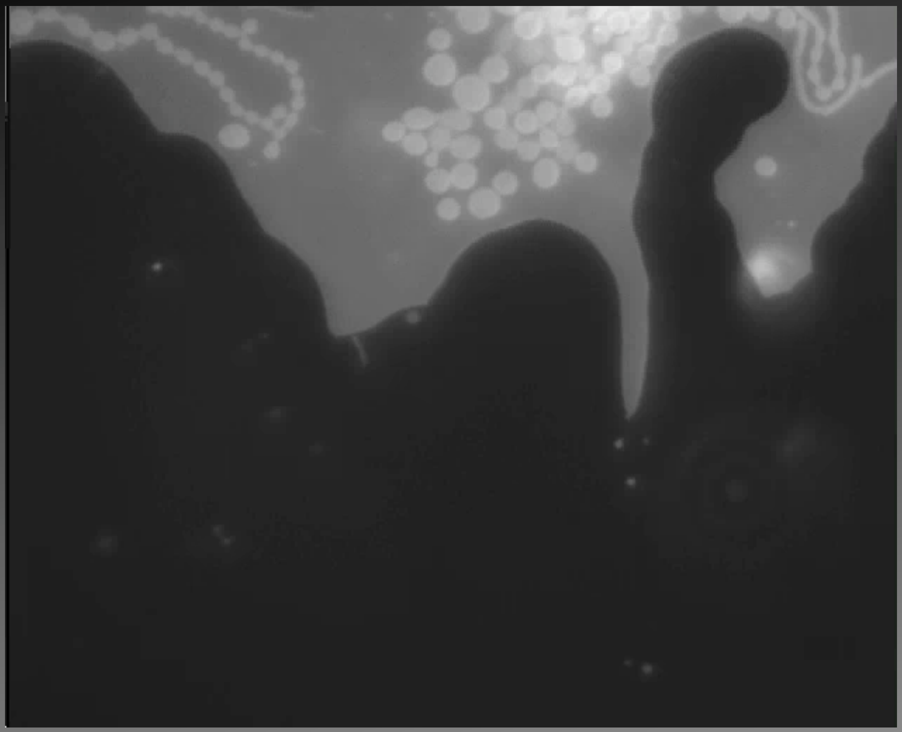
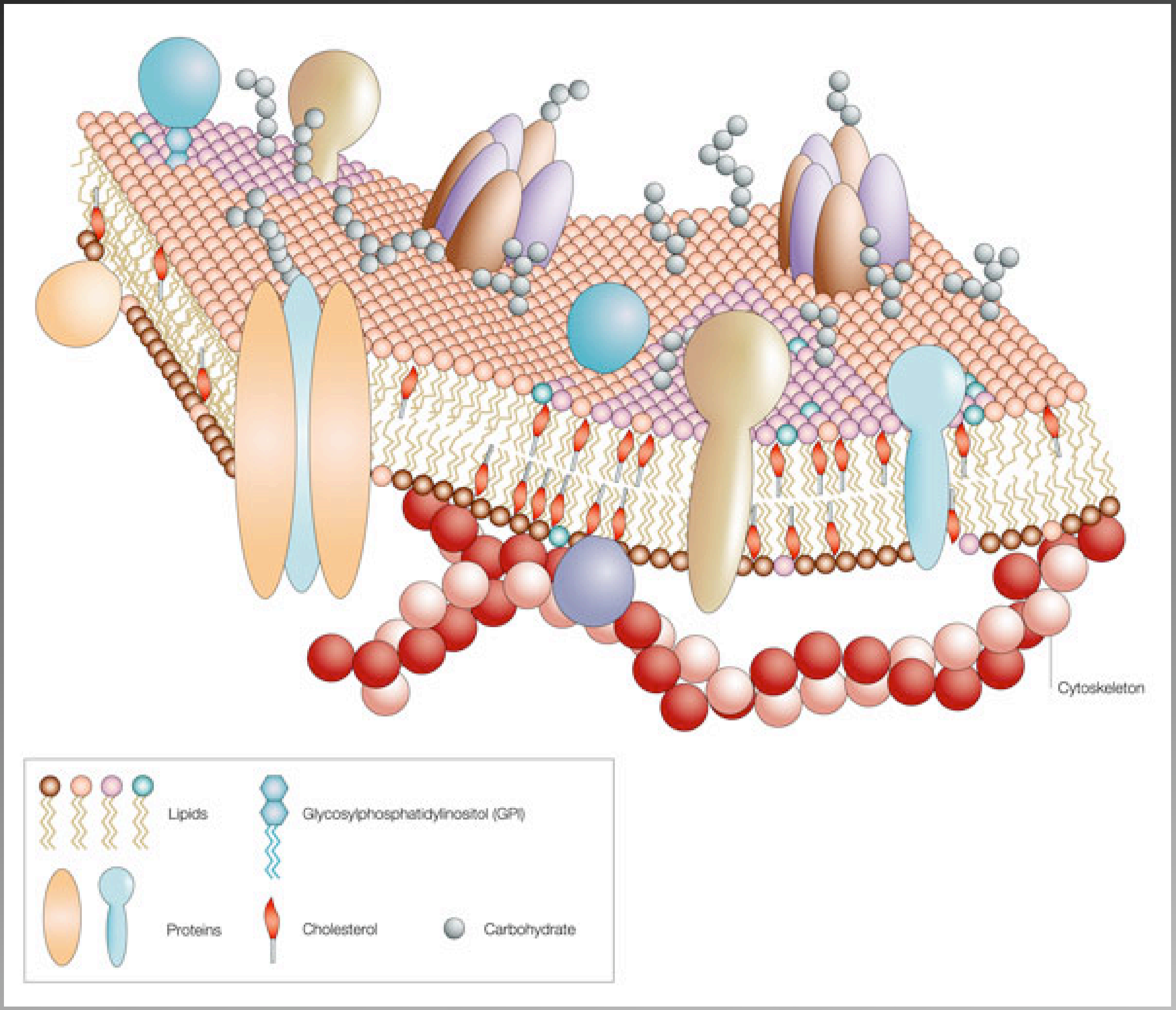


Phospholipid Bilayers as soft Materials

Complexity in Biological & Soft Matter,
CNLS Annual Meeting
May 22, 2007

Atul N. Parikh
University of California, Davis
email: anparikh@ucdavis.edu
http://parikh.ucdavis.edu

The Fluid-Mosaic-Model of the Biological membranes and Beyond…



4-6 nanometer thick

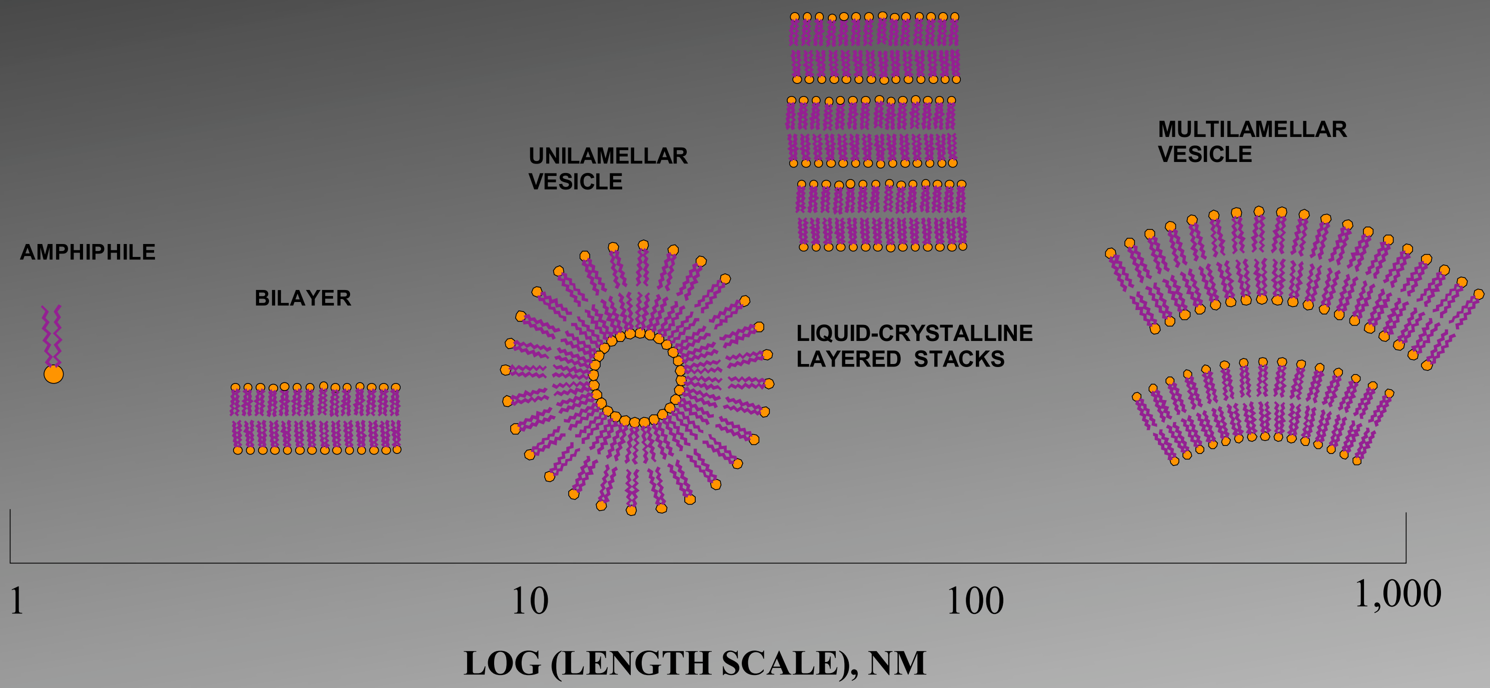
2-dimensional fluid

heterogeneous

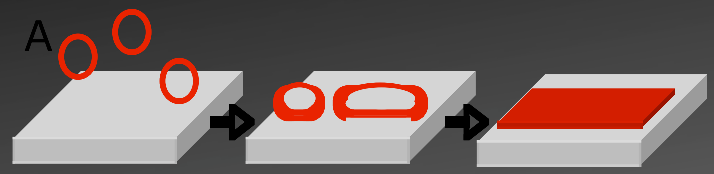
Highly Dynamic

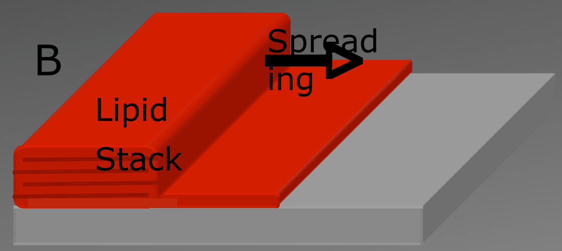
(Albert et al, Molecular Biology of the cell)

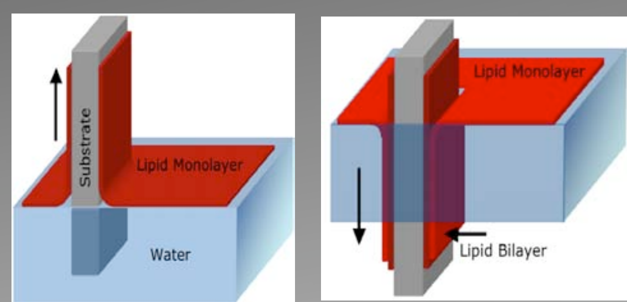
Self-Assembly of lipids and surfactants



Synthetic Membranes







(A. N. Parikh and J. T. Groves, MRS Bulletin, Editorial, 2006)

“Most membrane proteins do not enjoy the continuous unrestricted lateral diffusion....

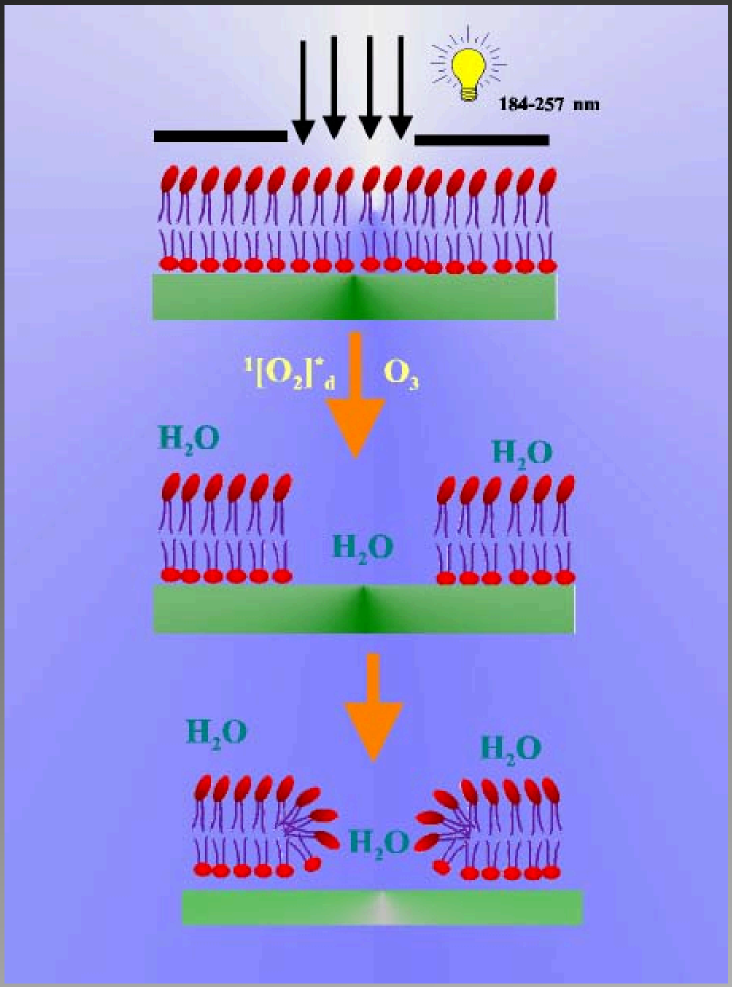
Instead, proteins diffuse in a more complicated way that indicates considerable lateral heterogeneity in membrane structure, at least on a nanometer scale”

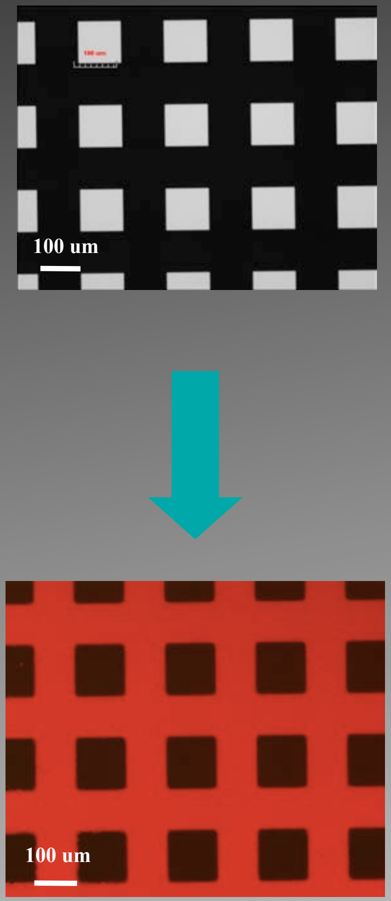
Jacobson, K., Sheets, E. D. & Simson, R. Science 268, 1441(1995)

Compartmentalizing Bilayer Fluidity

Supported membranes

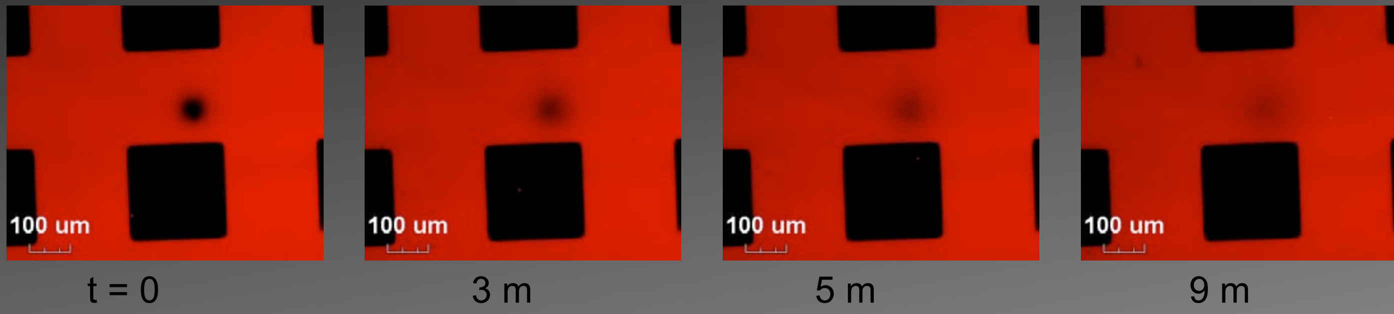
Membrane Photolithography

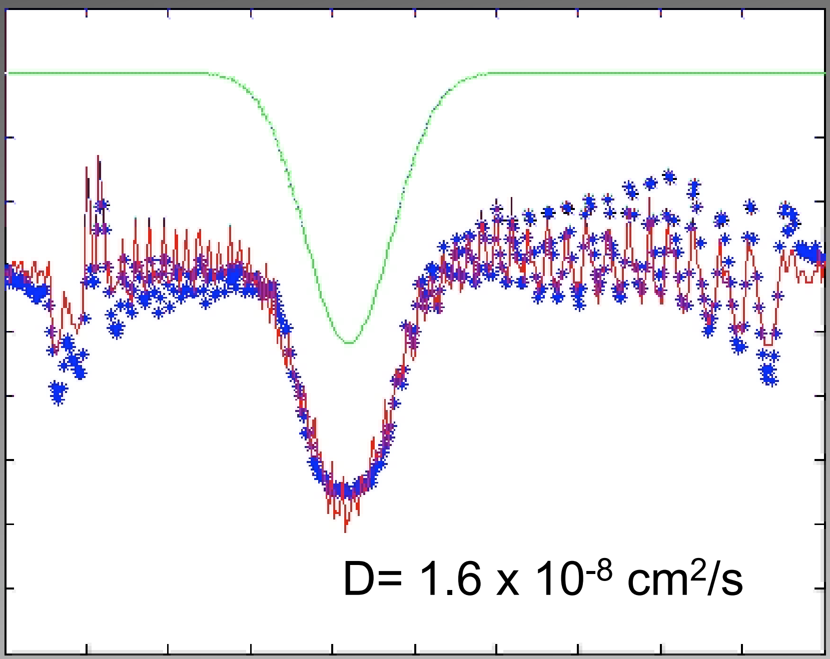


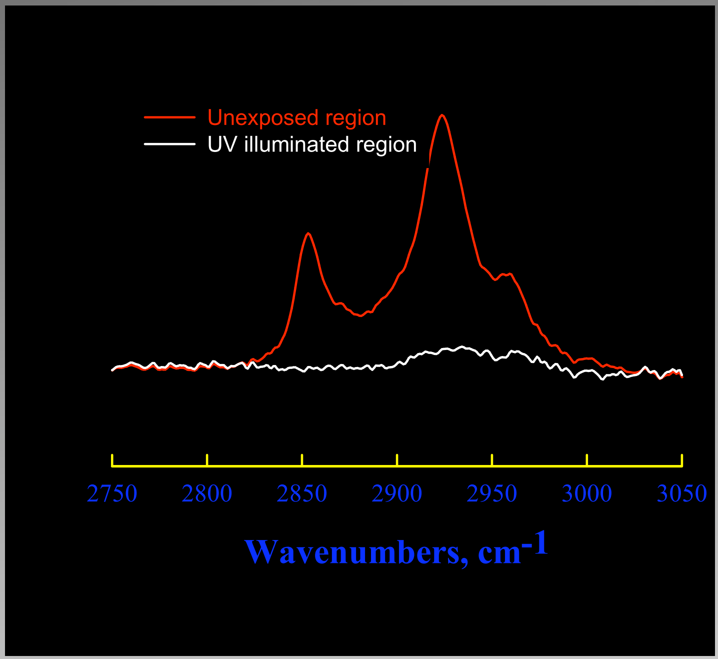


C. K. Yee, M. L. Amweg, A. N. Parikh, Adv. Mater. (2004)

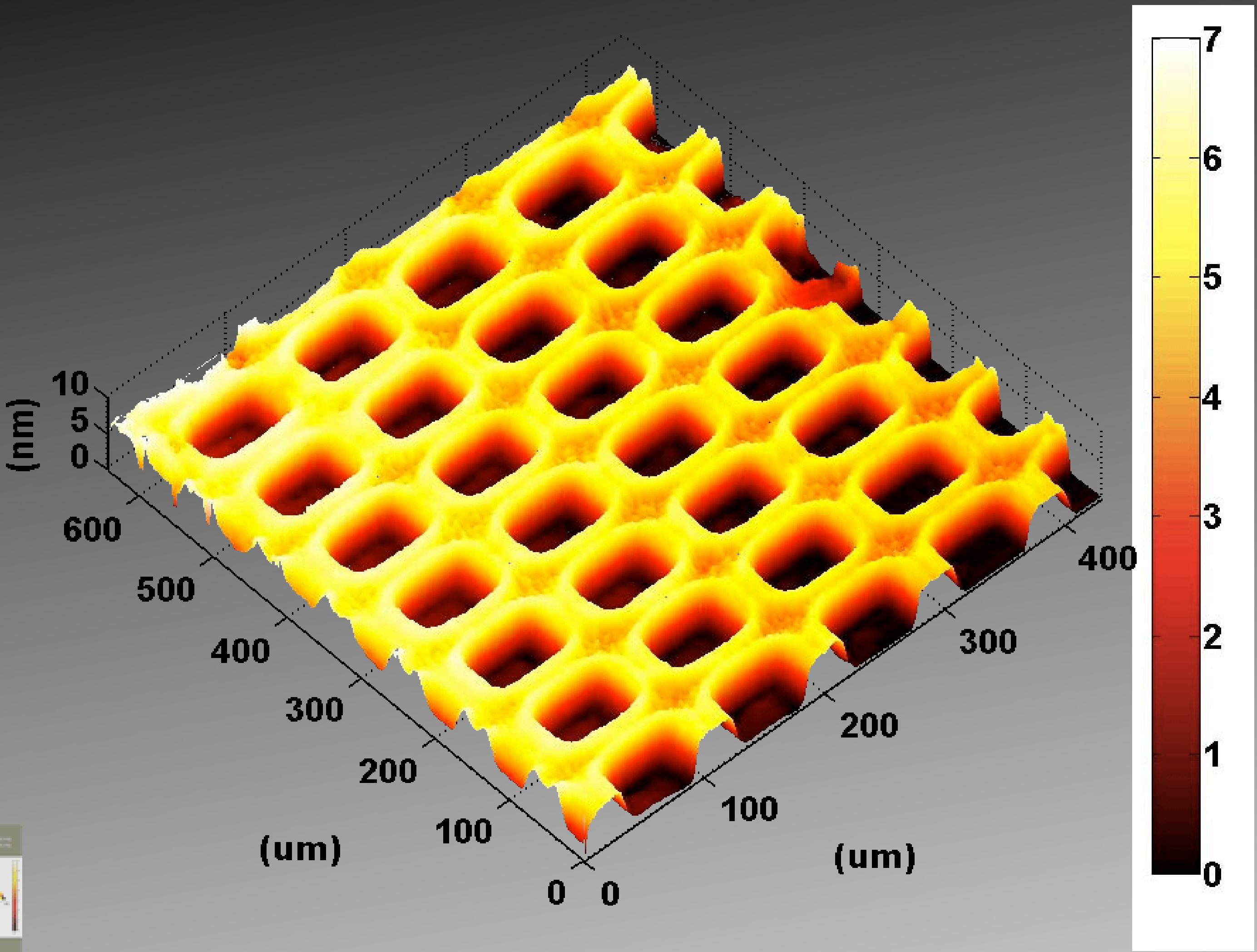
Membrane Photolithography





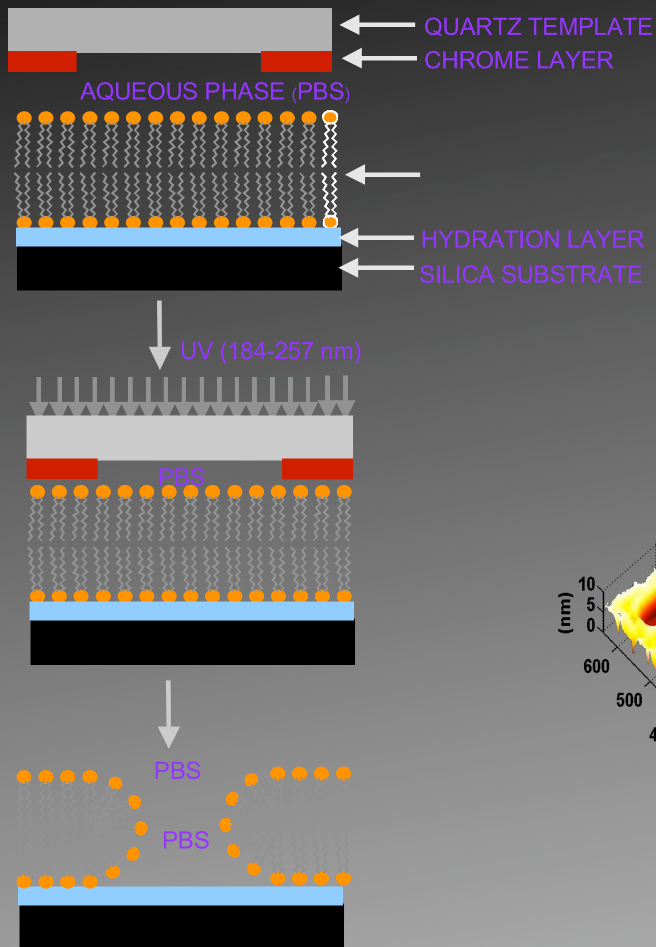


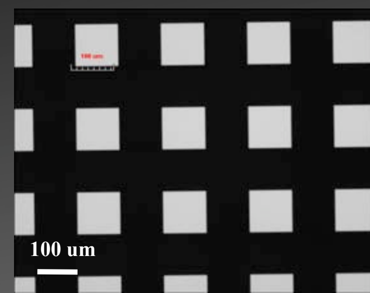
angstroms scale Height resolution by Imaging Ellipsometry

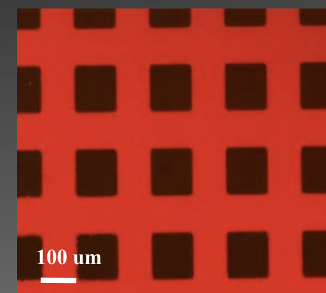


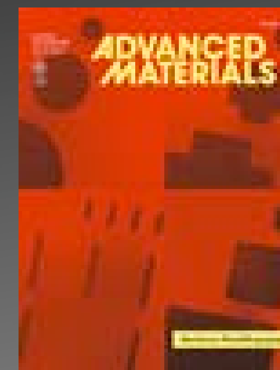
M. Howland, A. W. Szmodis, B. Sanii, A. N. Parikh, Biophys. J. 2007

