Laser Scissors and Tweezers: A Cell Biologist's Photonic Toolbox

Michael W. Berns

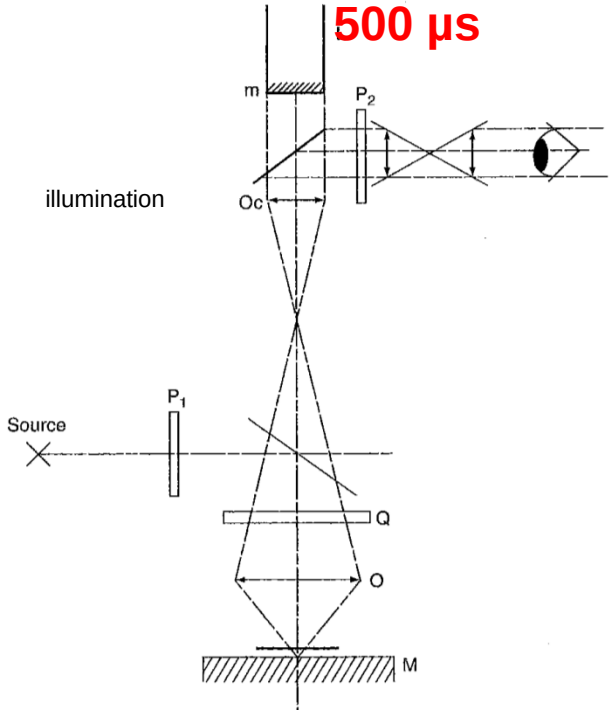
1st Laser microbeam was built by Marcel Bessis and Georges Nomarsky 1962

Marcel Bessis
(1917-1994)

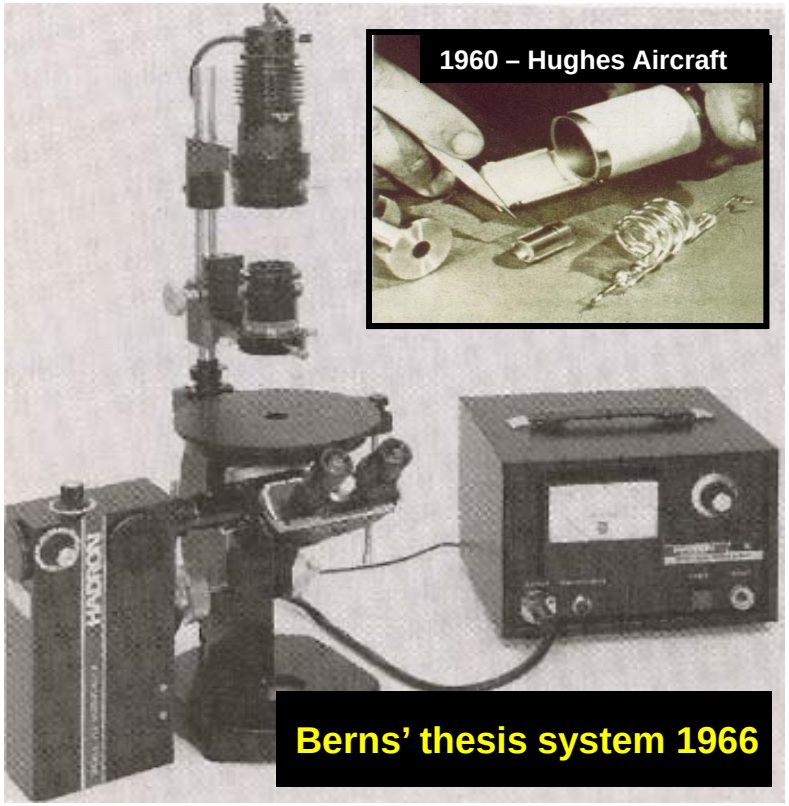
Georges Nomarski
(1919-1997)

