lab
Results:
Three Dimensional PSF
UIUC
x position [μm]
z position [μm]
≈ 2.5x resolution increase
FWHM=153 nm
≈ 3.4 x resolution increase
FWHM=357 nm


QLI Lab
Deconvolved Data
UIUC
1.12
1.96
2.8
z [µm]


QLI Lab
Deconvolved Data
UIUC
1.12
1.96
2.8
z [µm]


QLI Lab
Measurement of sub-cellular features
UIUC
x-z
x-y
Period of Feature [µm]
Length [µm]

QLI Lab

Conclusions

UIUC

i. Label Free, quantitative Imaging
ii. Femtogram sensitivity for dry mass
iii. Spatial ranges from submicron to millimeters
iv. Temporal ranges from milliseconds to days
v. Ability to quantify dynamics without tracking
vi. Multimodal
vii. Fully biocompatible
viii. Suitable for most cell types.

109

QLI Lab

Outlook

UIUC

- Specificity enabled applications
  - Synapse formation
  - Neuro-muscular junctions
  - Organelle dynamics
- Cellular Signatures
  - Cancerous cells
  - Differentiation stages
  - Heterotypic cultures
  - Modulation

110