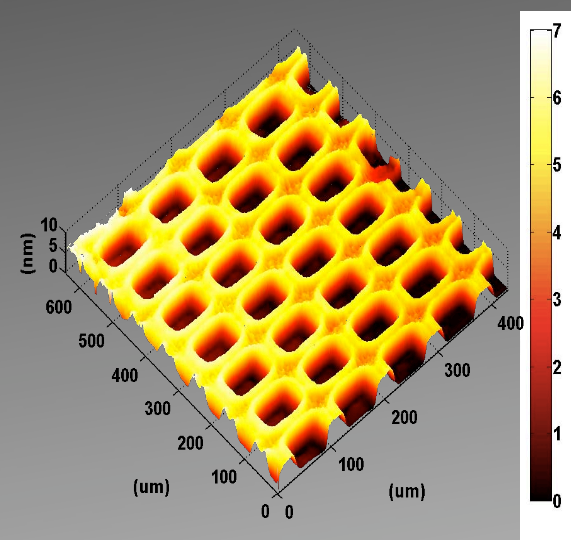
Membrane Photolithography

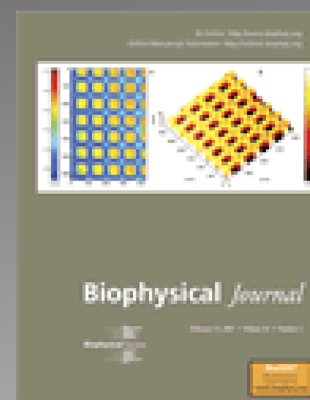






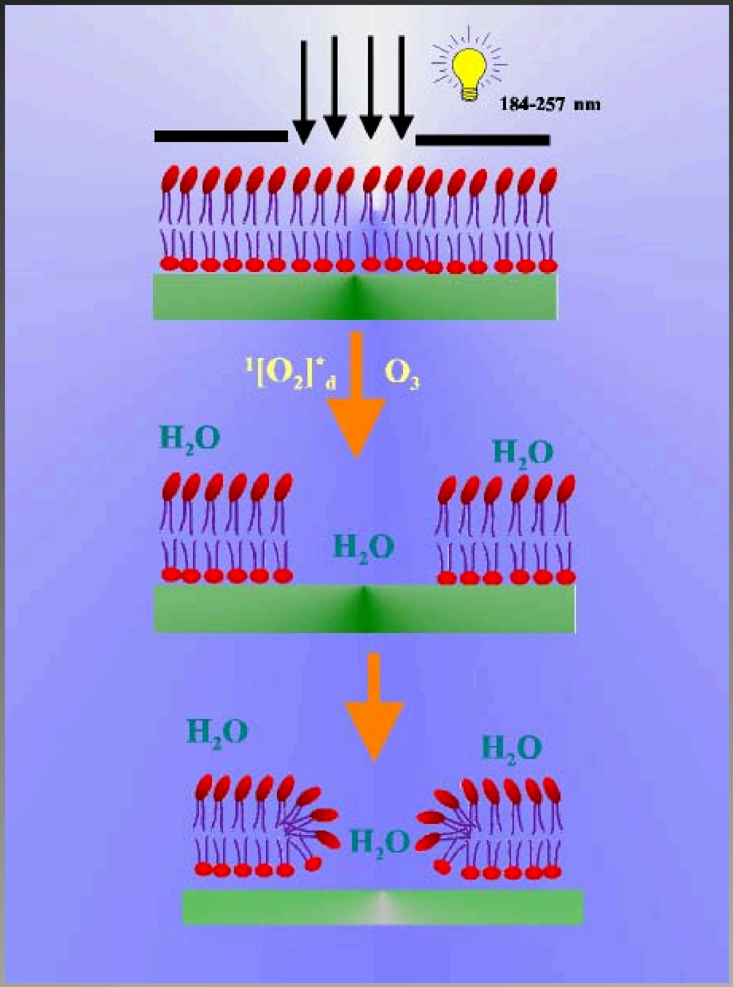


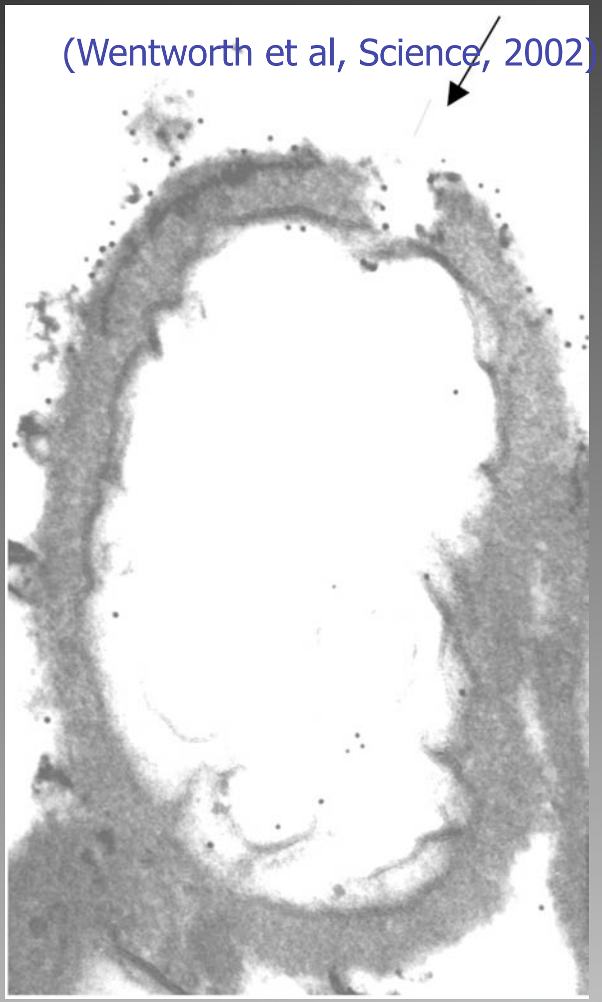




C. K. Yee, M. L. Amweg, A. N. Parikh, Adv. Mater. (2004)
C. K. Yee, M. L. Amweg, A. N. Parikh, J.Amer. Chem. Soc. (2004)
A. W. Szmodis, M. Howland, B. Sanii. A. N. Parikh, Biophys. J (2007)
Patent # 7,132,122 (2006)

Photochemistry of Membrane Photolithography





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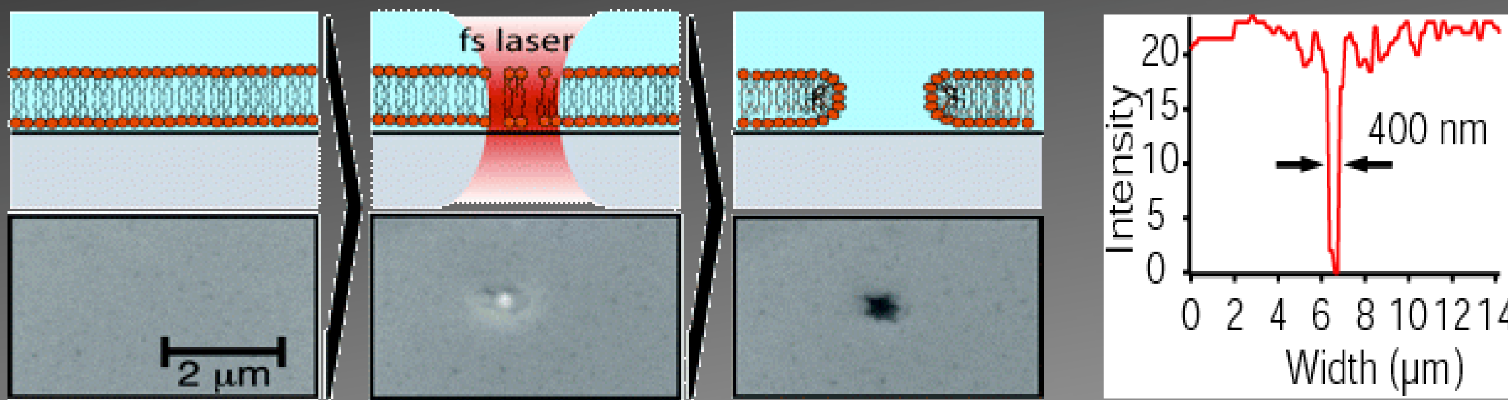
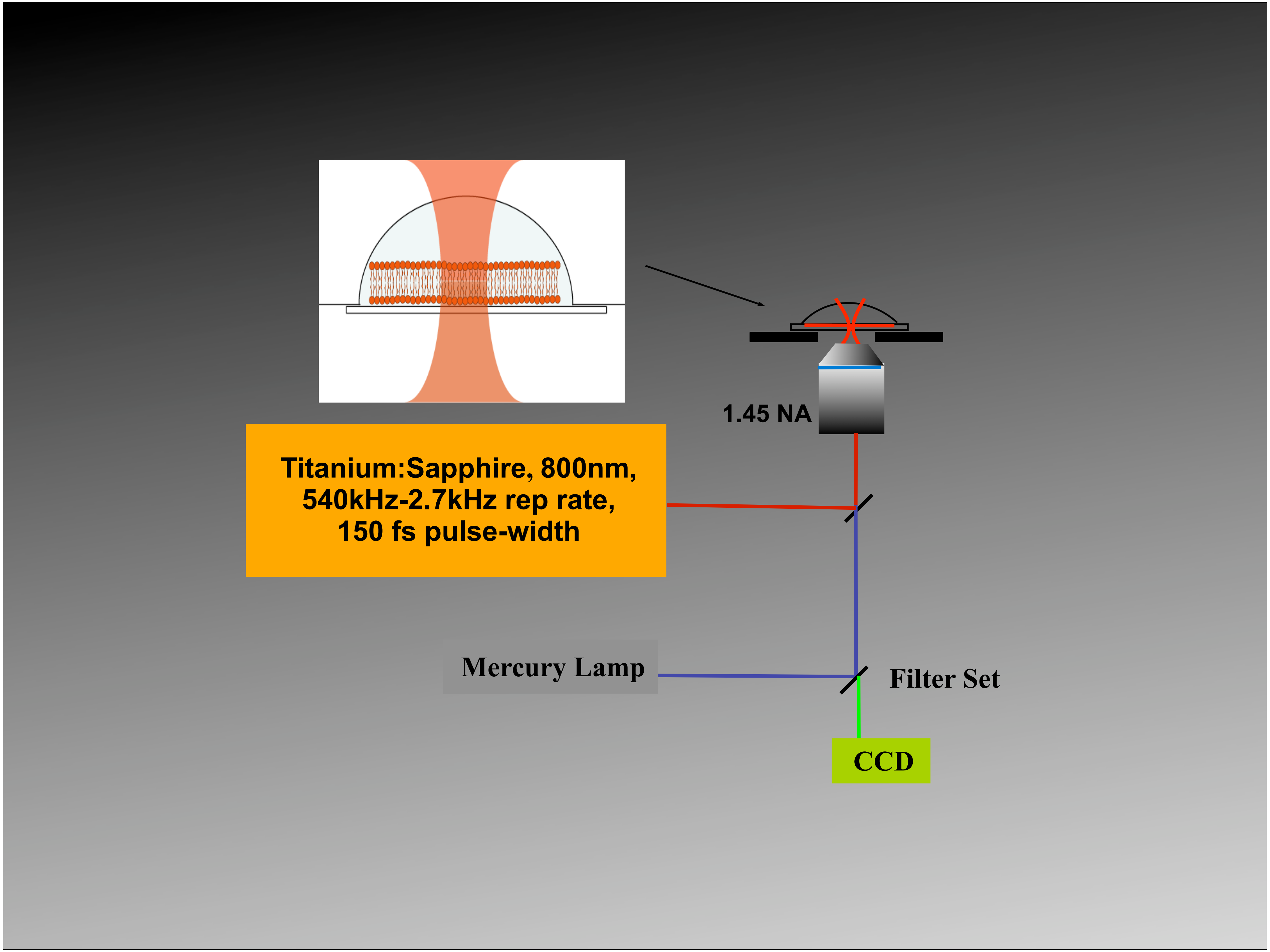
Jacobson, K., Sheets, E. D. & Simson, R. (1995)
Science **268,** 1441

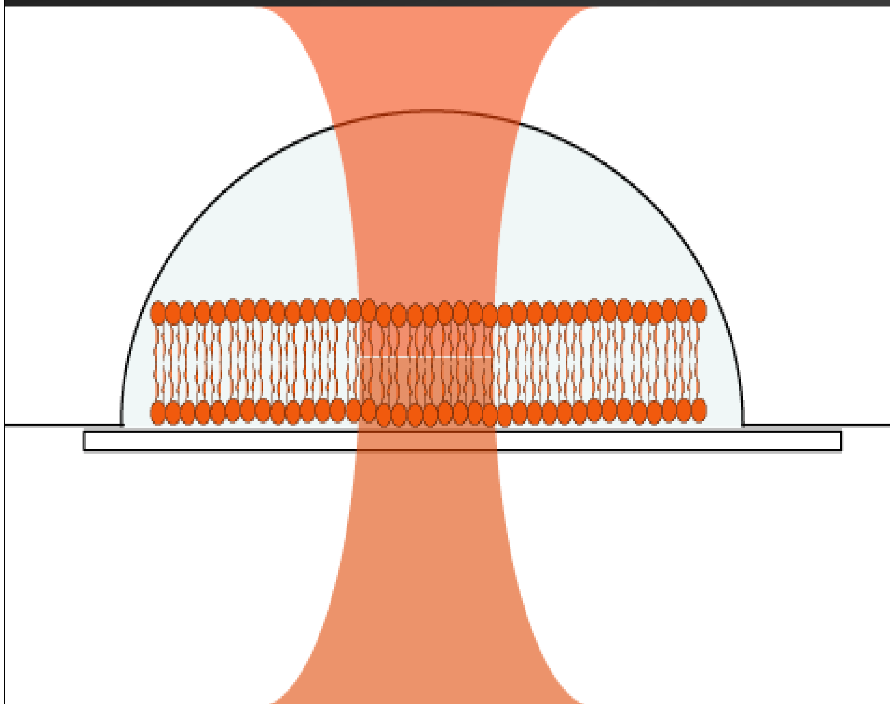
Femtosecond Bilayer Surgery at the Nanoscale

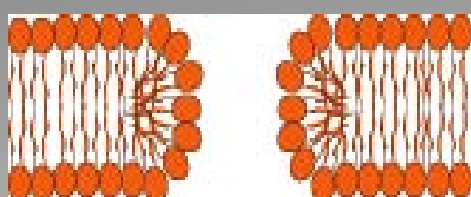


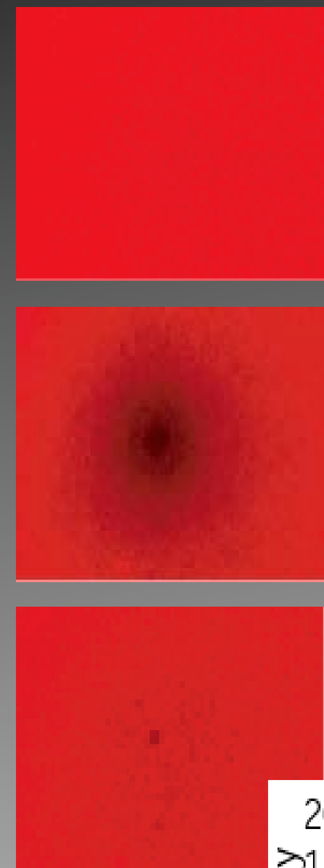
A. M. Smith, T. R. Huser, A. N. Parikh, J. Amer. Chem. Soc. (2007)

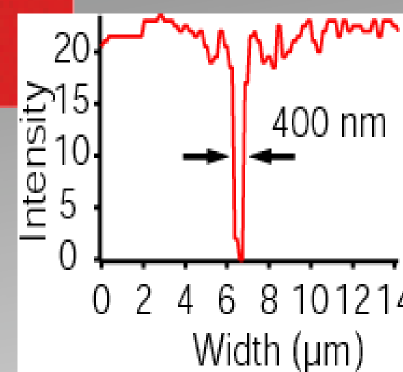

1.45 NA
Titanium:Sapphire, 800nm,
540kHz-2.7kHz rep rate,
150 fs pulse-width
Mercury Lamp
Filter Set
CCD

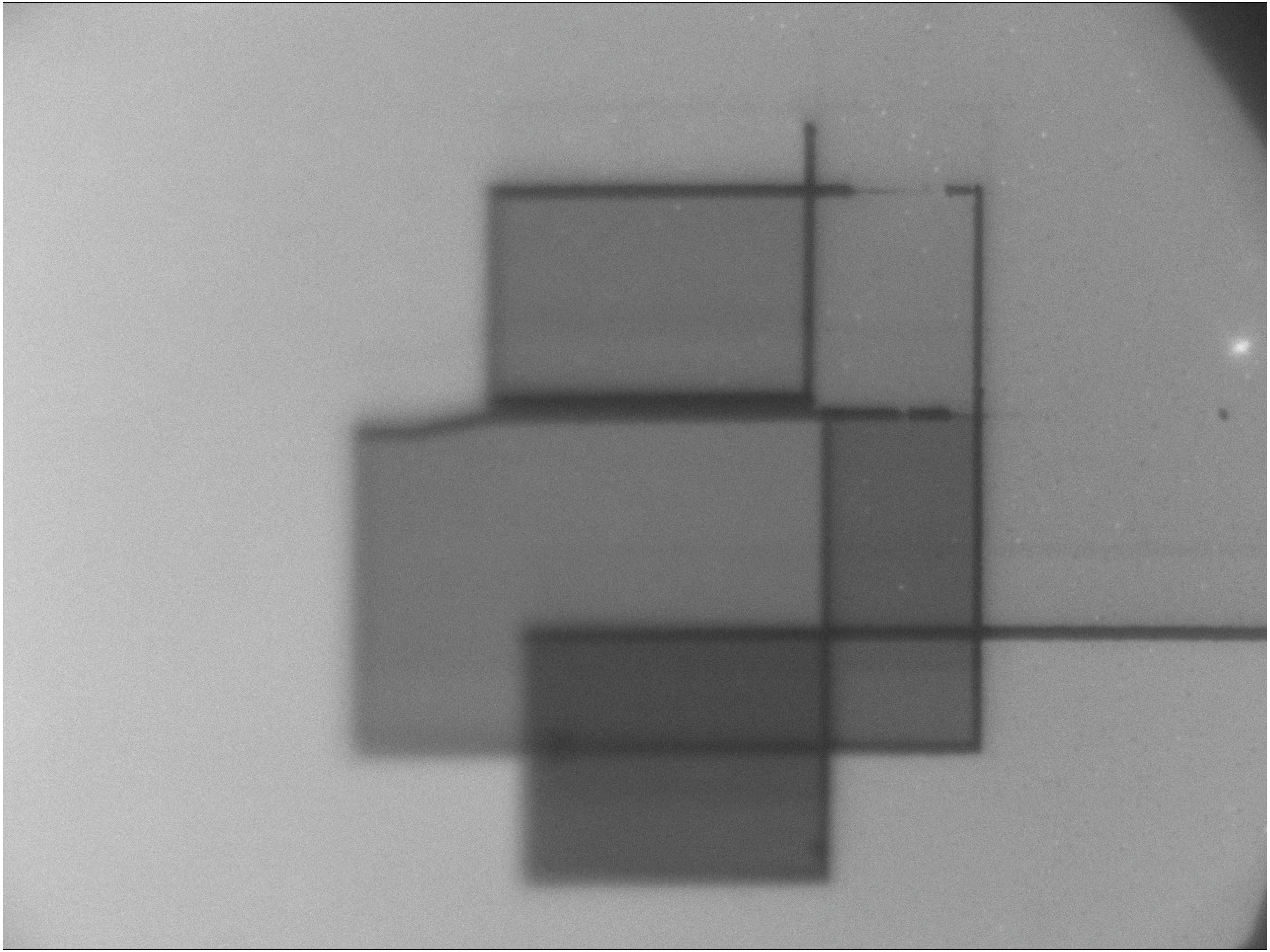
Femtosecond Bilayer Surgery !