Ruby Laser

500 µs

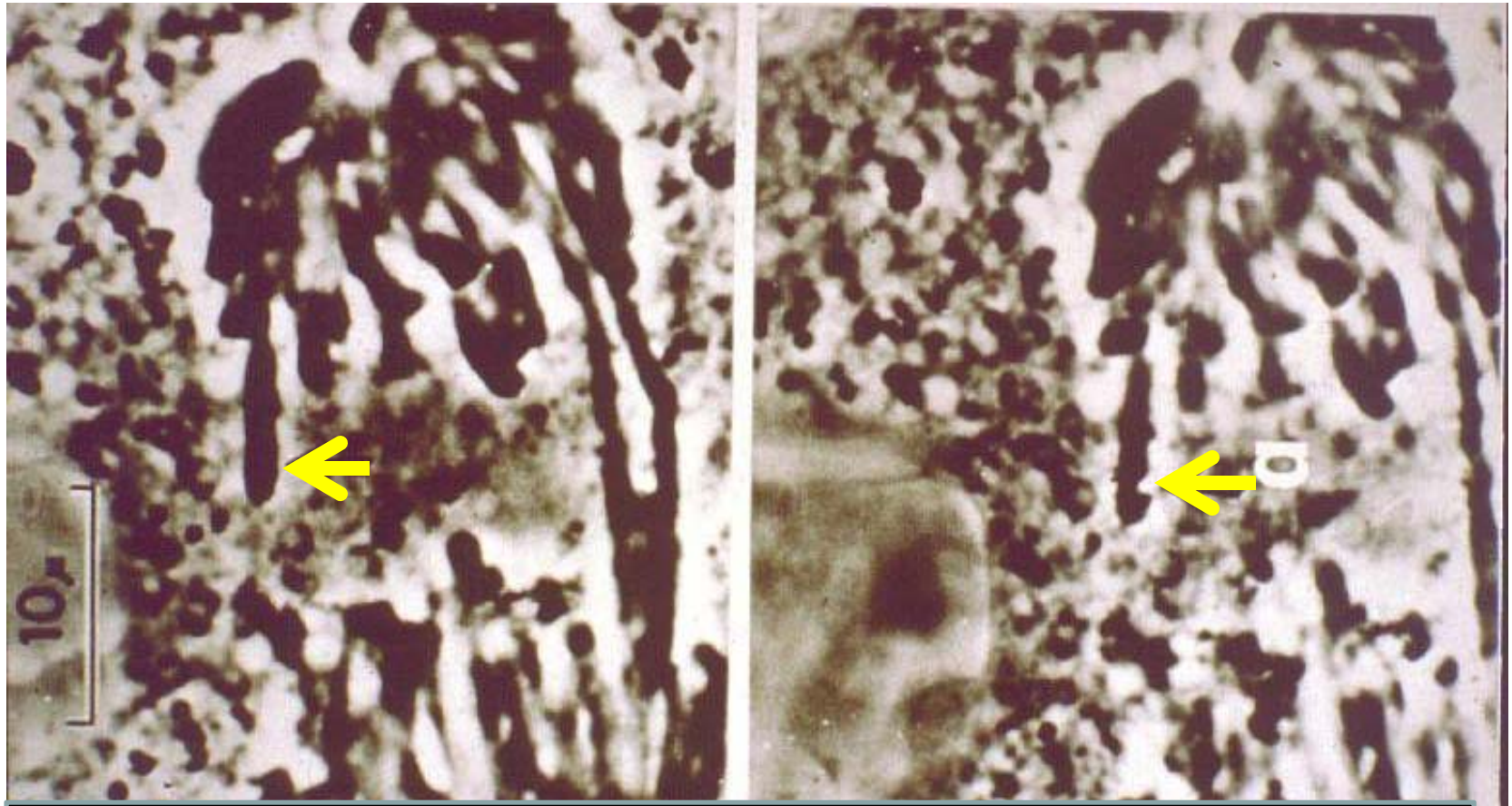


C. R. Acad,. Sci **225**:1010-1012. 1962

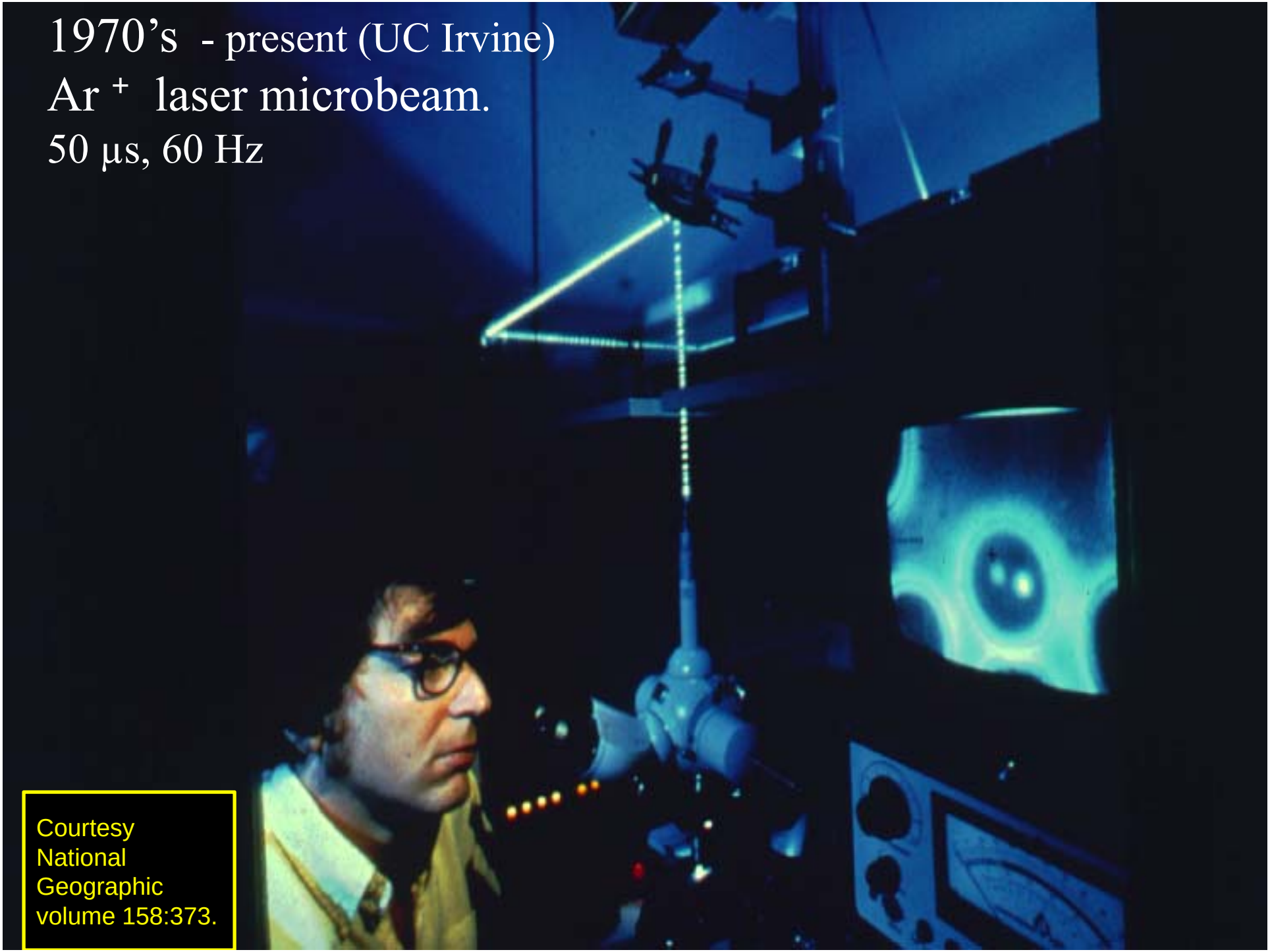


Became a Cell Biologist

Berns, M., Olson R., D. Rounds, (1969) *Nature* 221: 74-75.
Cell Surgery by Laser: *Sci. Amer*. (1970)



Removed 0.5 μm piece of a chromosome in live epithelial lung cell (salamander)


1970's - present (UC Irvine)
Ar + laser microbeam.
50 μs, 60 Hz
Courtesy National Geographic volume 158:373.