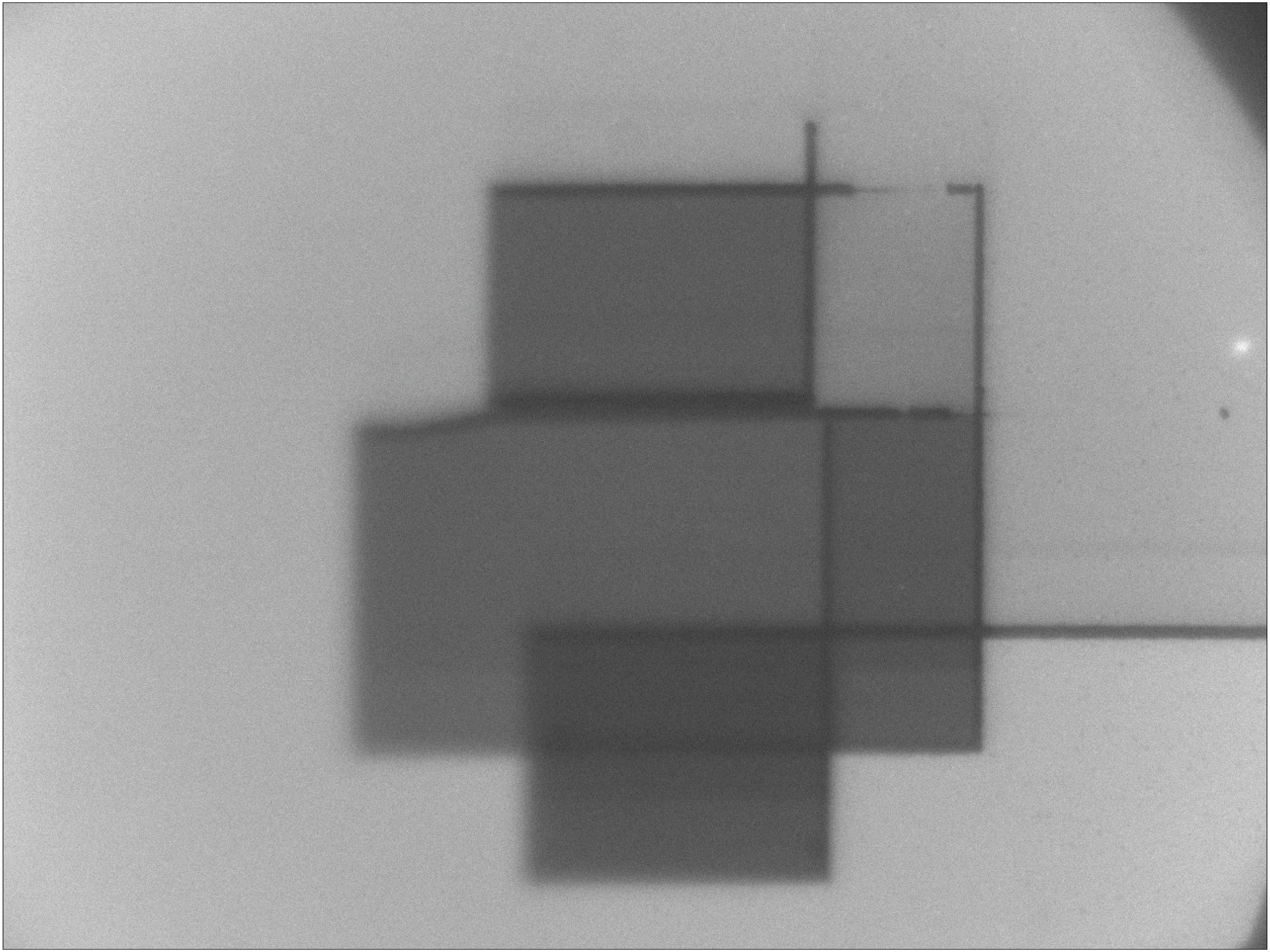


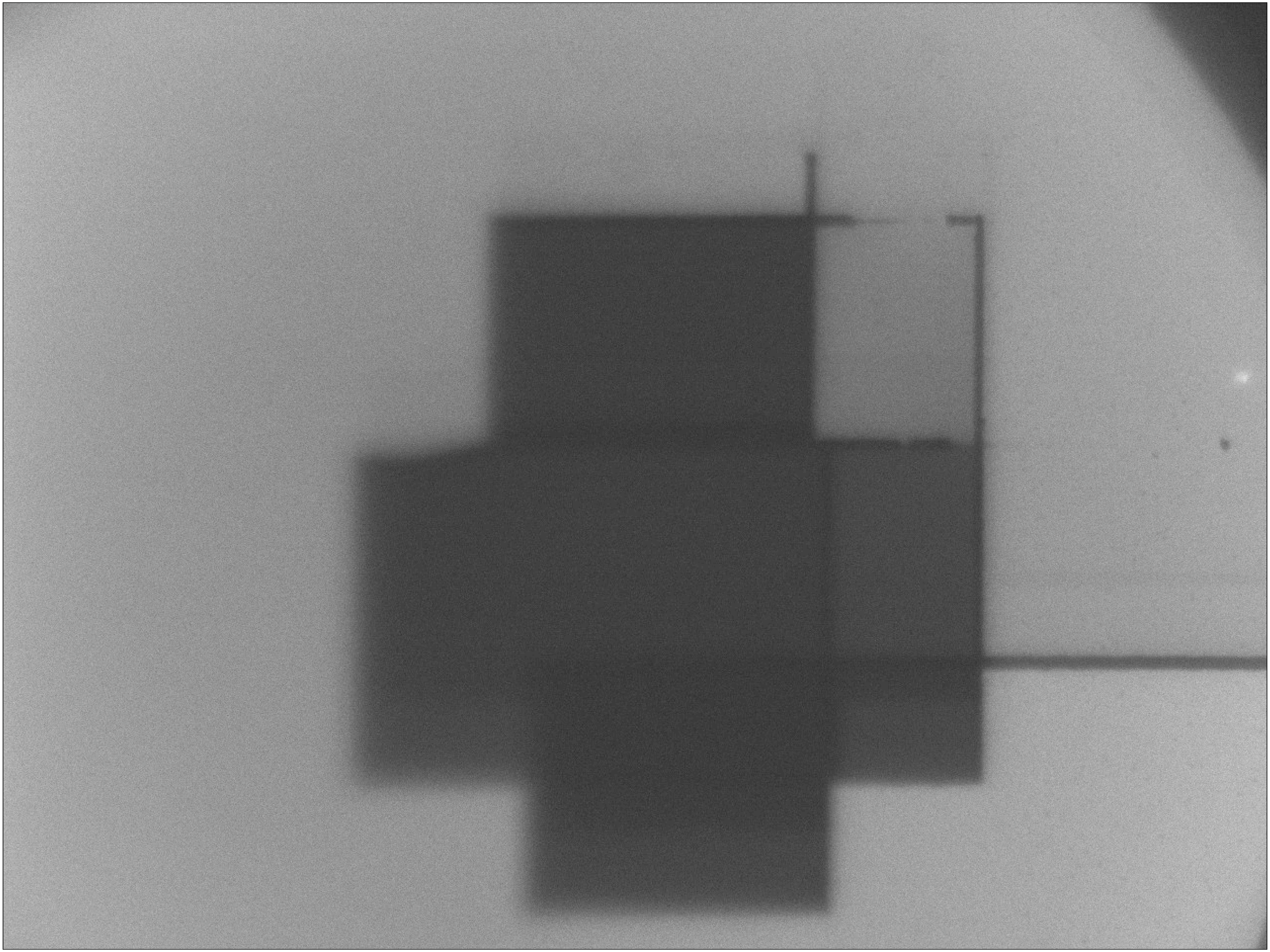


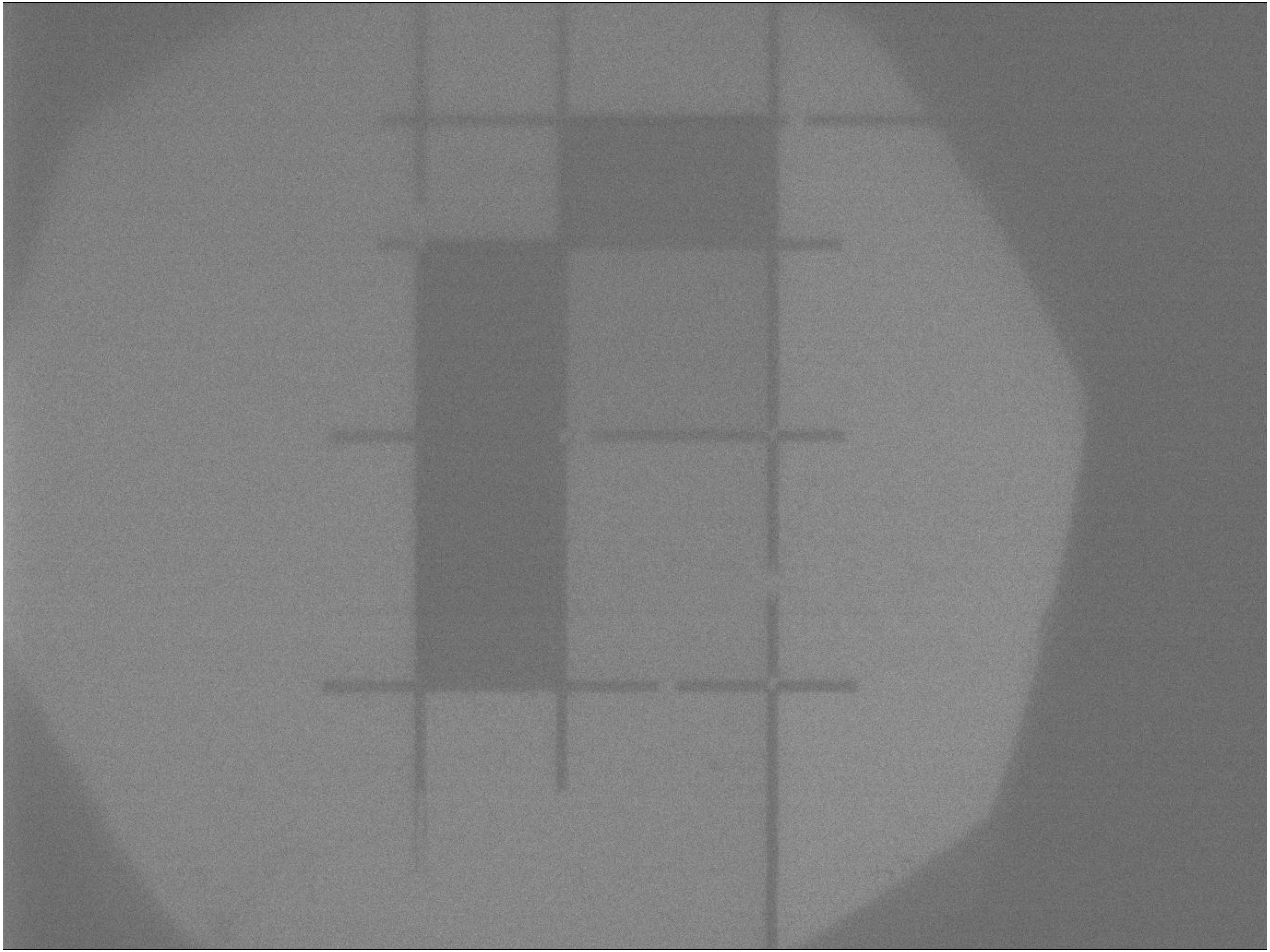


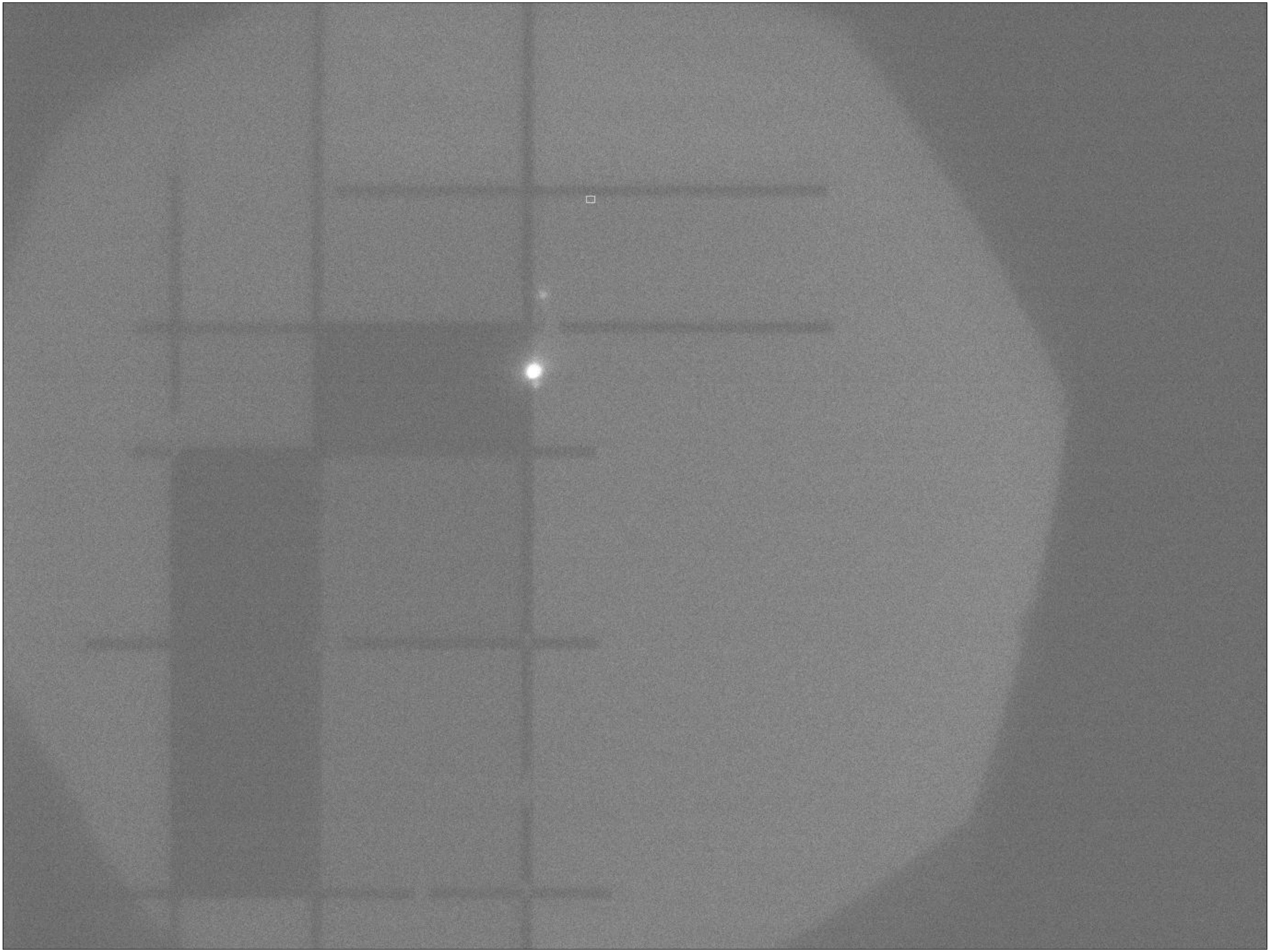


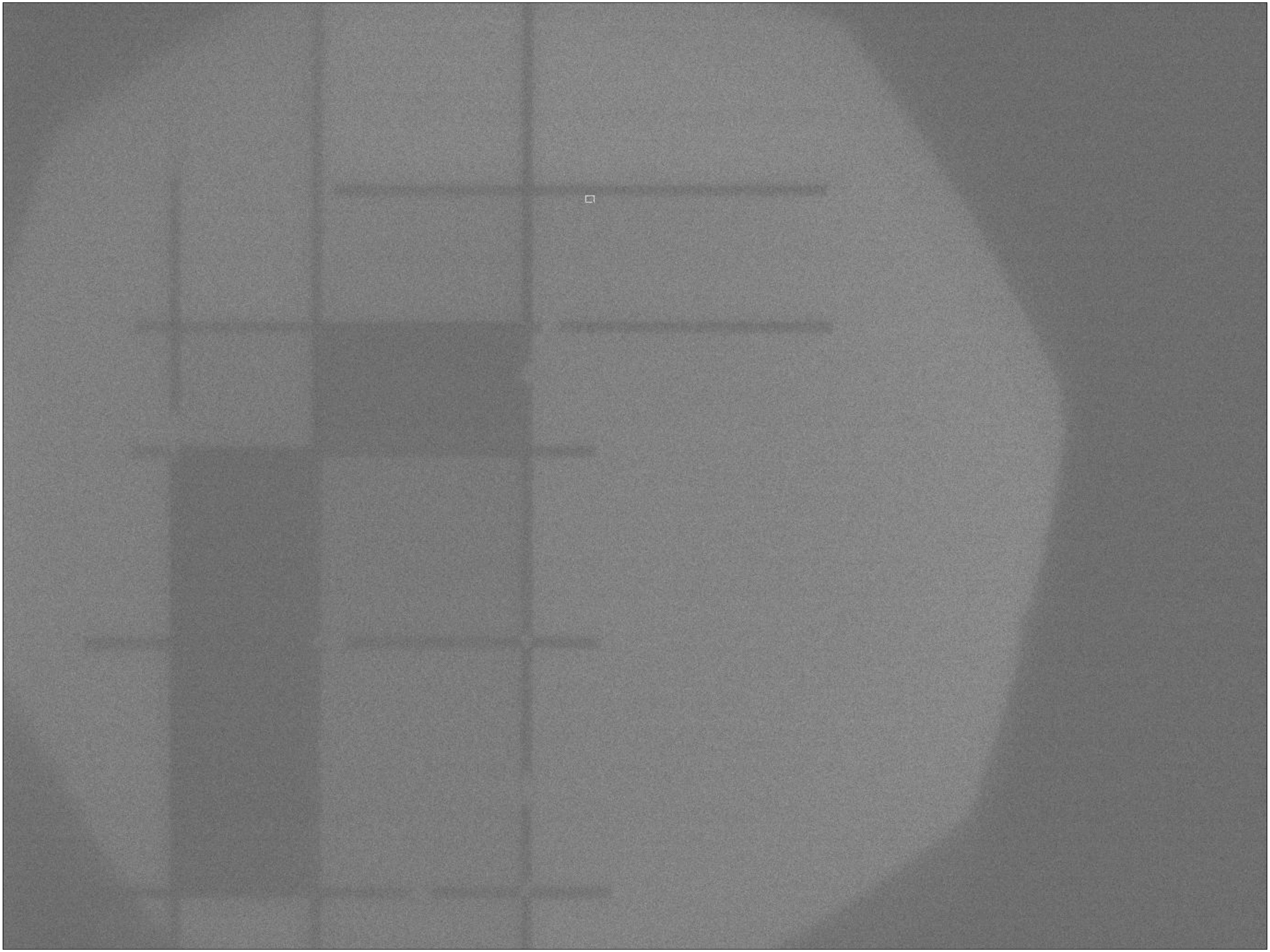




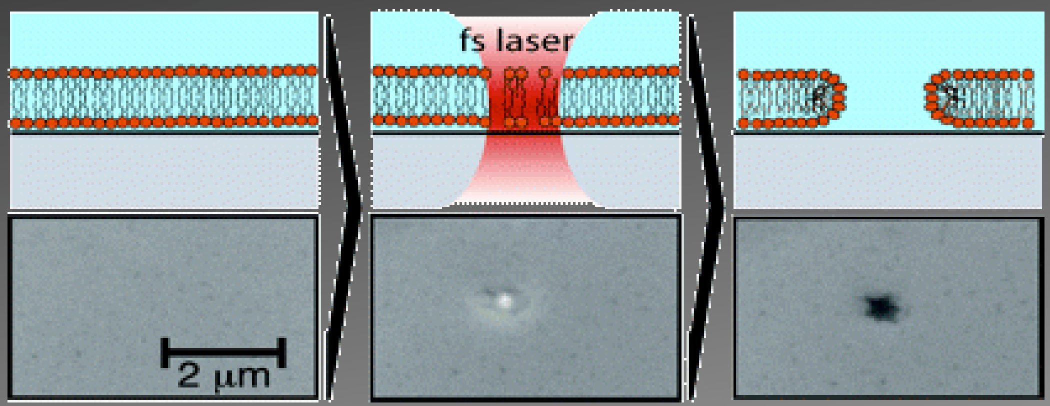


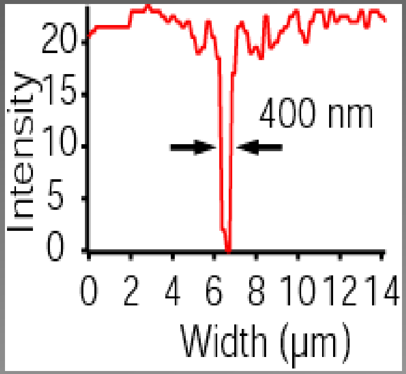






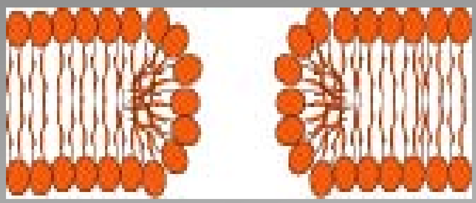
Femtosecond Bilayer Surgery at the Nanoscale



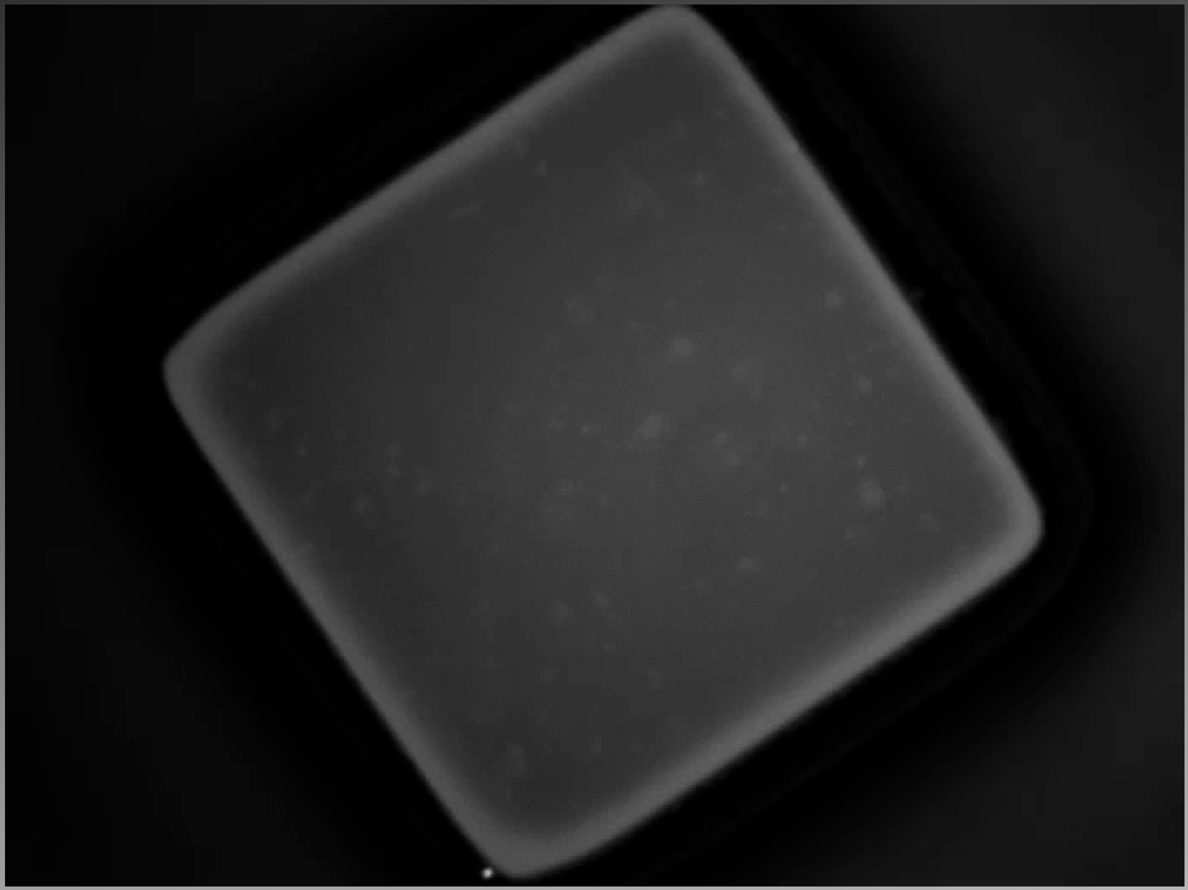


A. M. Smith, T. R. Huser, A. N. Parikh, J. Amer. Chem. Soc. (2007)

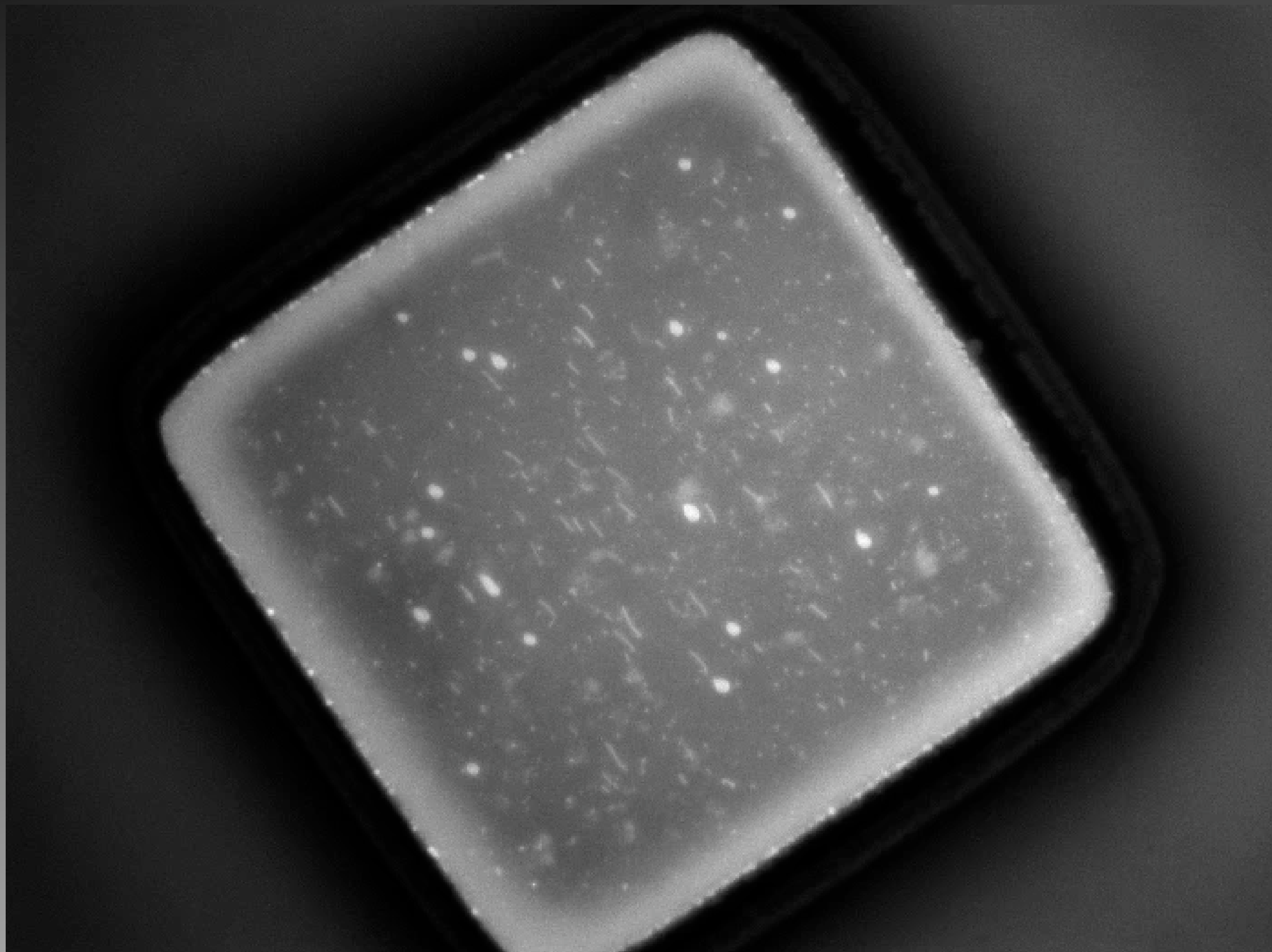
The edge
pores, holes, and permeability



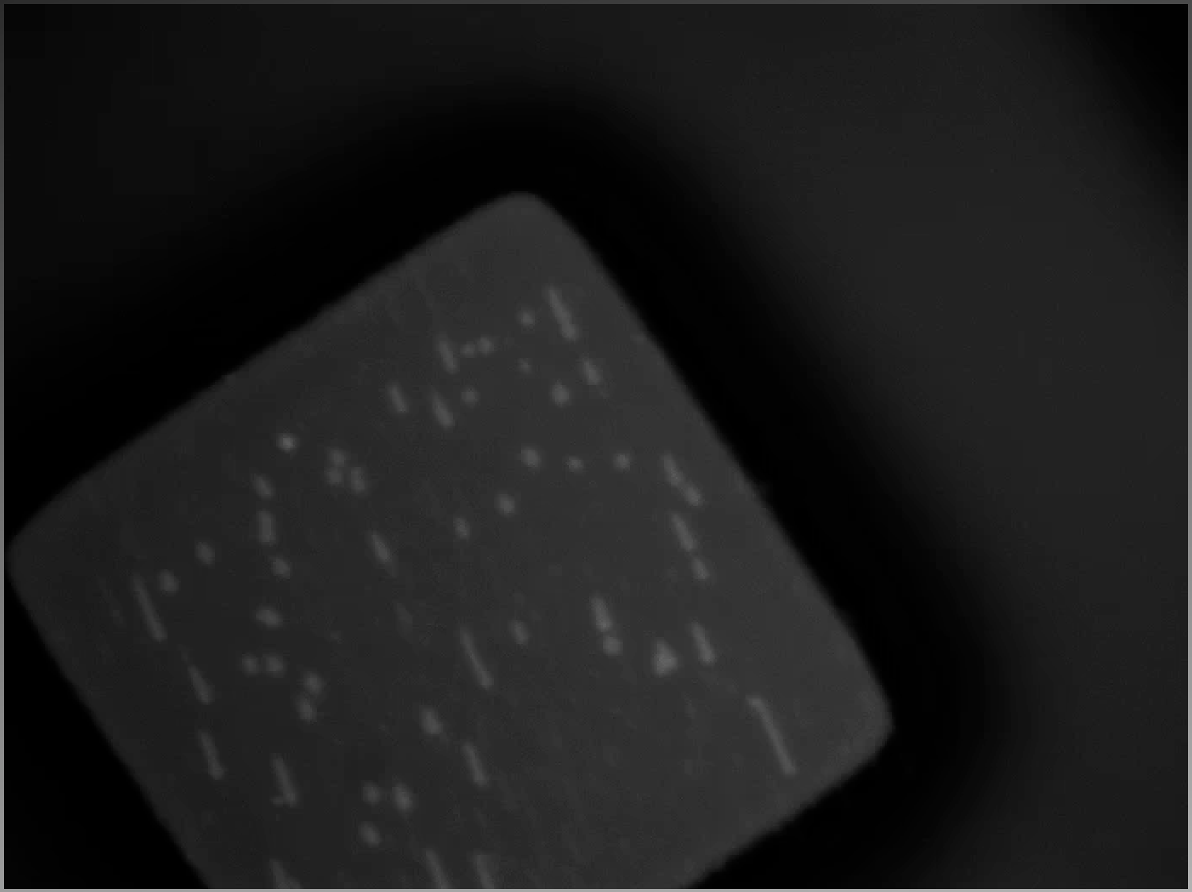
DiI behaviour near the edge of a DMPC bilayer



25- 45 degree celcius

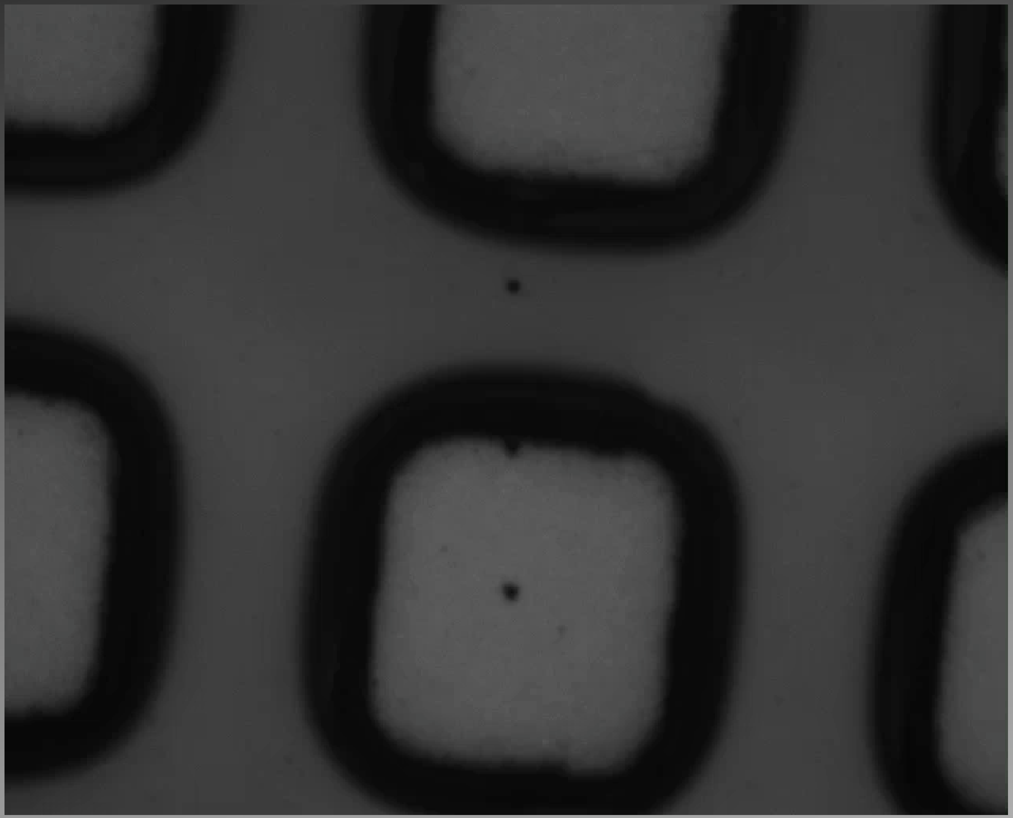


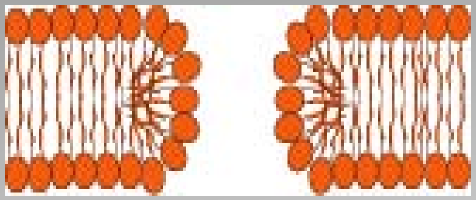
45- 55 degree celcius



65- 25 degree celcius

Melting FRAP spots via Heating

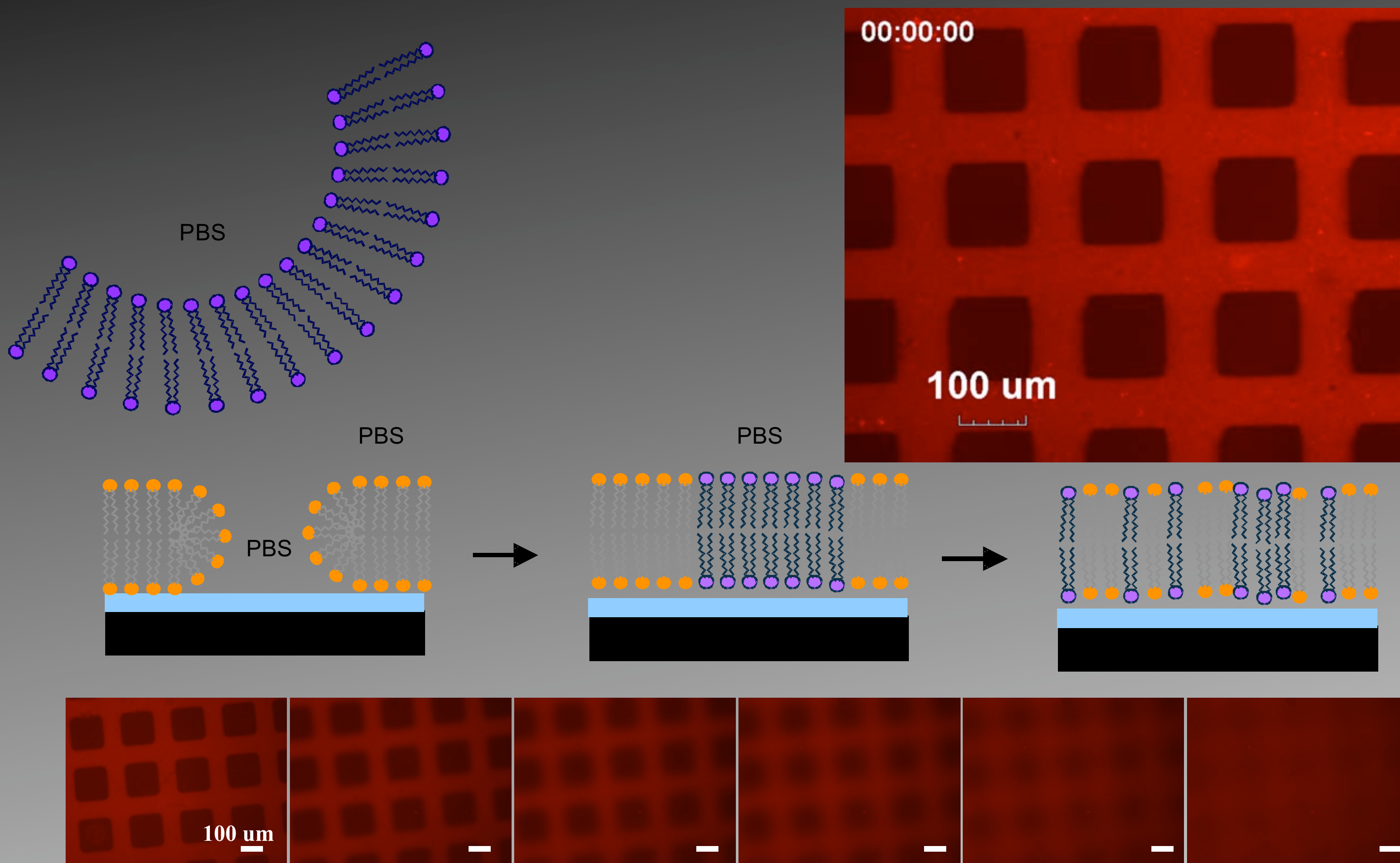




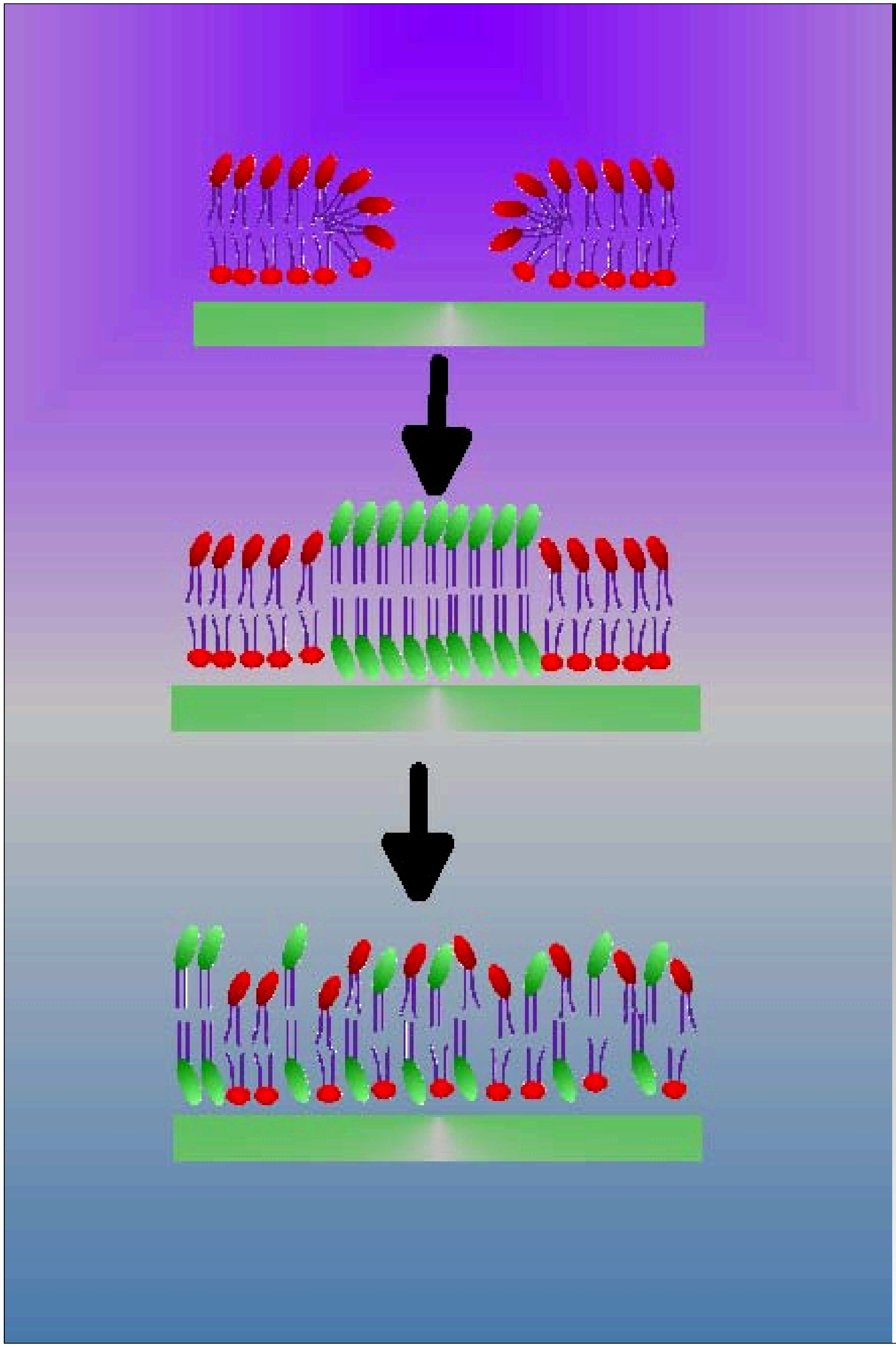
controlling compositions

studying lipid rafts?

Probing Membrane Heterogeniety and Dynamics using model bilayers



Before Backfilling | T=0 min. | T=1 min. | T=5 min. | T=10 min. | T=20 min.



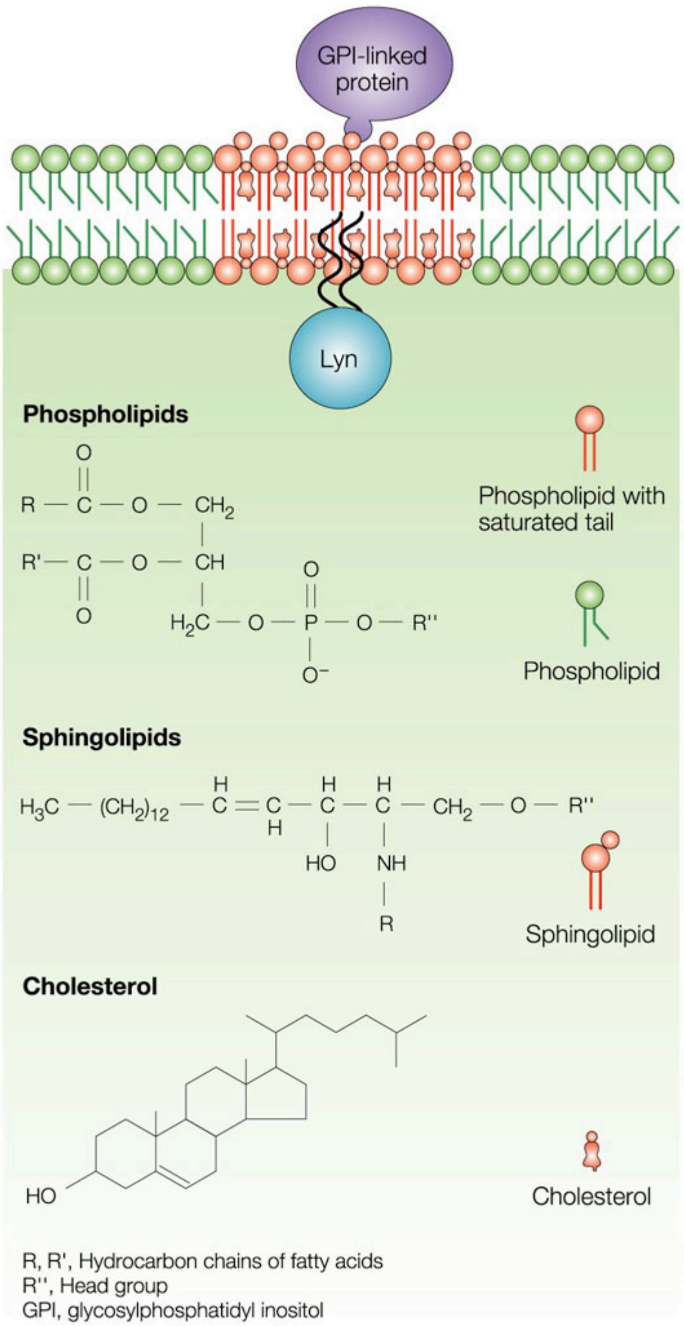
A Biophysical tool for Understanding Lipid heterogeneity

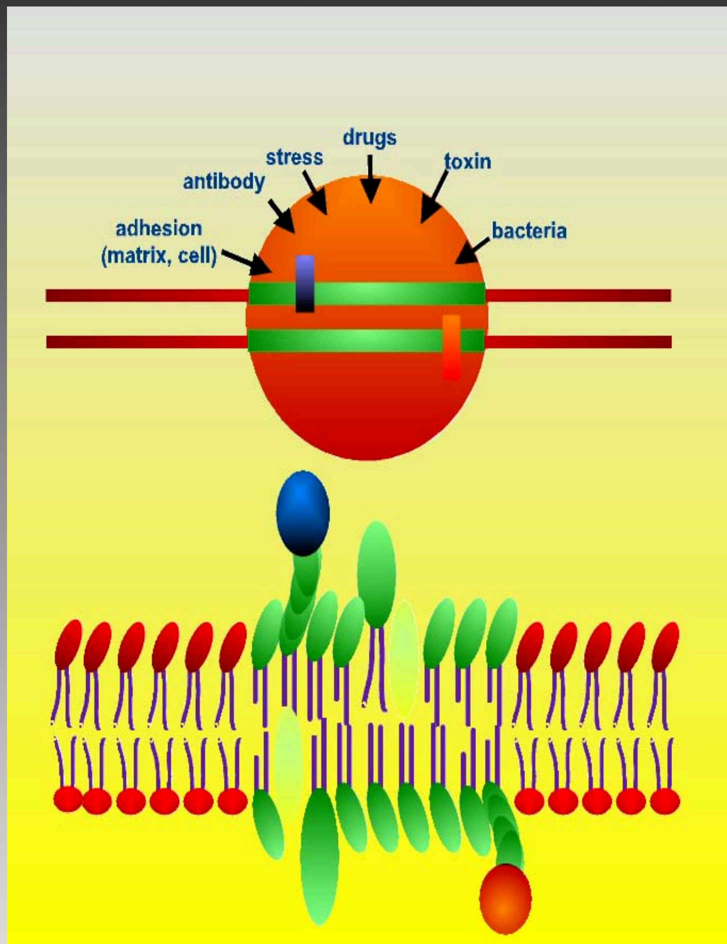
Designed reactive-diffusive fronts

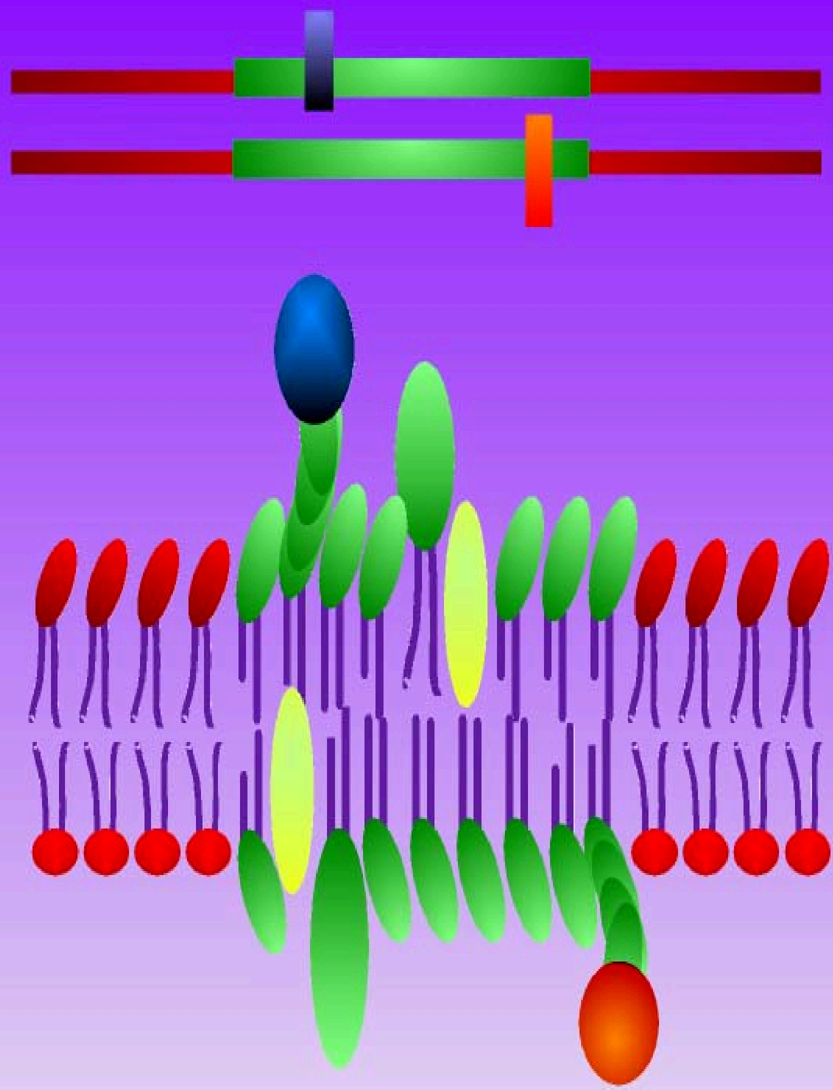
Lipid-lipid interdiffusion, compositional manipulation
Phase dynamics and stability
Engineering arrested diffusion

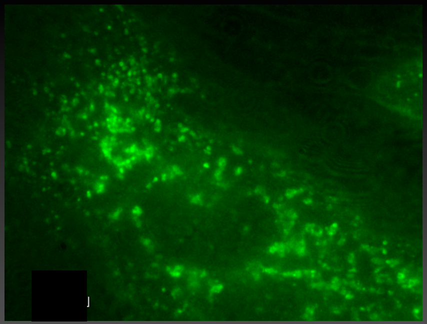
Kinetically and chemically arrested

Mixing for functional patterning

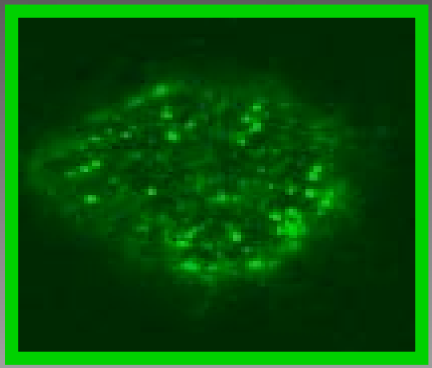

GPI-linked protein
Lyn
Phospholipids
Phospholipid with saturated tail
Phospholipid
Sphingolipids
Sphingolipid
Cholesterol
Cholesterol
R, R', Hydrocarbon chains of fatty acids
R'', Head group
GPI, glycosylphosphatidyl inositol


drugs
stress
toxin
antibody
adhesion (matrix, cell)
bacteria

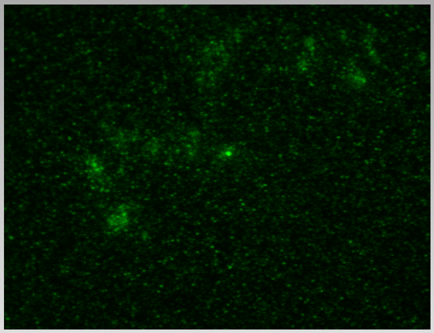




HAEC
Gm1

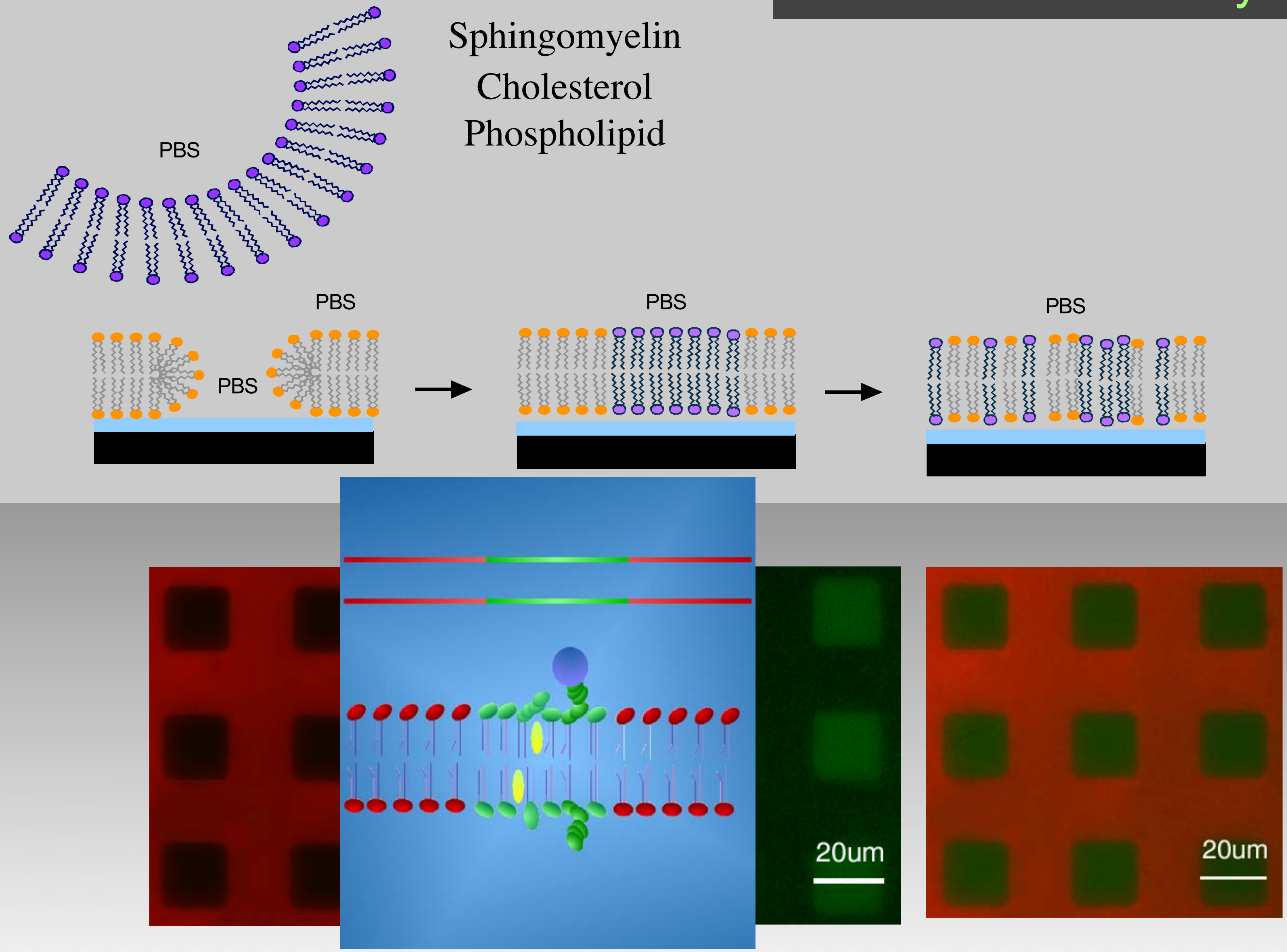


HL-60
CTB



Bilayers
CTB

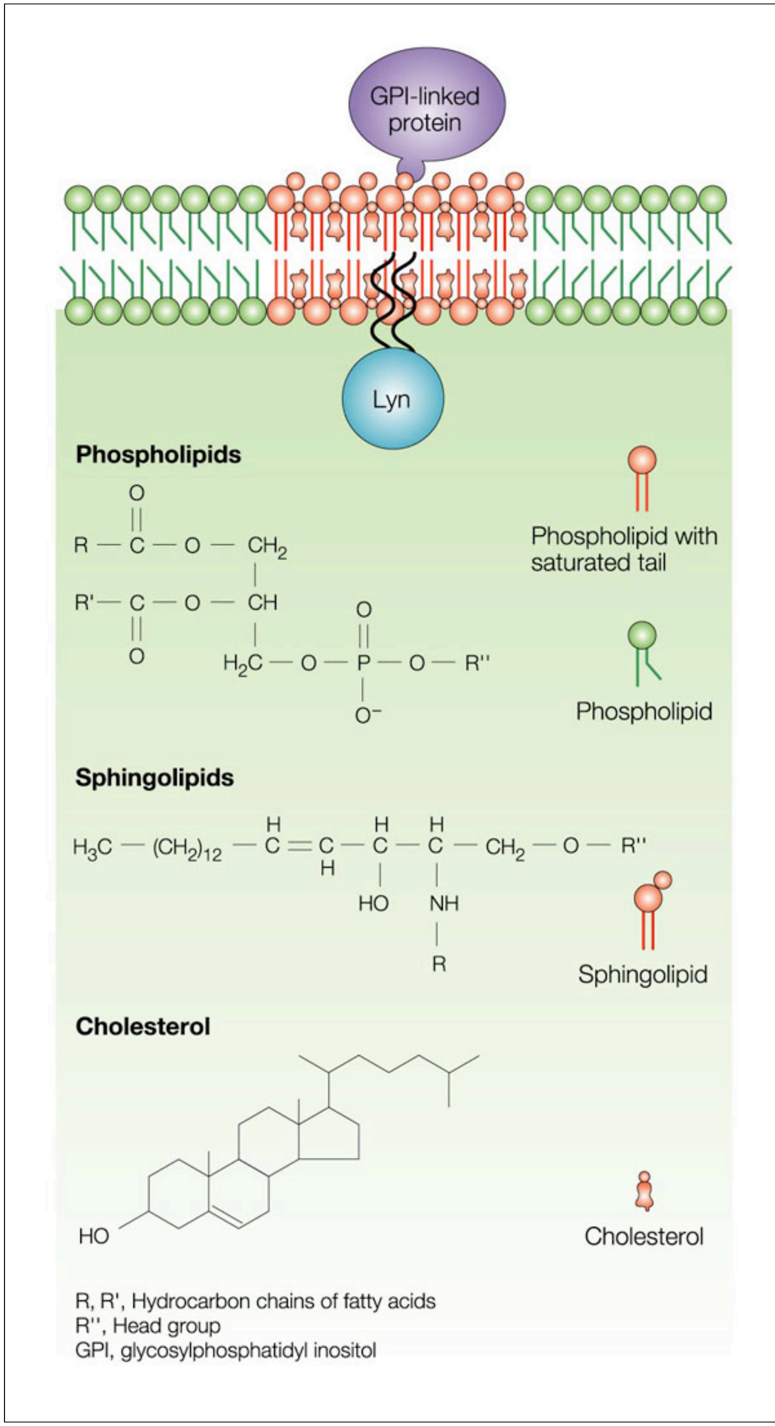
Raft Microarrays

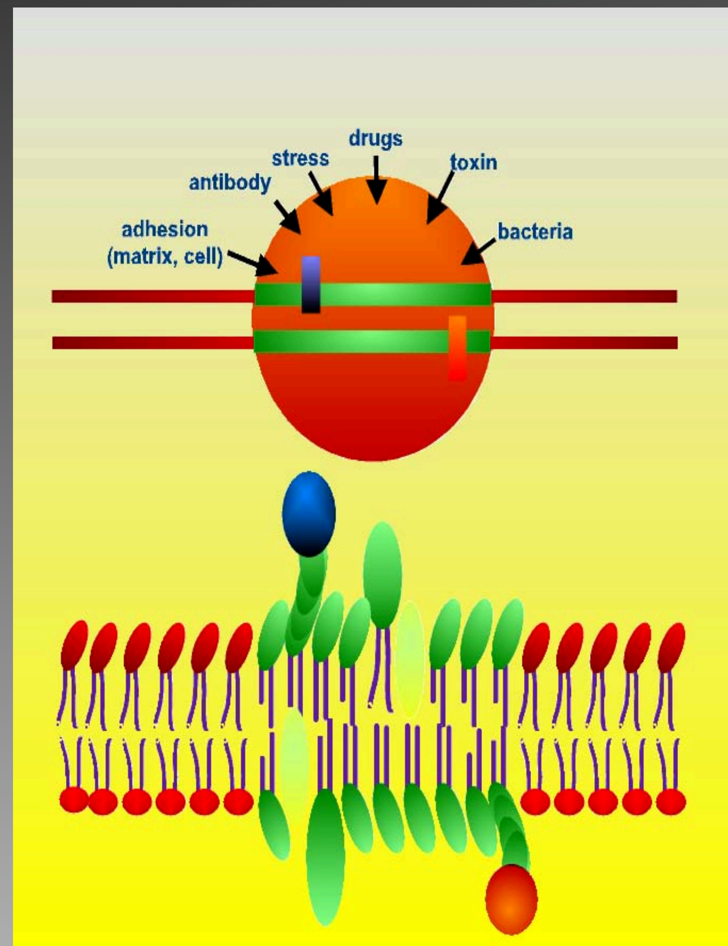


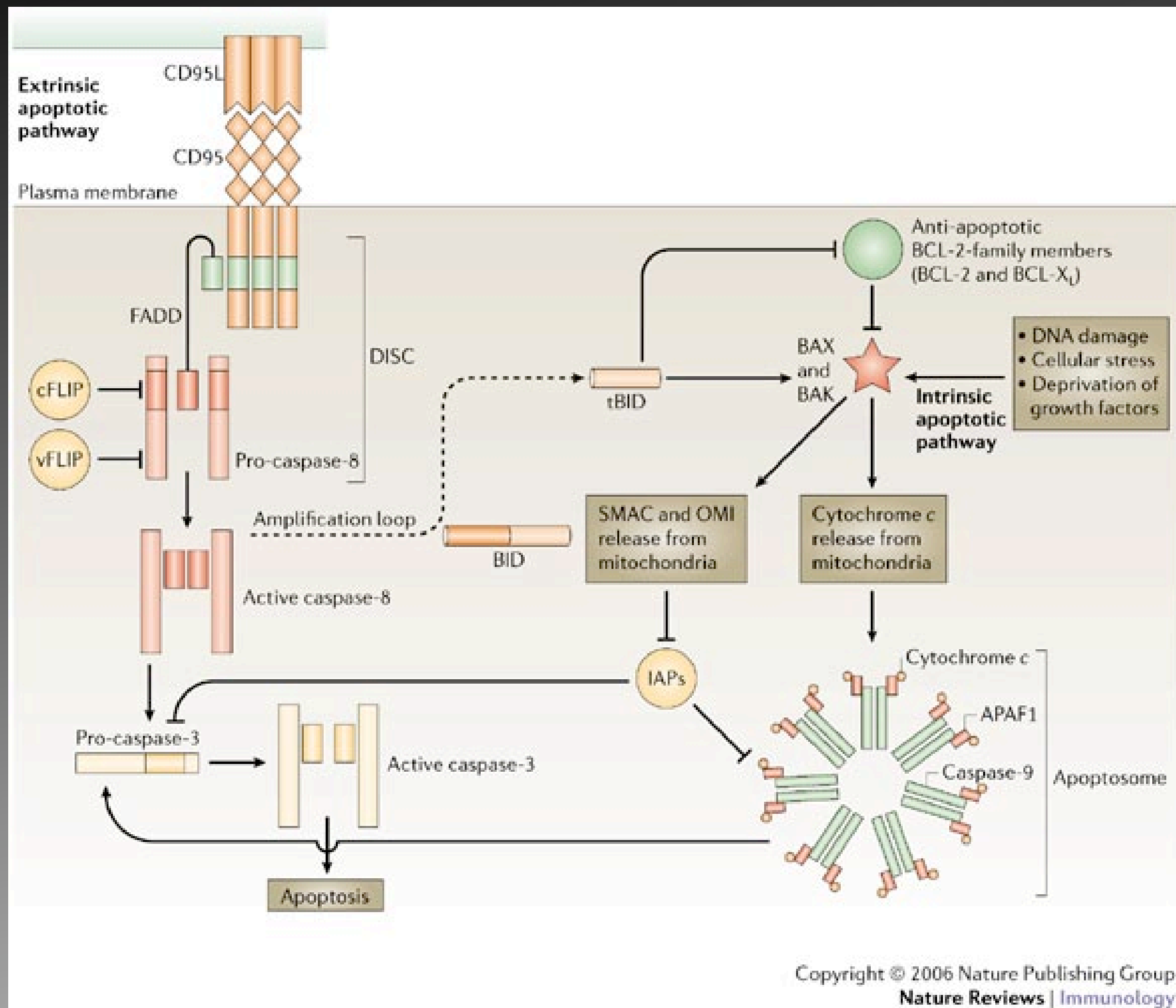
A. R. Sapuri, J. T. Groves, A. N. Parikh, *Langmuir* (2006)

functional dynamics at cellular surfaces

Cellular apoptosis

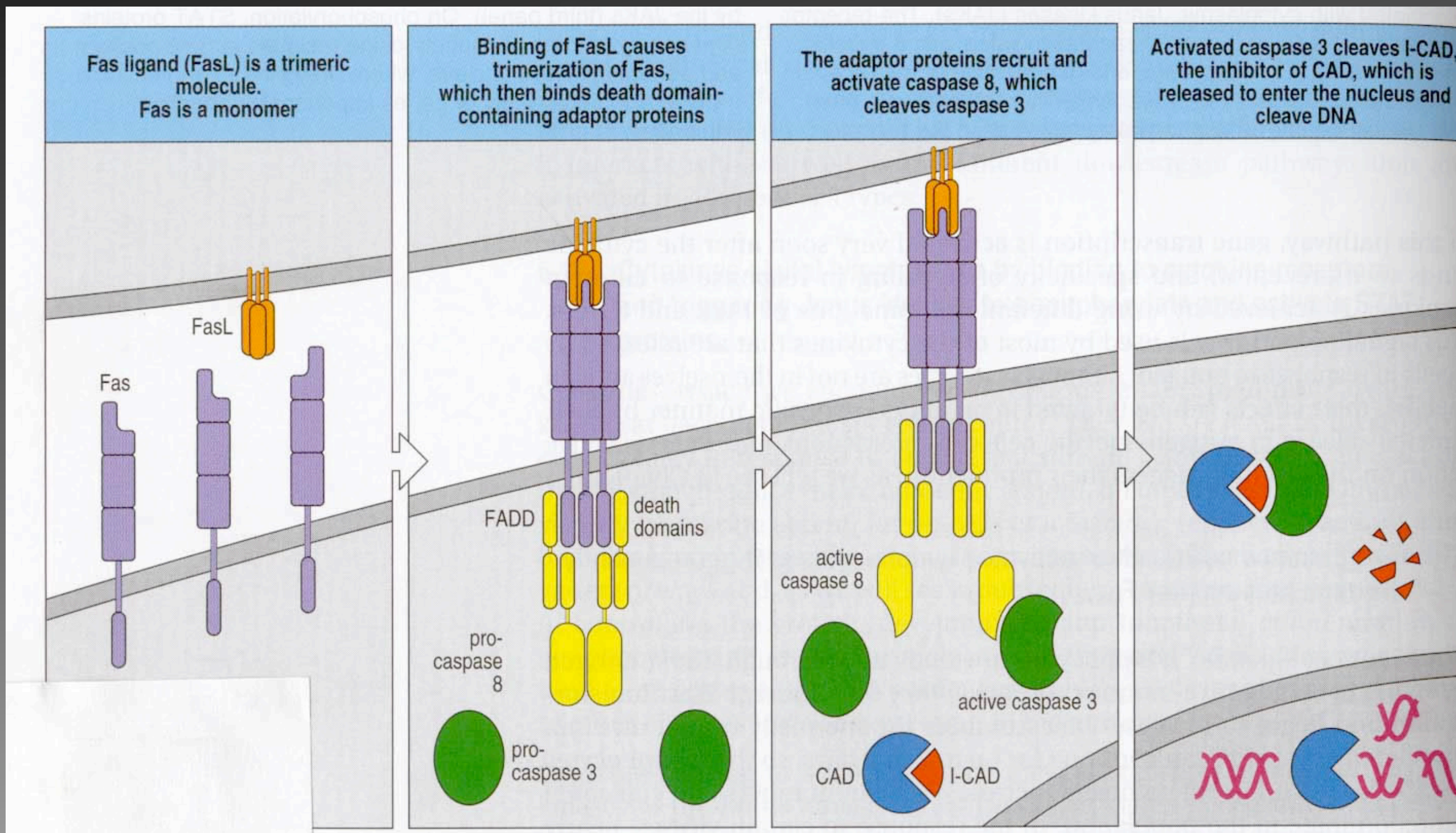

GPI-linked protein
Lyn
Phospholipids
Phospholipid with saturated tail
Phospholipid
Sphingolipids
Sphingolipid
Cholesterol
Cholesterol
HO
R, R', Hydrocarbon chains of fatty acids
R'', Head group
GPI, glycosylphosphatidyl inositol


drugs
stress
toxin
antibody
adhesion
(matrix, cell)
bacteria

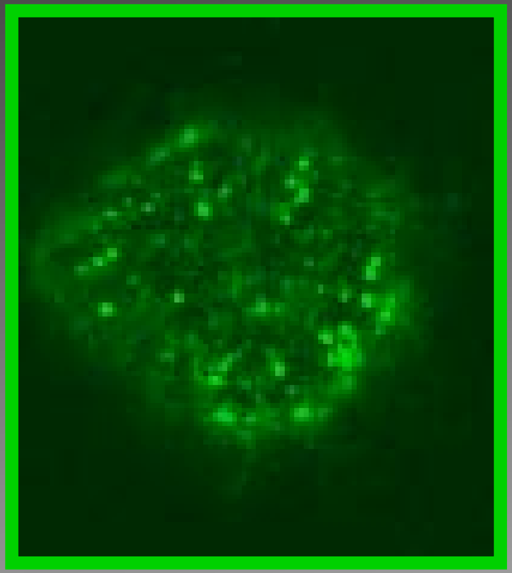


Siegel *Nature Reviews Immunology* **6**,308–317 (April 2006) | doi:10.1038/nri1809

nature REVIEWS IMMUNOLOGY

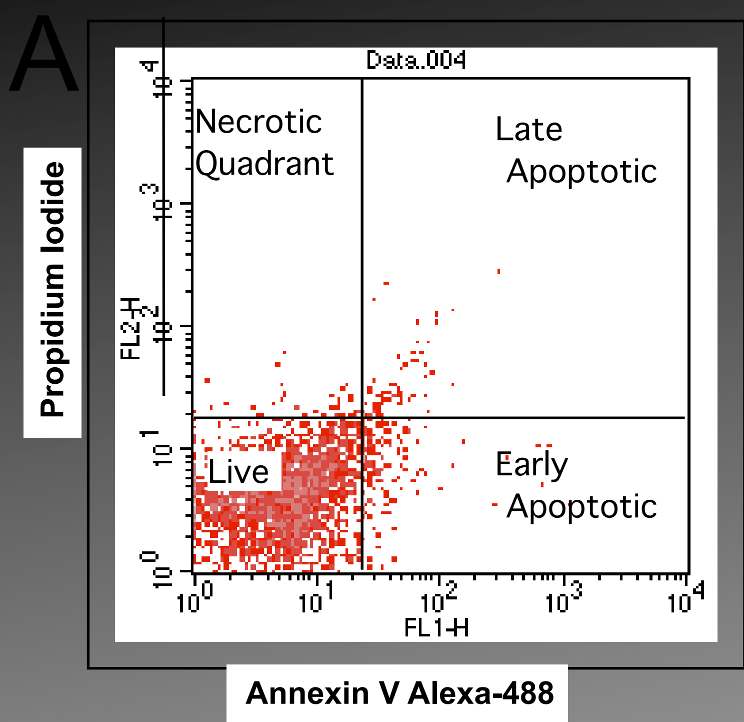

Fas ligand (FasL) is a trimeric molecule.
Fas is a monomer
Binding of FasL causes trimerization of Fas, which then binds death domain-containing adaptor proteins
The adaptor proteins recruit and activate caspase 8, which cleaves caspase 3
Activated caspase 3 cleaves I-CAD, the inhibitor of CAD, which is released to enter the nucleus and cleave DNA
FasL
Fas
FADD
death domains
pro-caspase 8
pro-caspase 3
active caspase 8
active caspase 3
CAD
I-CAD

Human Retina Pigment Epithelial Cells



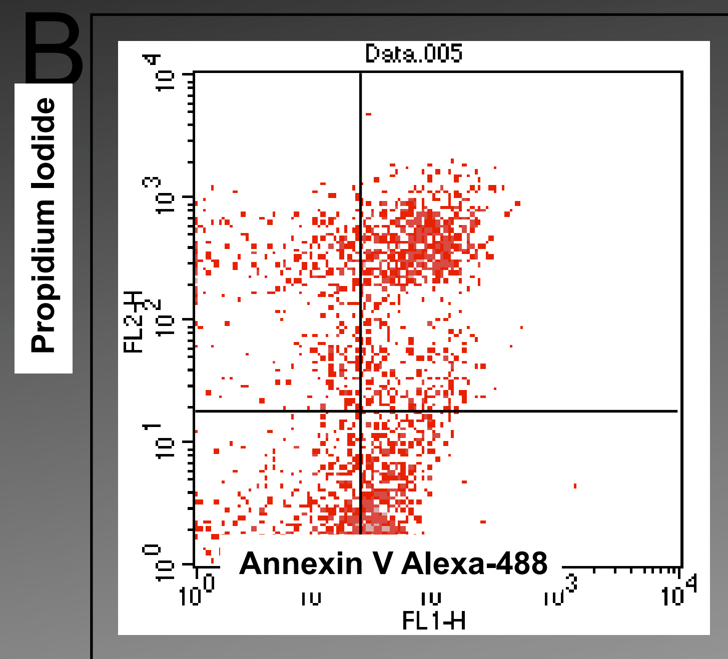
Lincoln, Boling, Parikh, Yeh, Gilchrist,Morse IOVS, 2006

uninduced



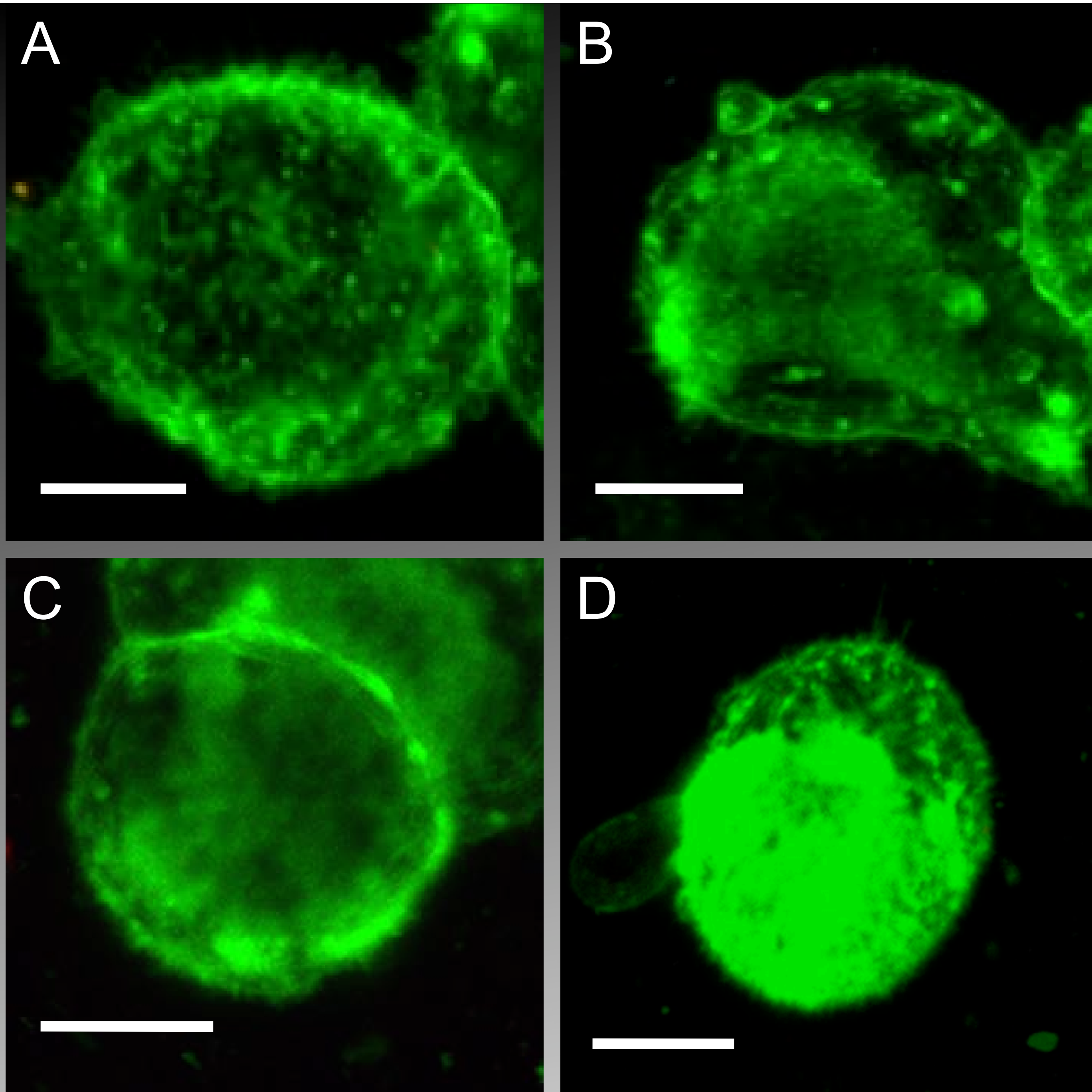
Cell State	% of gated
Live	92.54%
Early Apoptotic	3.99%
Necrotic/Late Apoptotic	3.46%

Induced



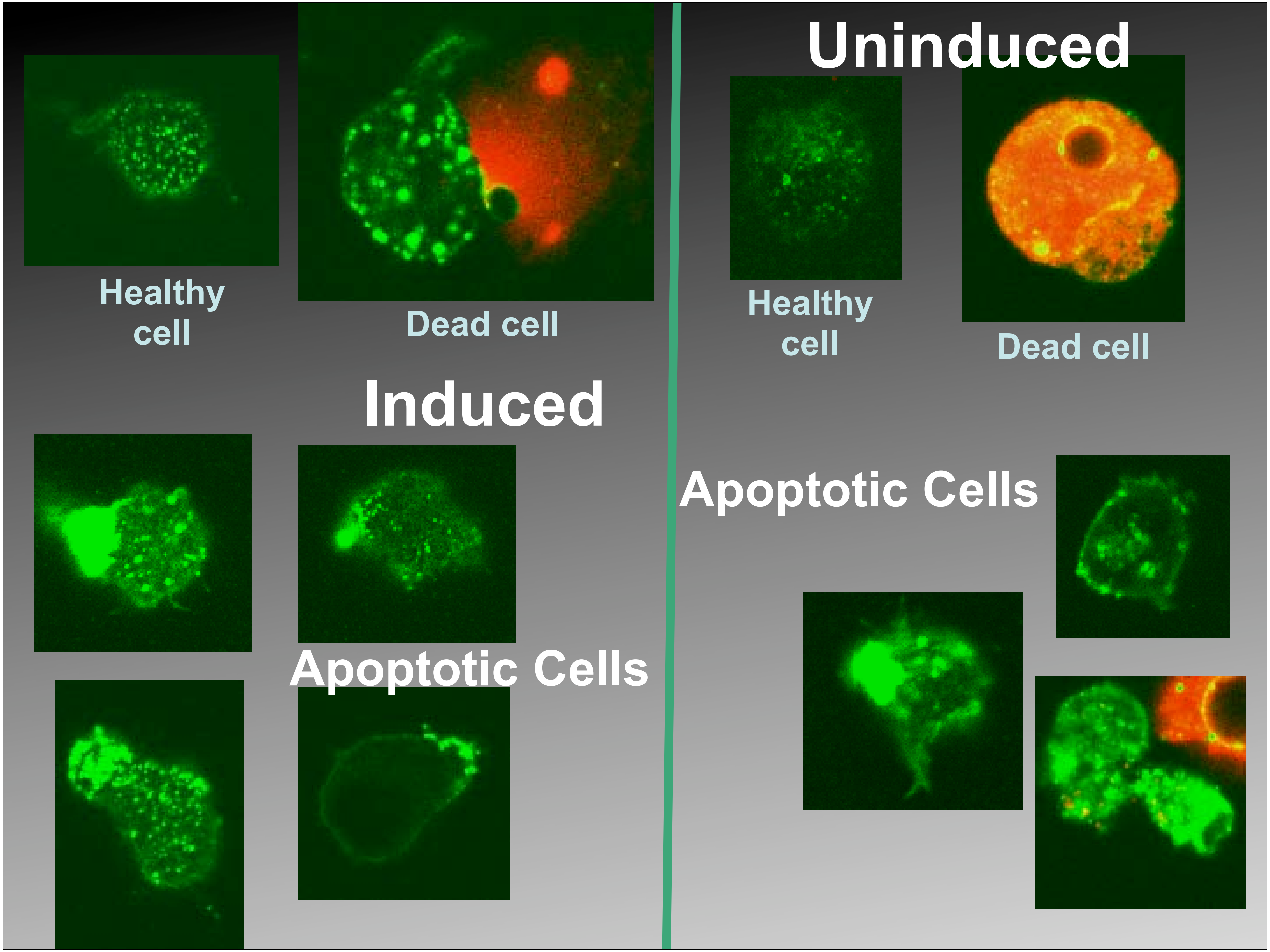
Cell State	% of gated
Live	24.60%
Early Apoptotic	22.74%
Necrotic/Late Apoptotic	52.66%

5ng/mL Fas ligand for 24 hrs


A
B
C
D

Tert-butyl hydroperoxide

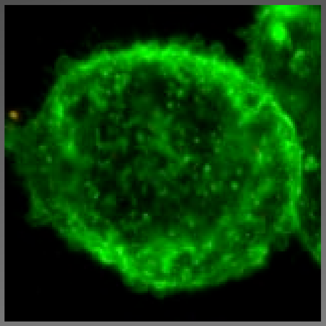
Chemical AIF


Uninduced
Healthy cell
Dead cell
Apoptotic Cells
Induced
Healthy cell
Dead cell
Apoptotic Cells

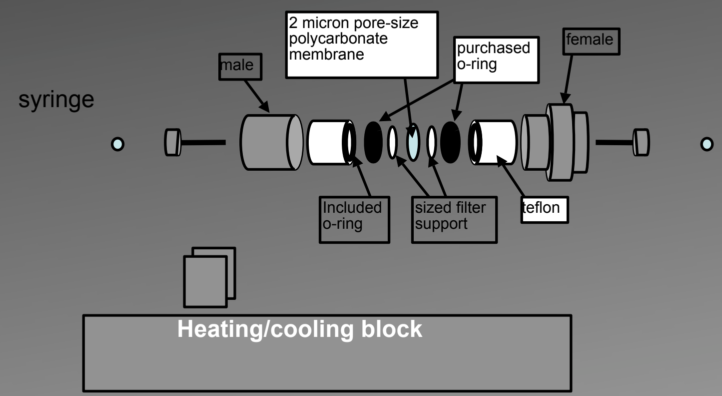
chemical reorganizations

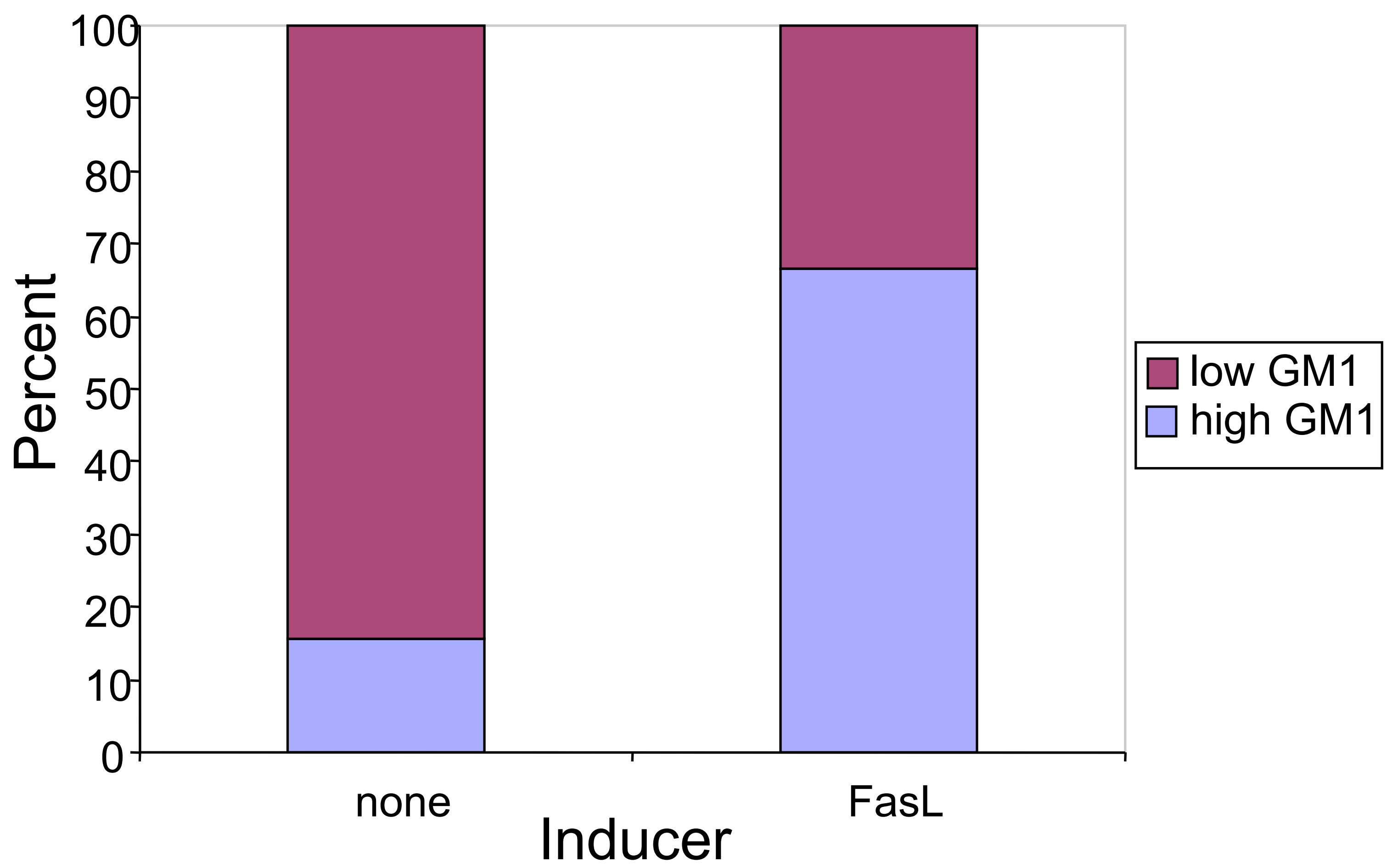
Cellular apoptosis

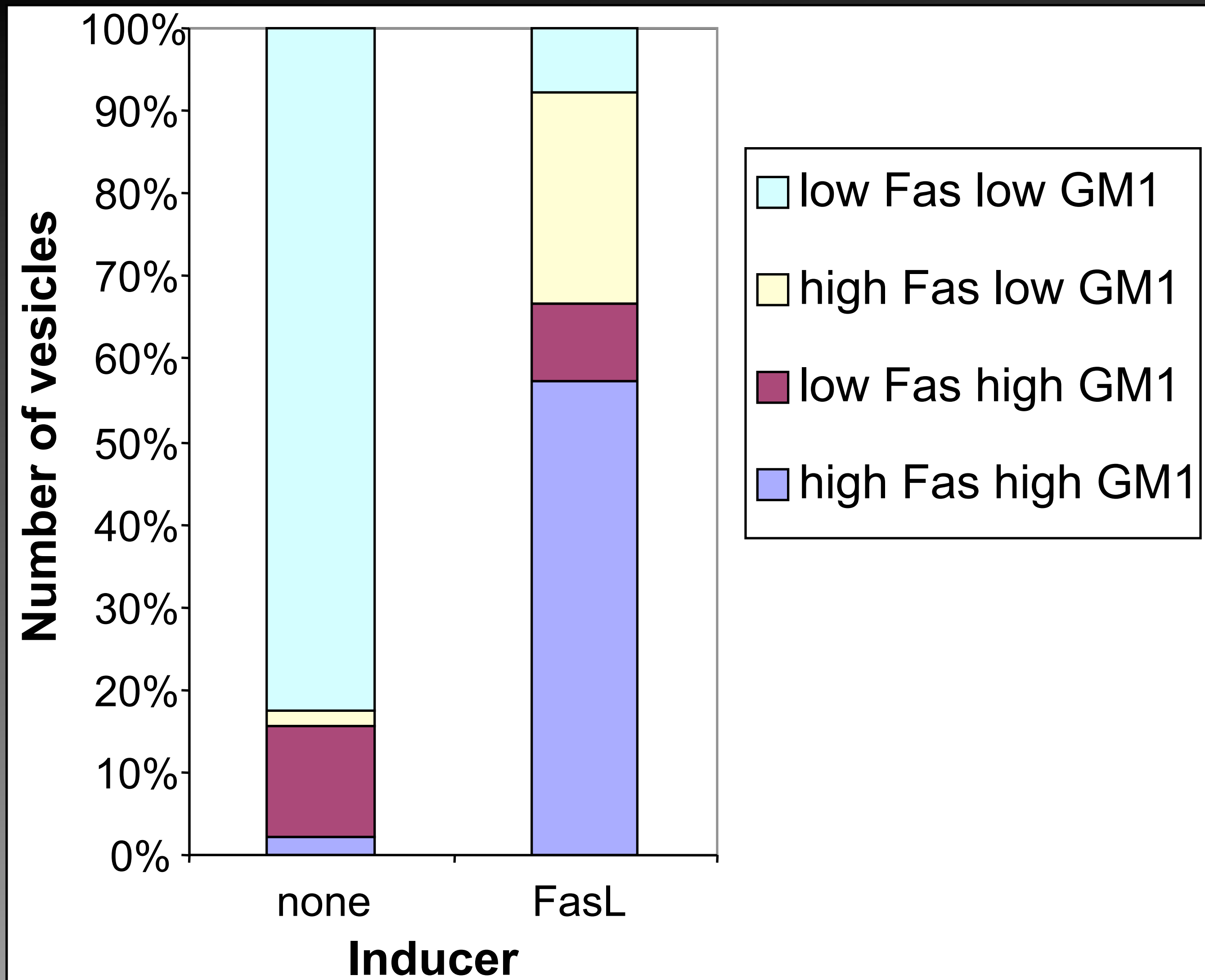
HAEC
300.19
RPE
HL-60



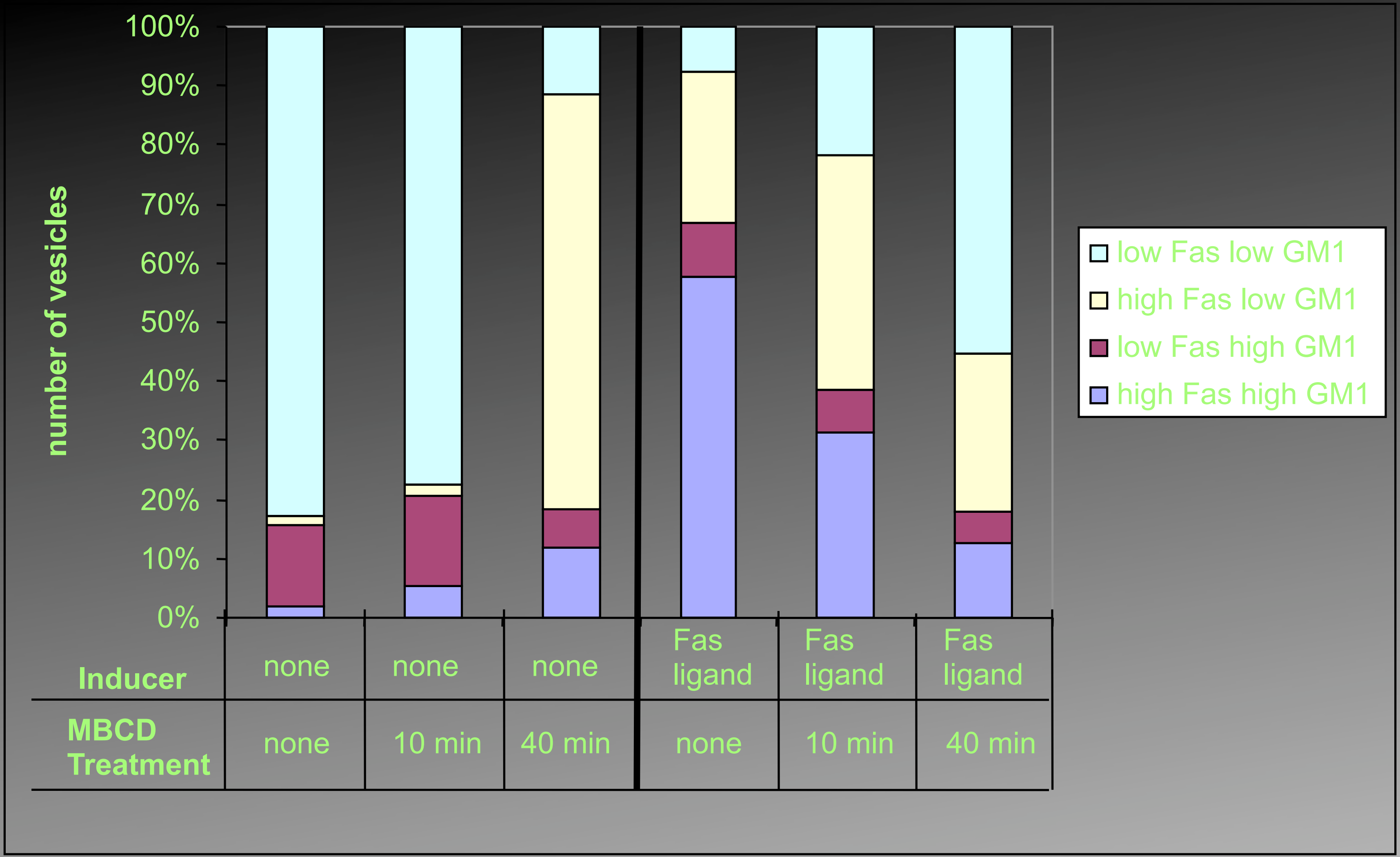
Native vesicles




100
90
80
70
60
50
40
30
20
10
0
Percent
none
FasL
Inducer
low GM1
high GM1



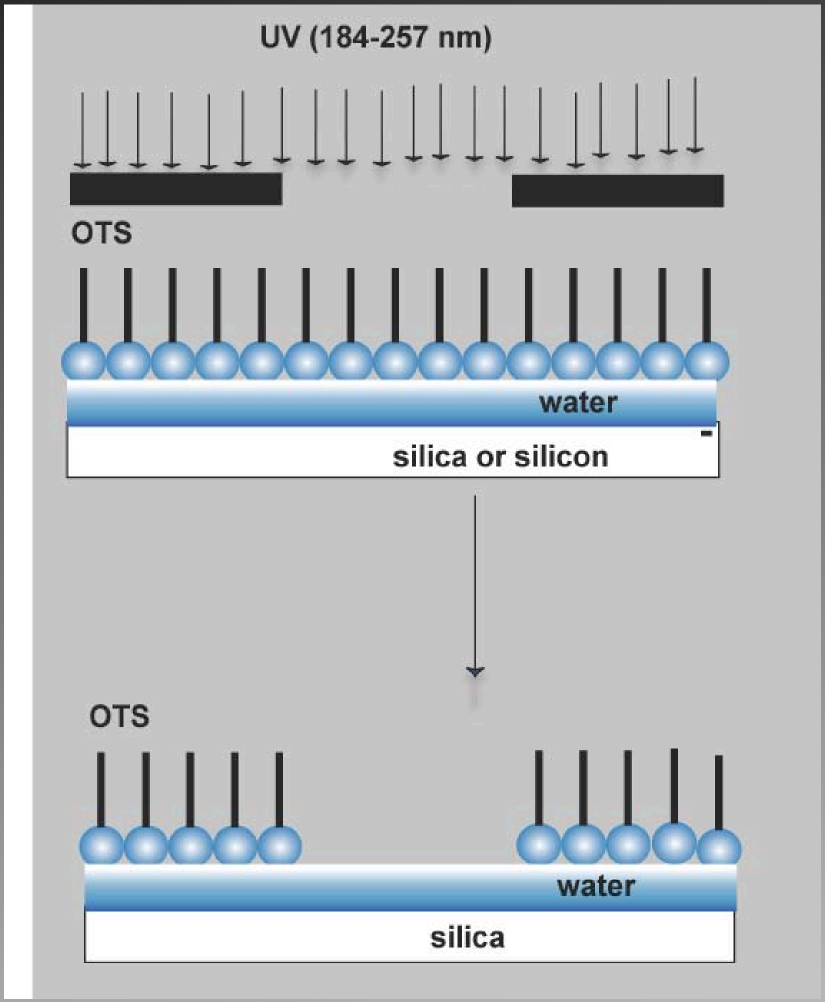
Fas receptor proteins are recruited to raft microenvironments following induction of apoptosis.

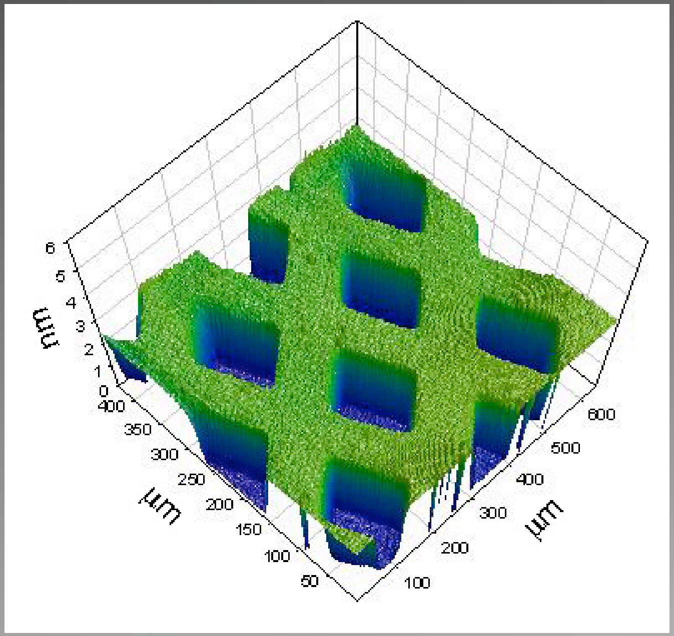


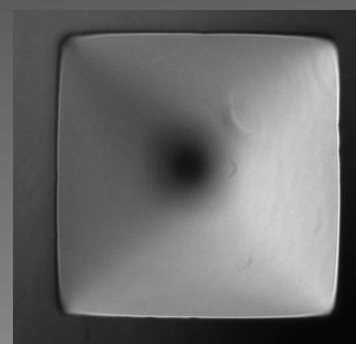
Raft formation is inhibited by depletion of cholesterol from RPE cell membranes.

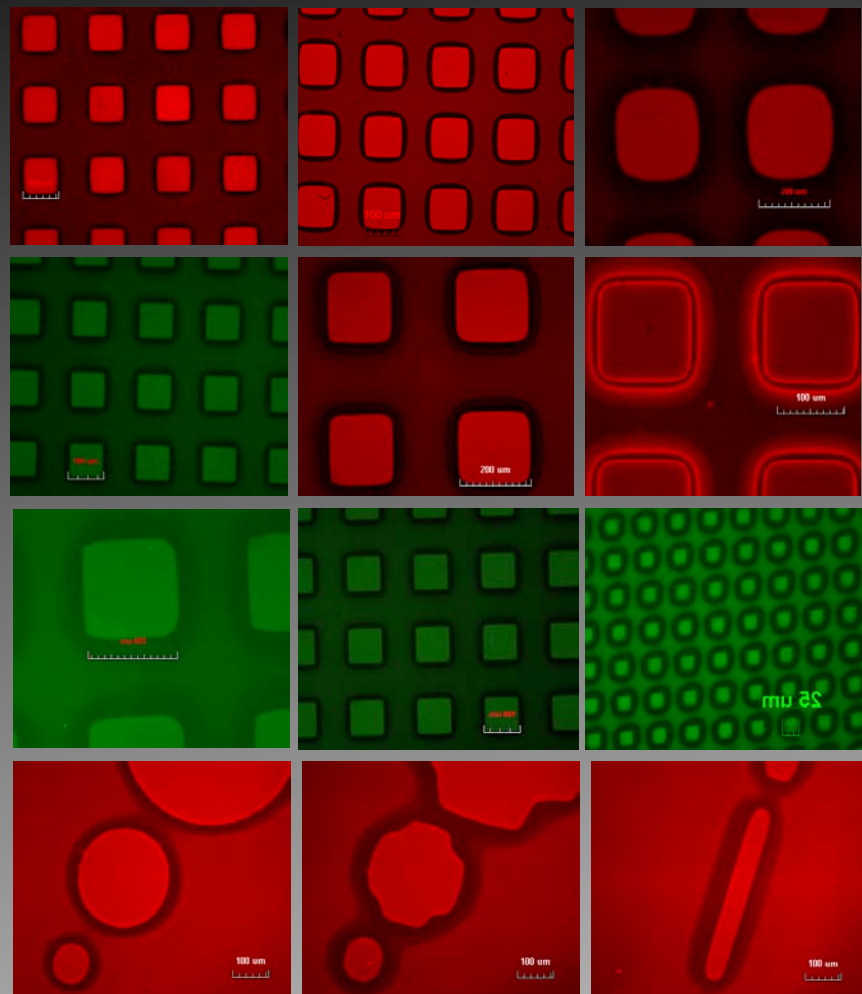
Templating membranes Using Structured Surfaces

Surface energy patterns Using Self-Assembled Monolayers









DLPC
DMPC
POPC

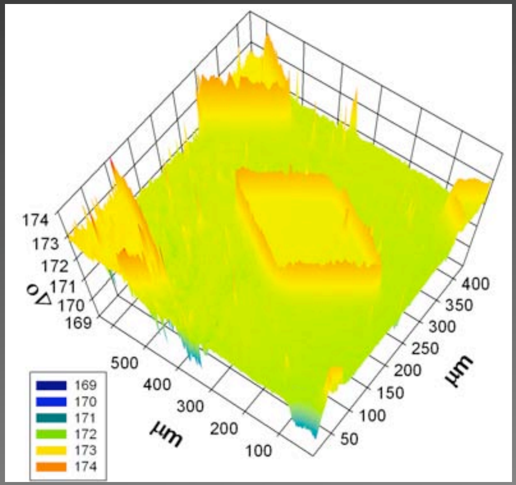
NBD
Rhodamine
Dil

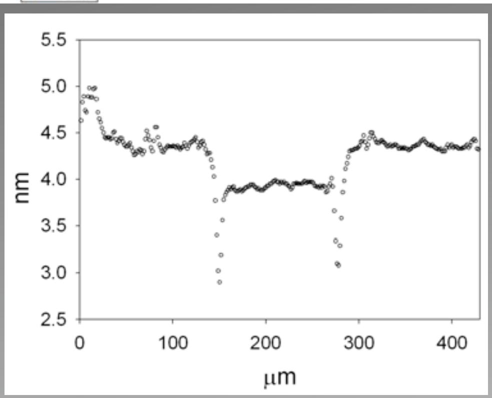
size

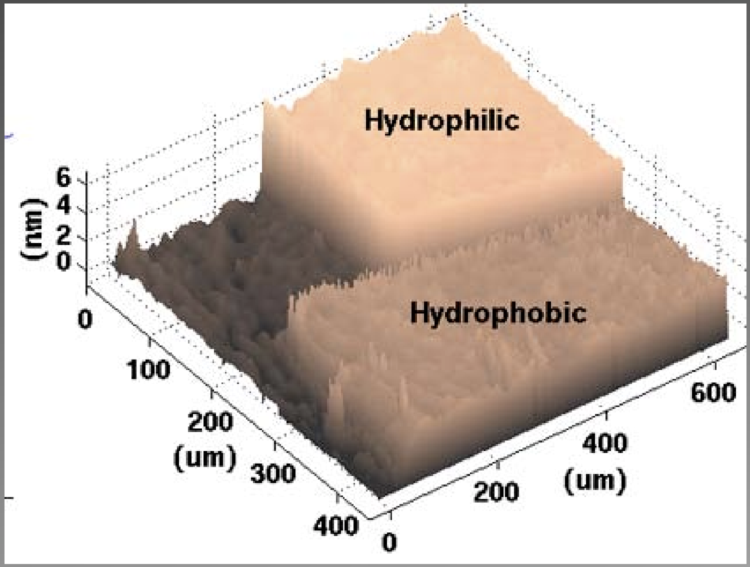
shape

(Howland, Butti, Dattelbaum, Shreve, Parikh, J. Amer. Chem. Soc. 2005)

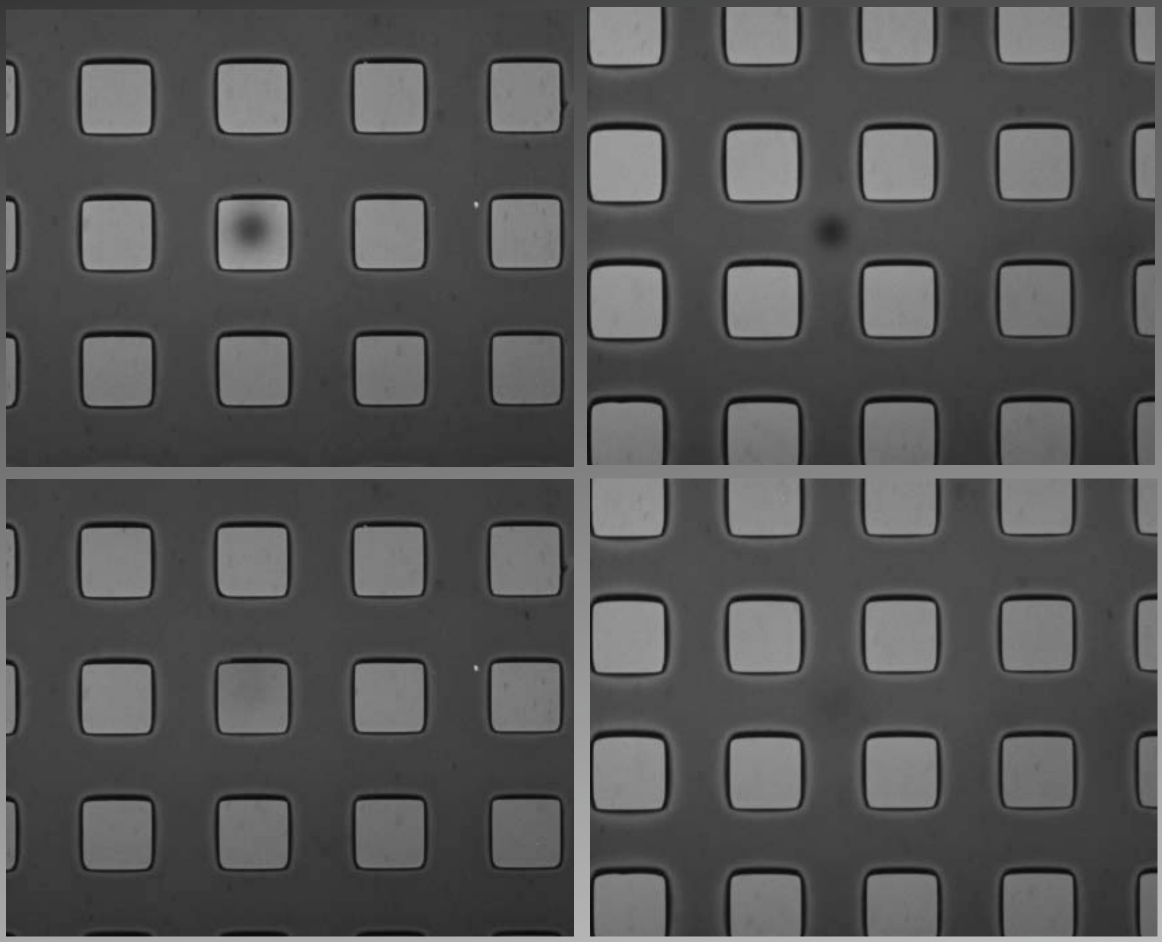
Imaging Ellipsometry confirms single monolayer and Bilayer formation



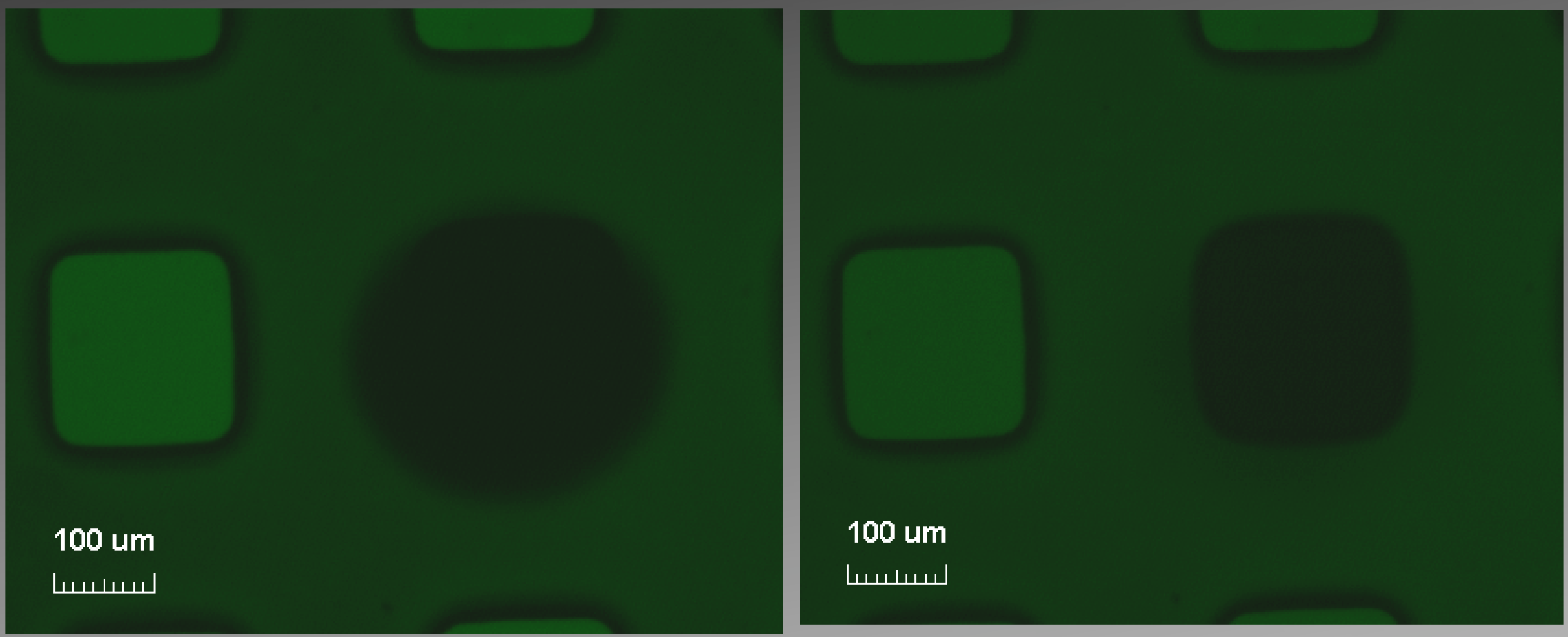




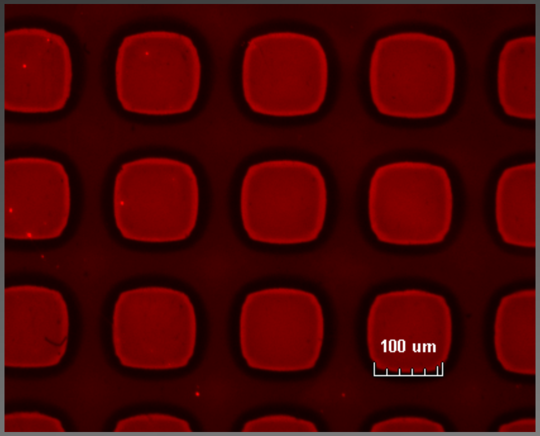
Both Mono- and Bilayer exhibit typical long-range fluidity

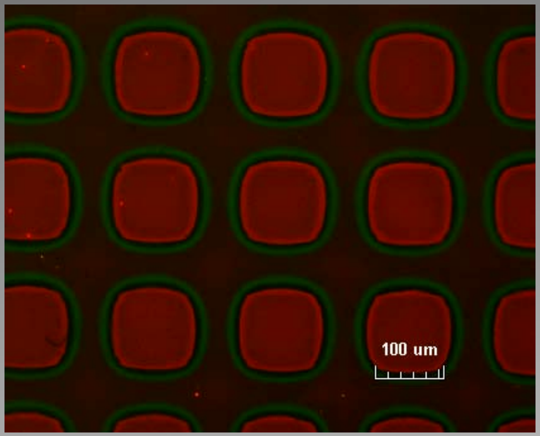


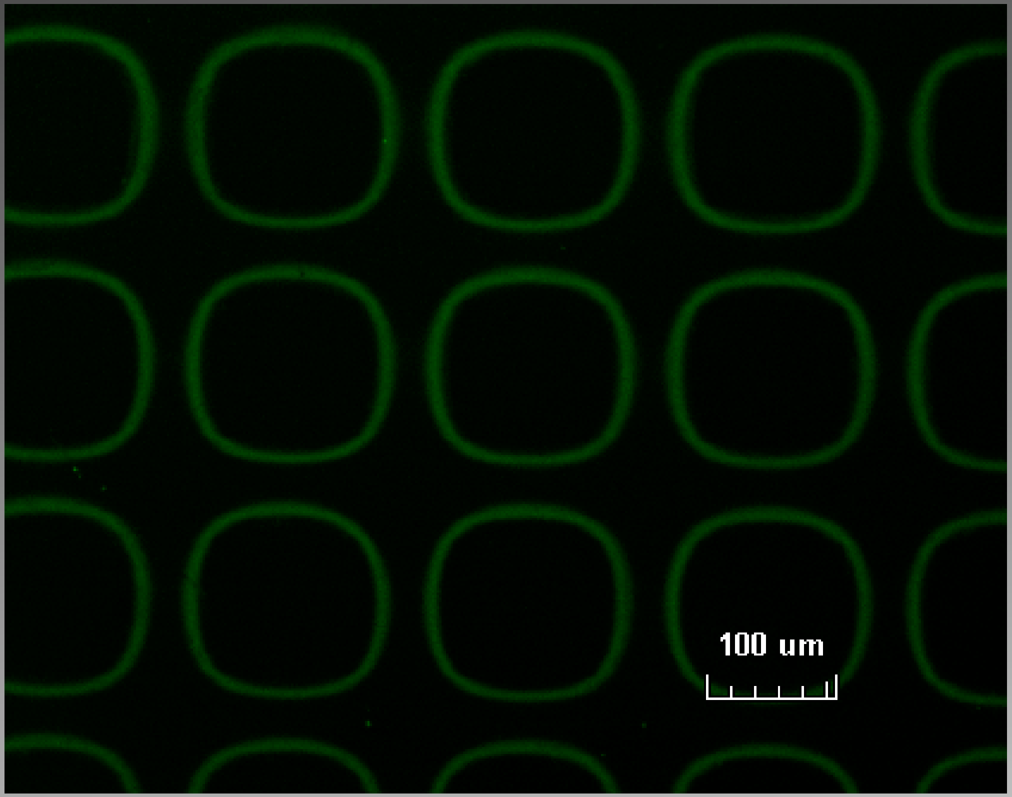
The two membrane fluids are disconnected



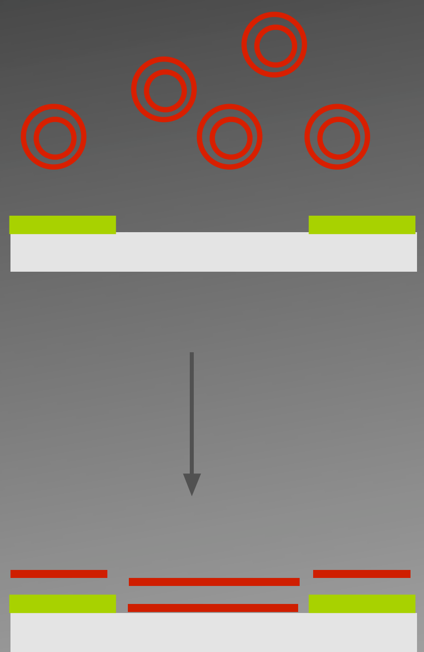
Protein patterns within membrane moats
(nanoscale dimensions using microscale masks)

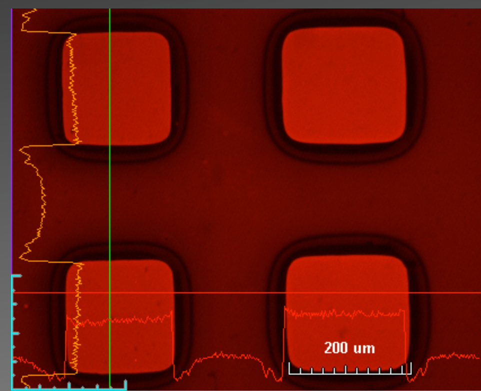


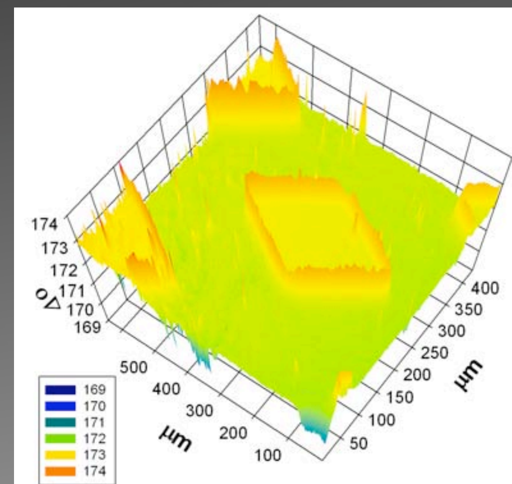


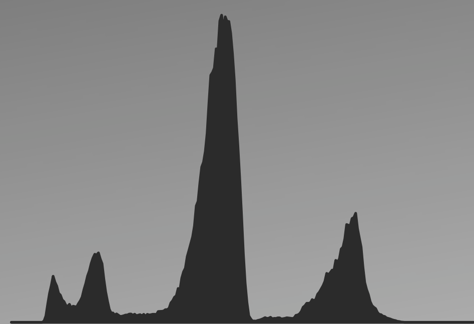


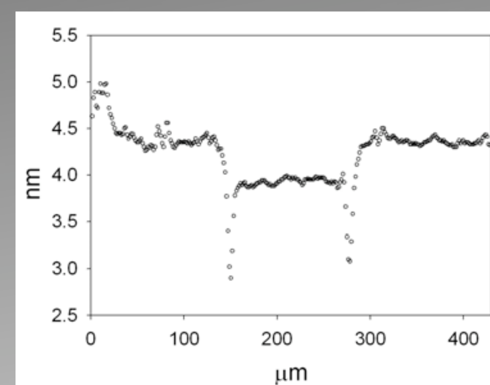
Membrane morphologies are templated by the Patterns of surface energy









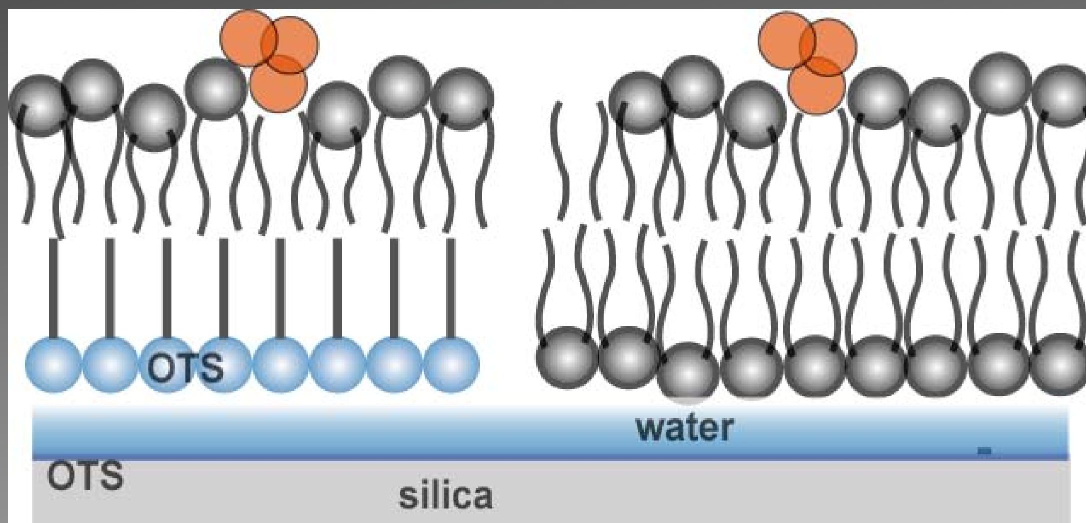


Howland et al, J. Amer. Chem. Soc., 2005

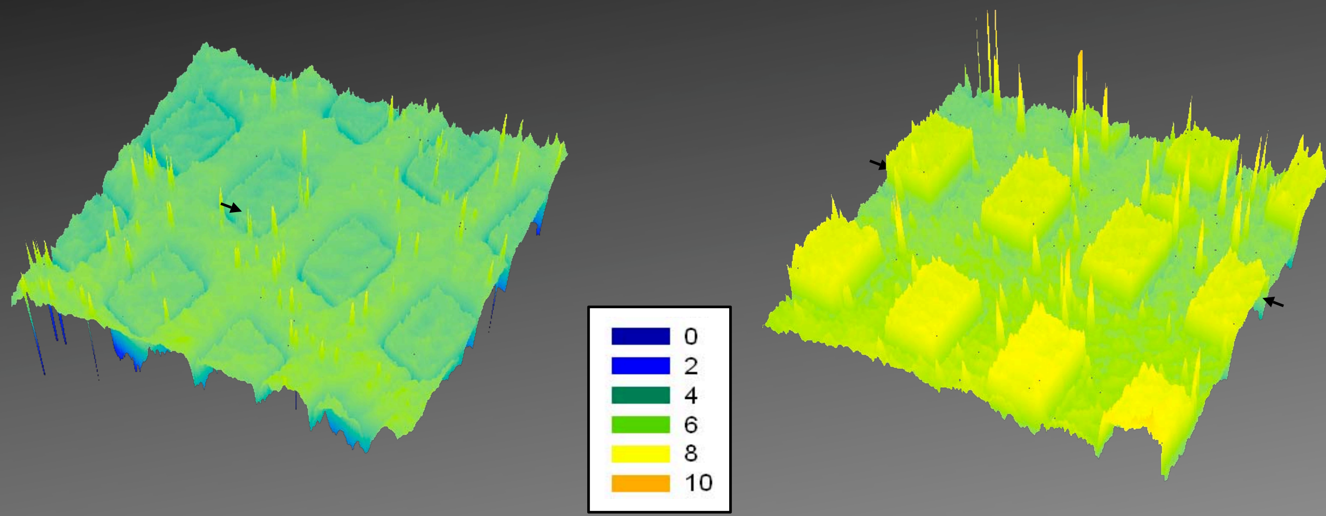
Asymmetric Distribution of Charged lipids

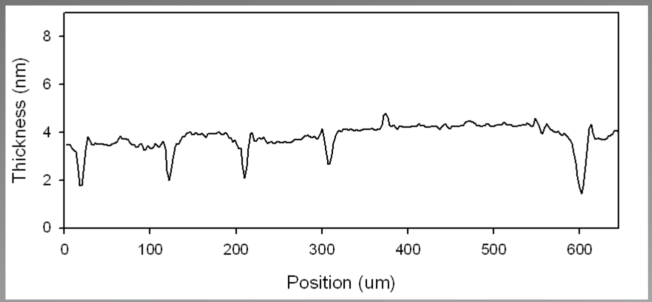
Negatively charged Texas-red and Gm1 lipids

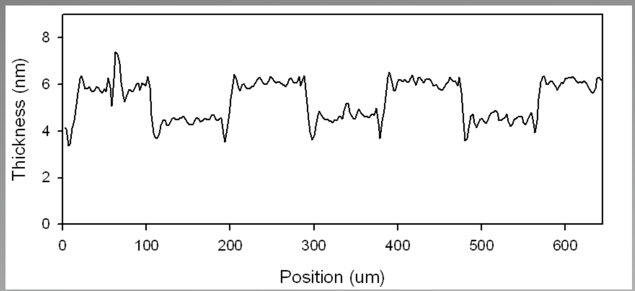
membrane asymmetry



Cholera toxin (FITC-CTB) binding to 1% Gm1 containing POPC membrane patterns

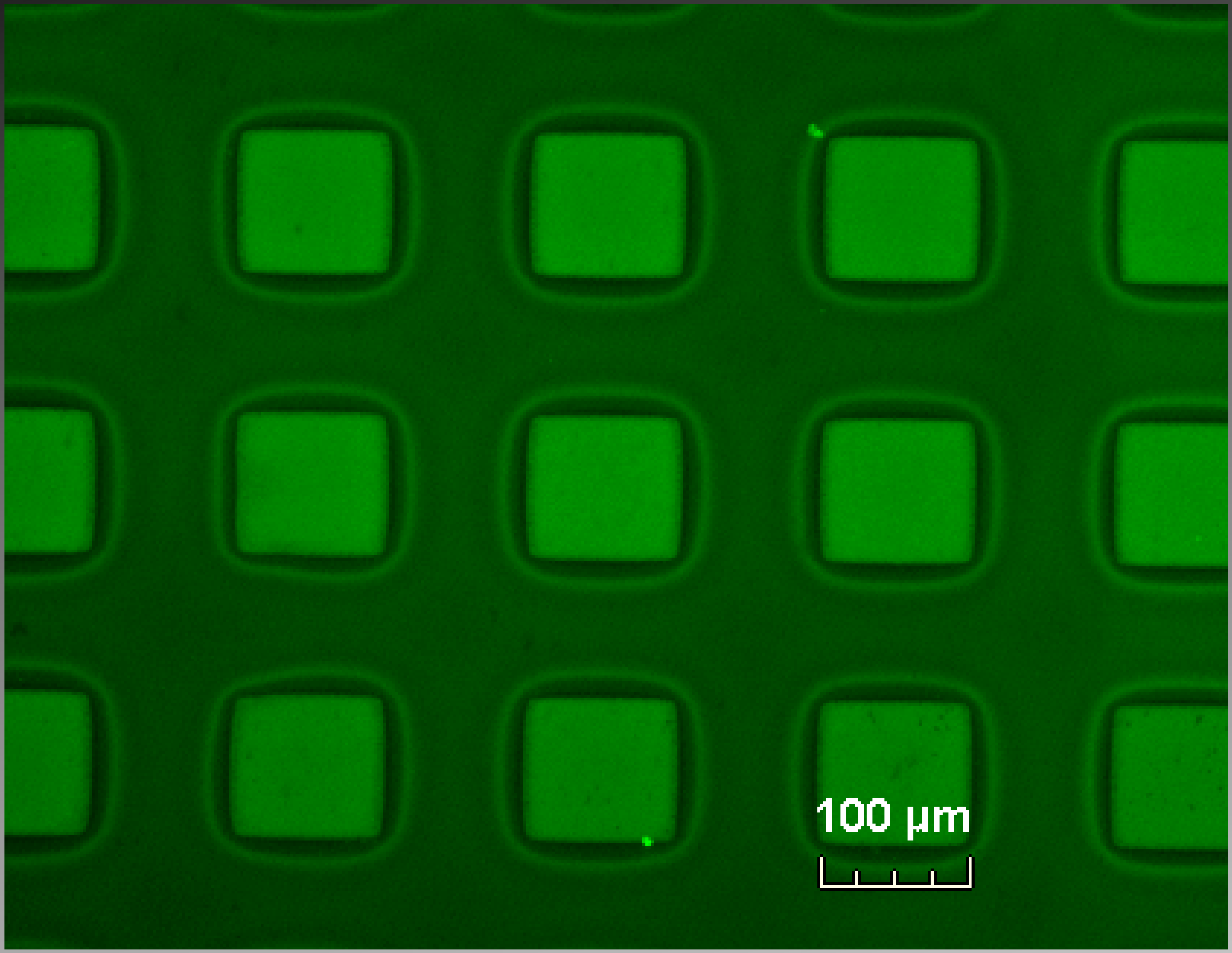






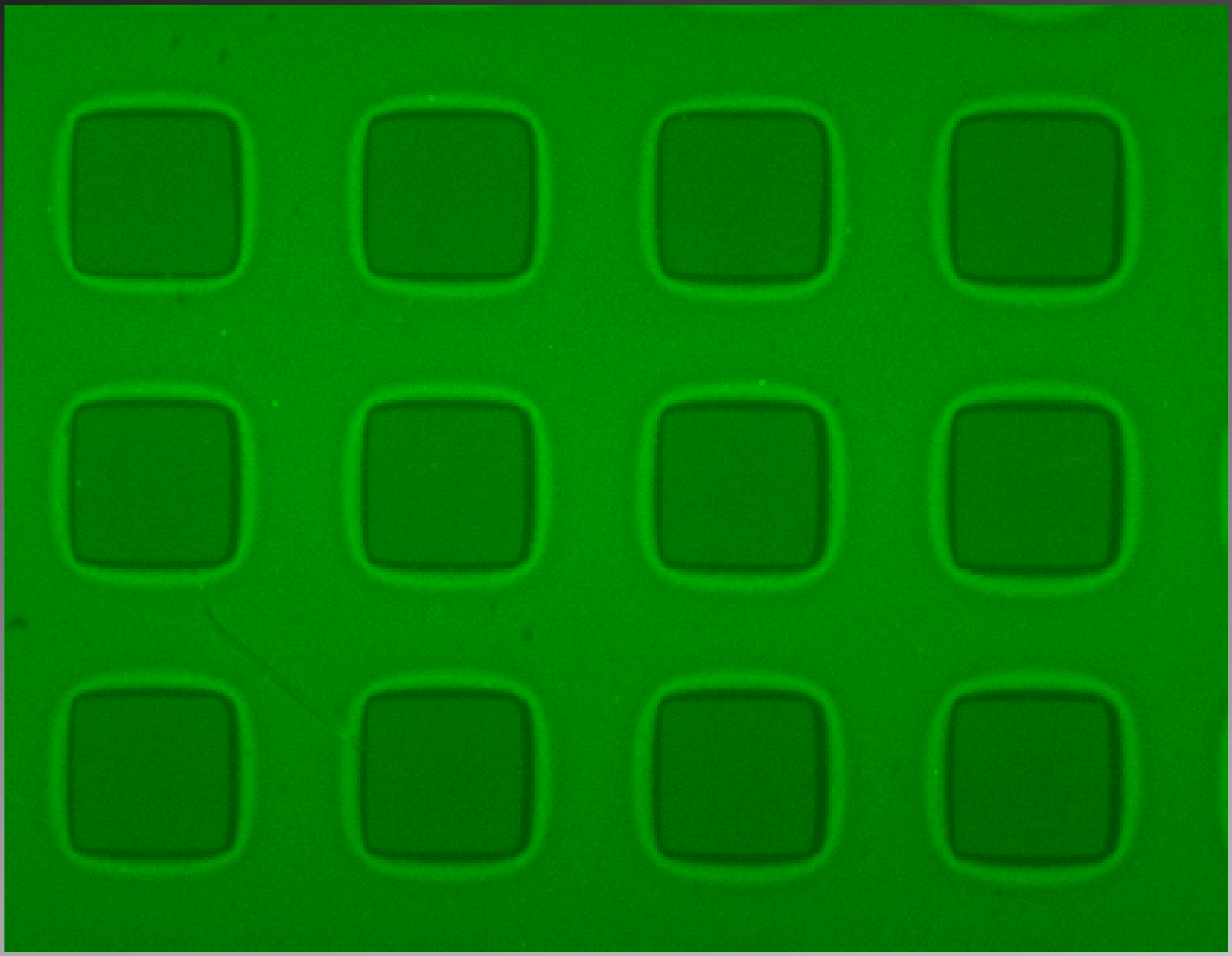
2 to 2.5 times higher binding in the bilayer region

FITC-CTB fluorescence pattern



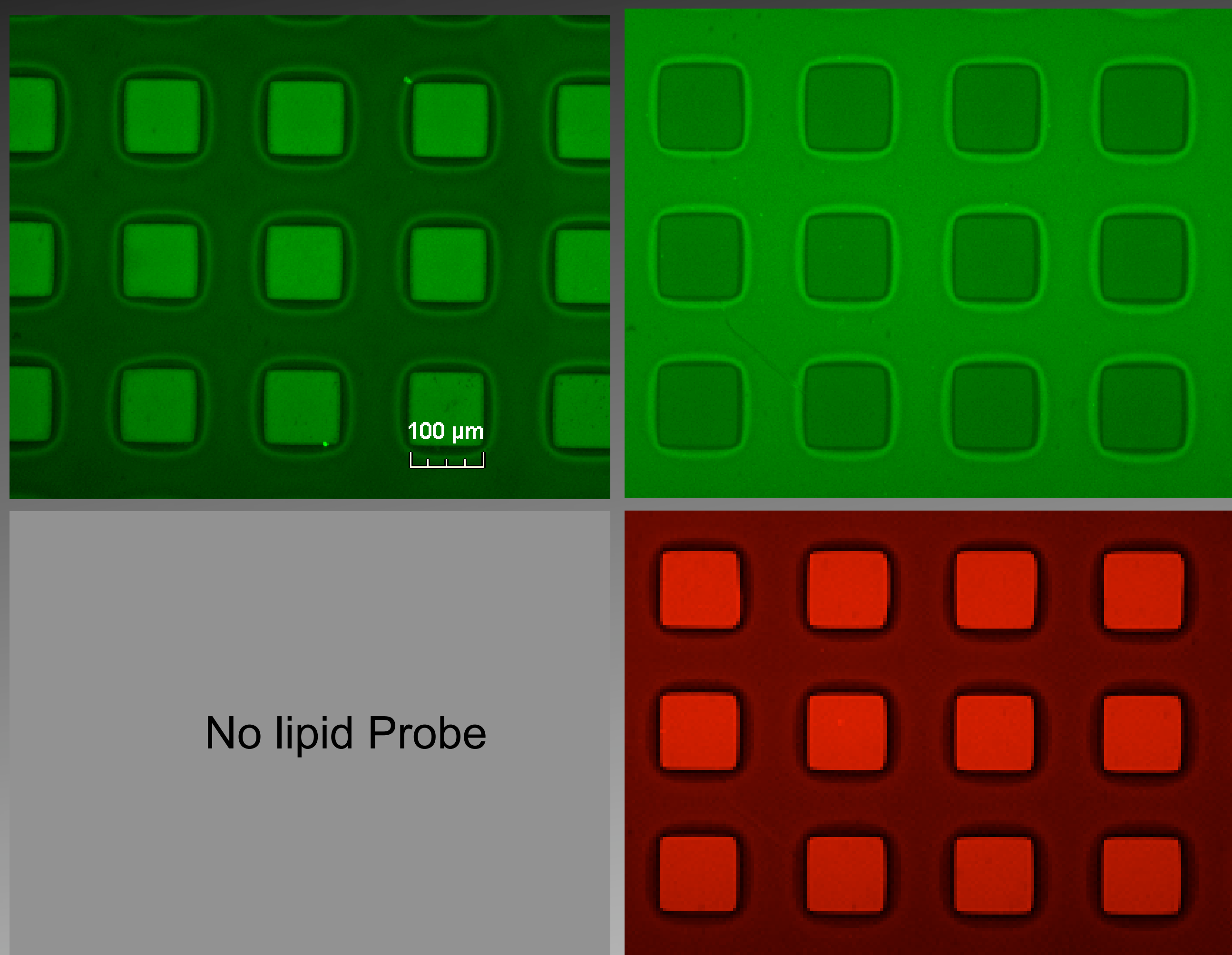
1 % Gm1 and 99% POPC

FITC-CTB fluorescence pattern

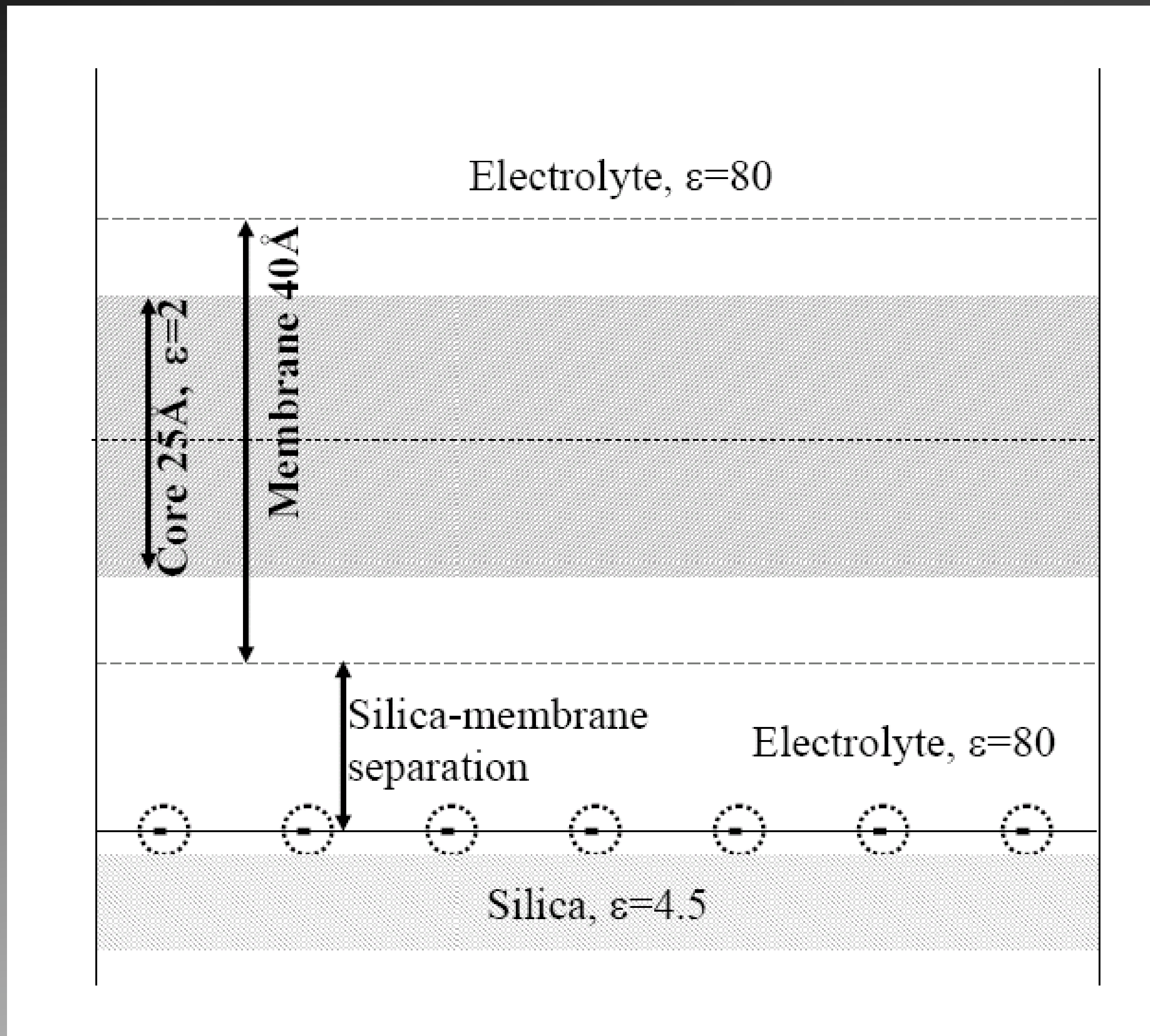


1 % Gm1, 1% Texas-red DHPE, 98% POPC

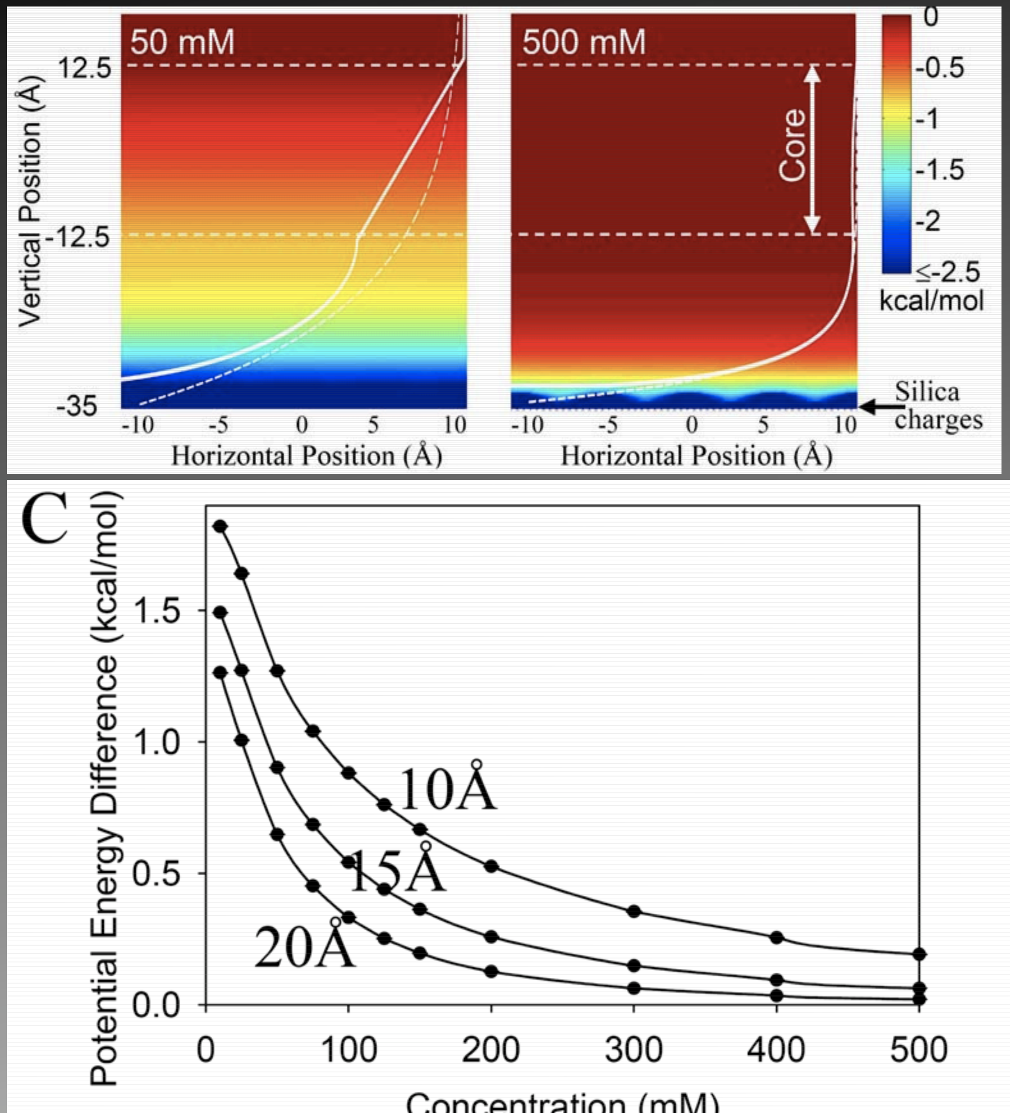
The contrast reversal suggests a significant enhancement Of Texas-red probe in the distal leaflet



Electrostatic considerations



Collaboration: Toby W. Allen



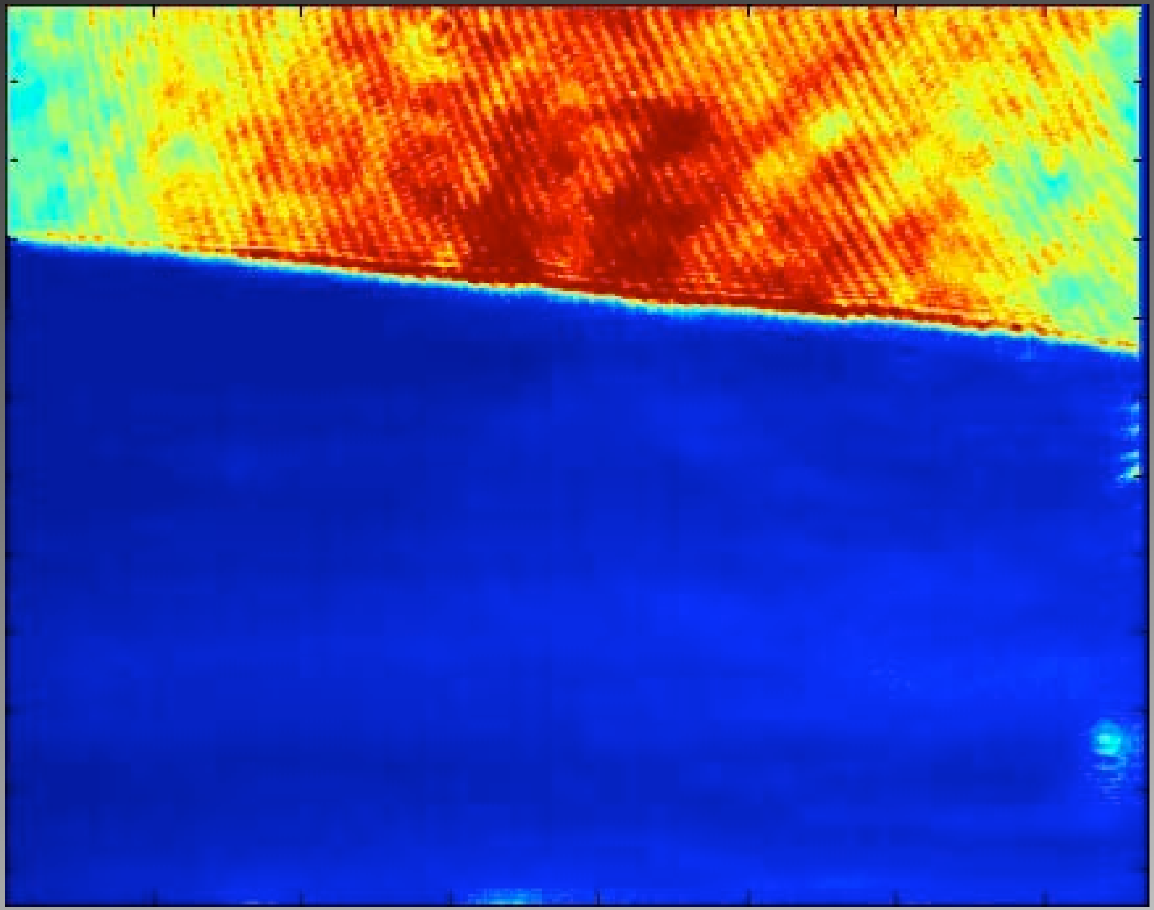
Collaboration: Toby W. Allen

Lipid spreading dynamics

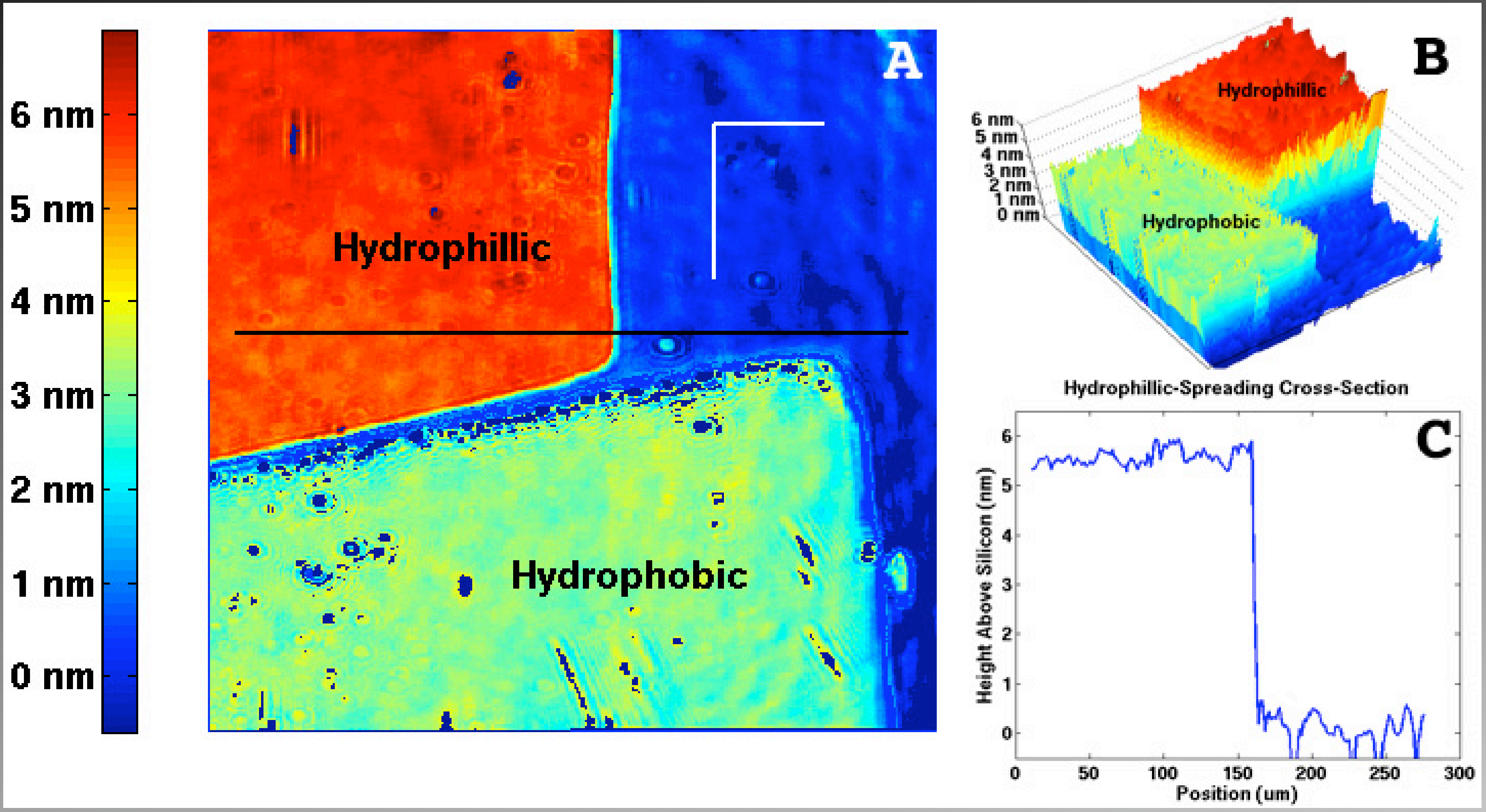
Surface energy patterns

spreading

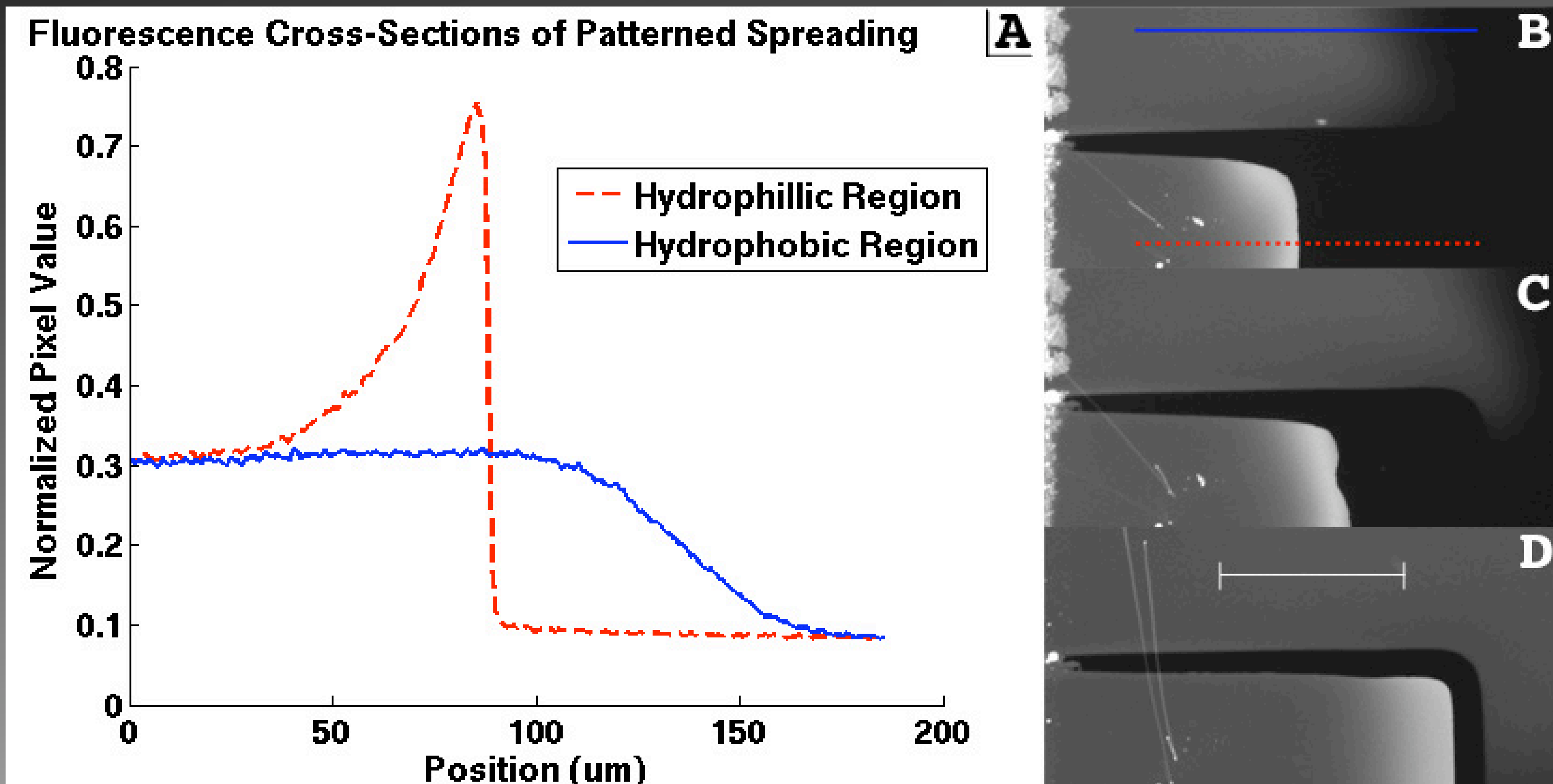
the thickness of the spreading foot



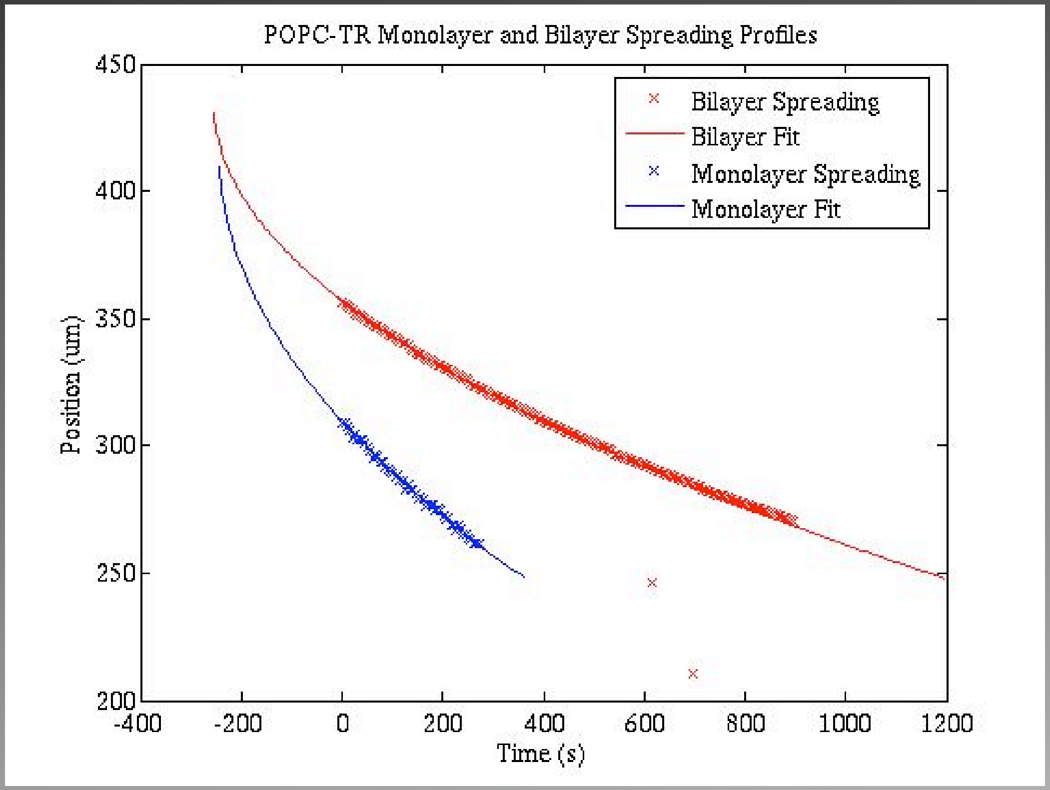
Ellipsometric confirmation of the spreading membrane morphologies



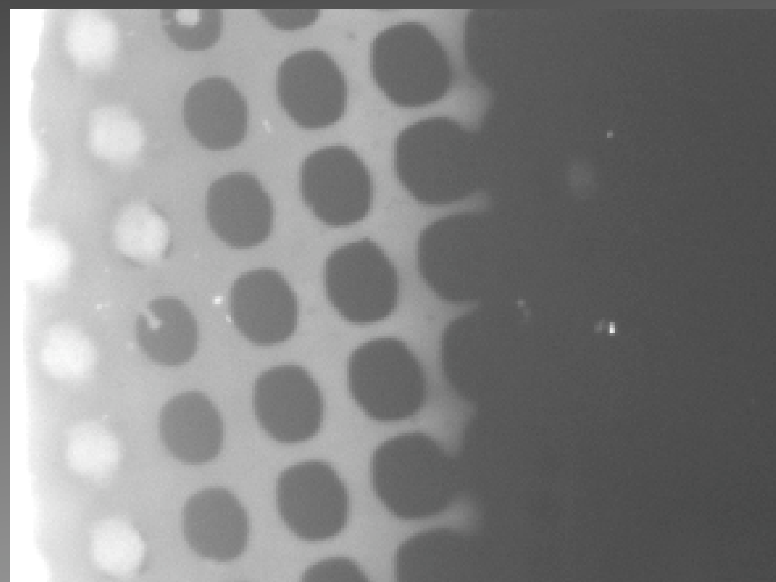
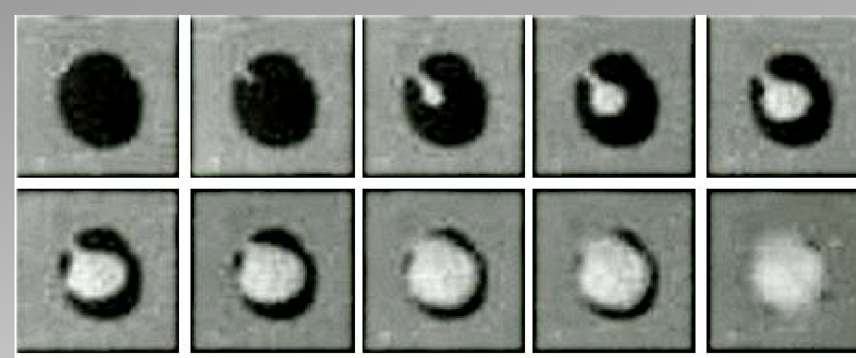
spreading fronts are tense



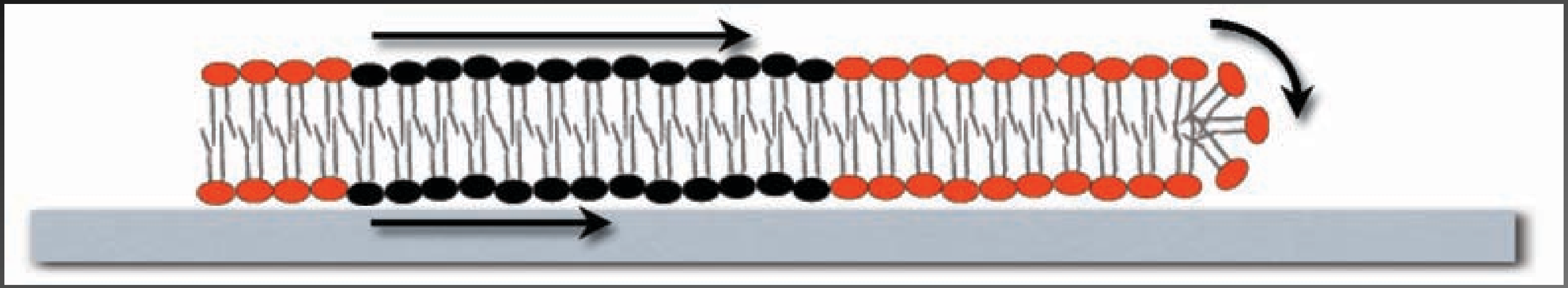
Monolayer spreads faster than the bilayer both spread with square root of time kinetics

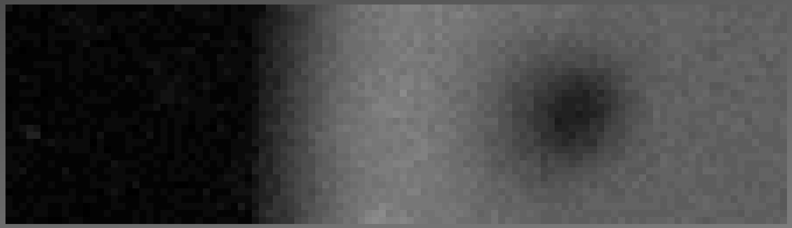


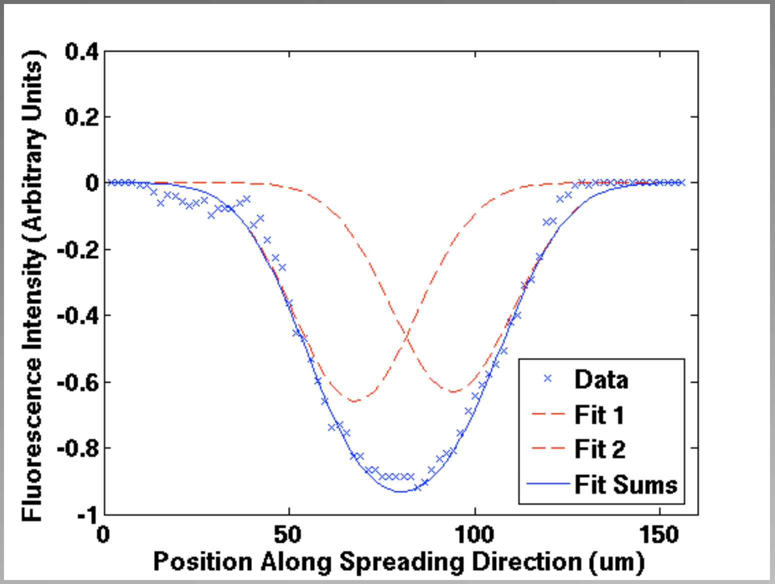
B. Sanii and A. N. Parikh, Soft Matter, in press (2007)

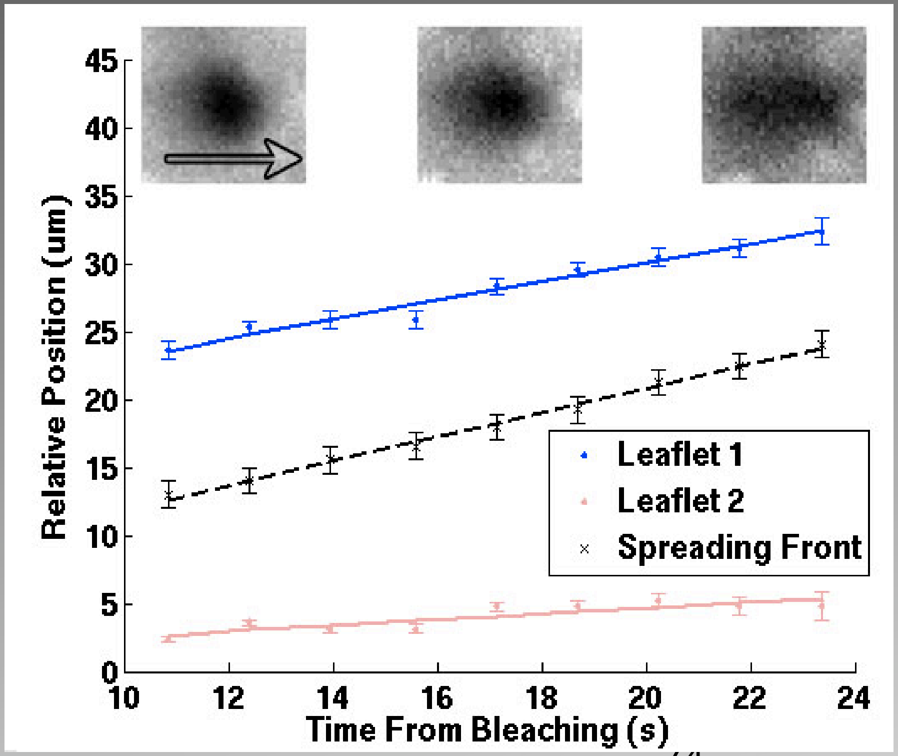



FRAP-ping a Spreading Bilayer



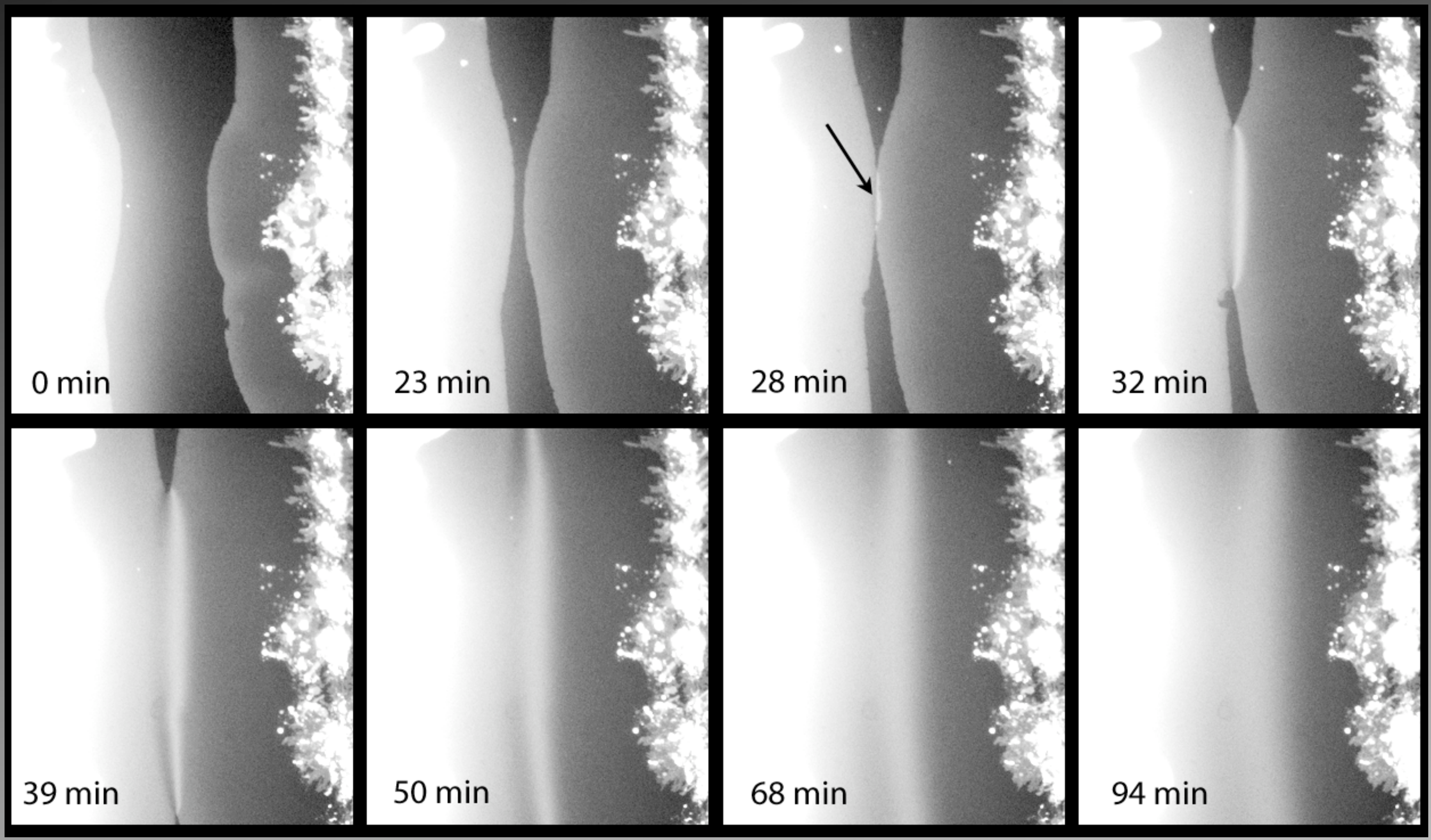




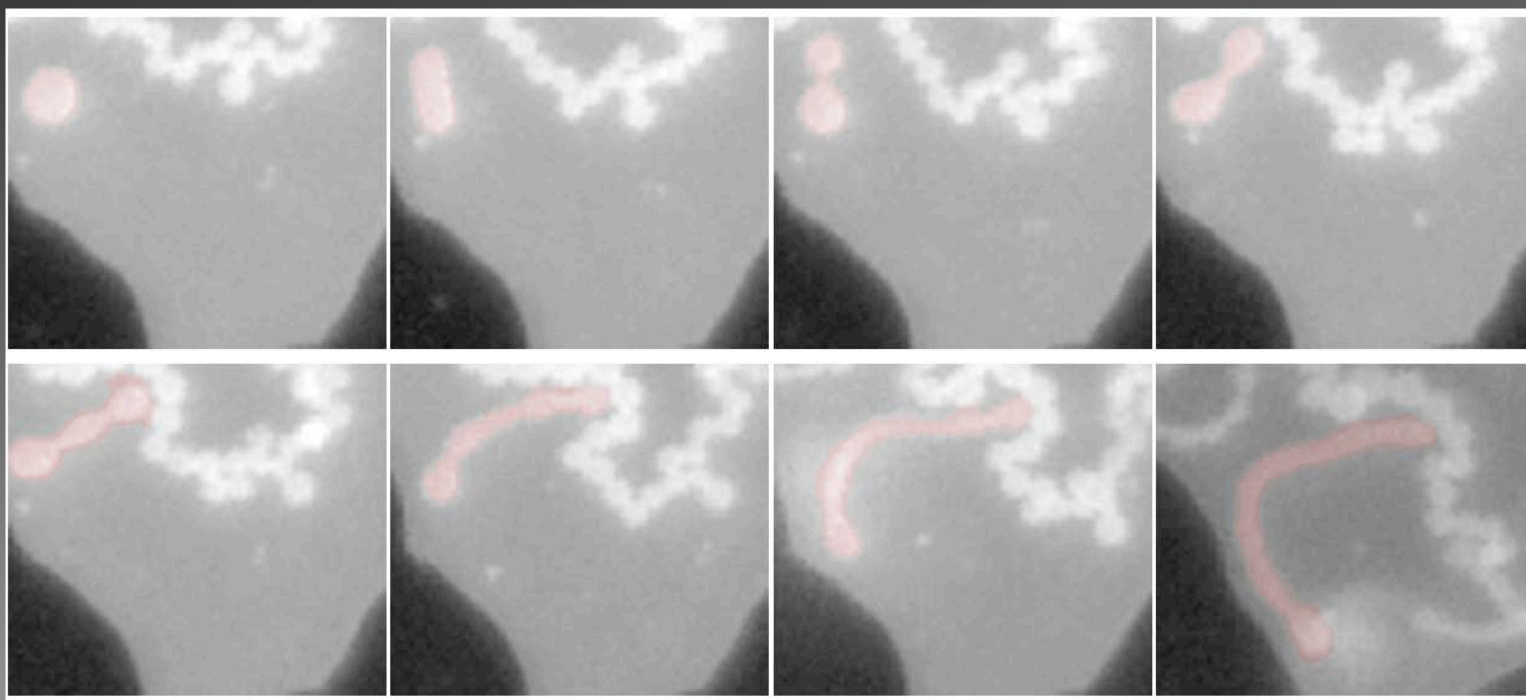


Bilayer Collisions

FRET Channel



Building Blocks of life



Corrugated surfaces

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