1971

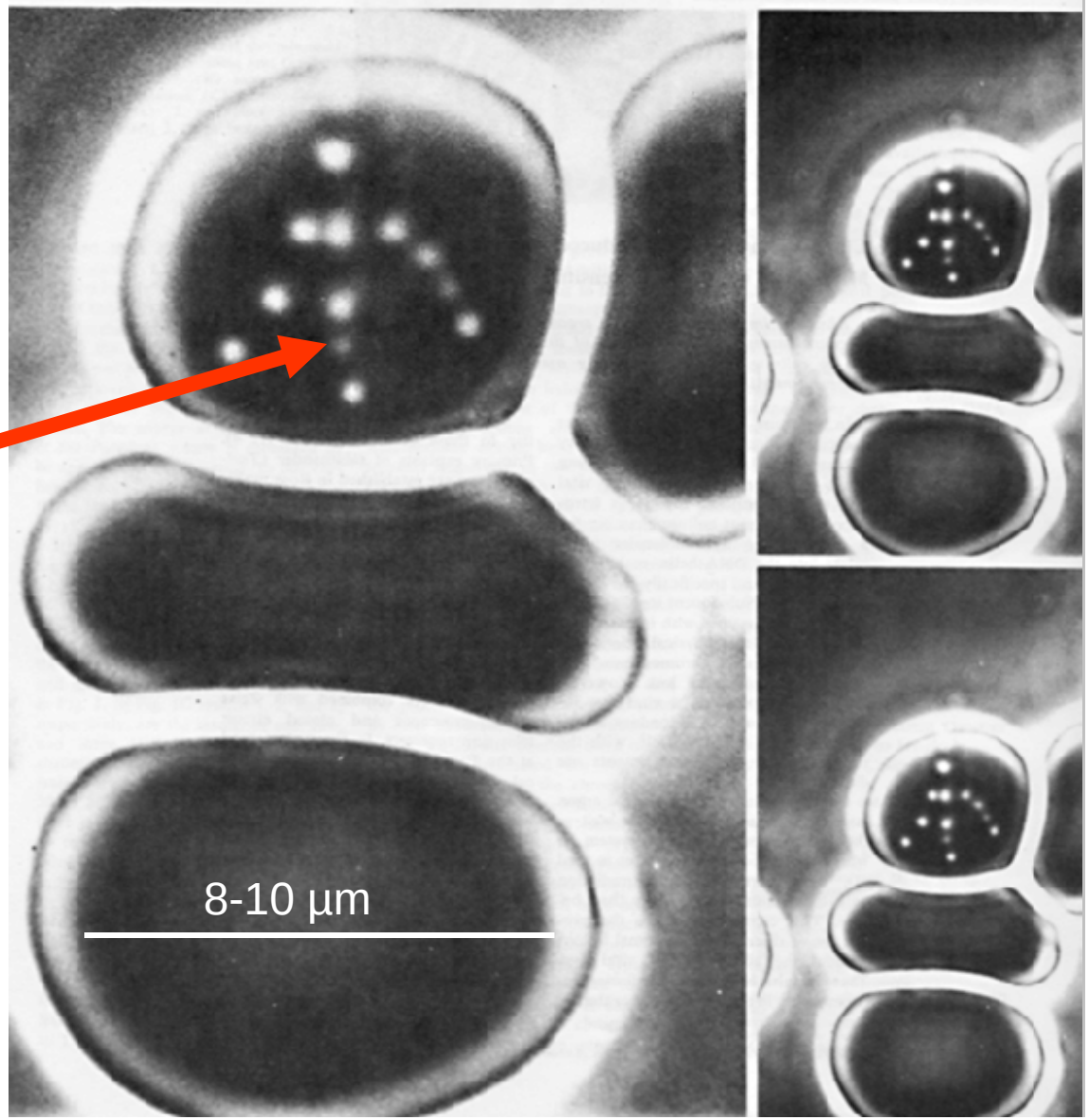
Reprinted from
5 March 1971, Volume 171, pp. 903-905

SCIENCE

Chromosome Lesions Produced with an Argon Laser Microbeam without Dye Sensitization

Michael W. Berns, Wanny K. Cheng, Alton D. Floyd and Yasushi Ohnuki

Argon ion laser
µsec. pulses
0.5 – 1µm lesion

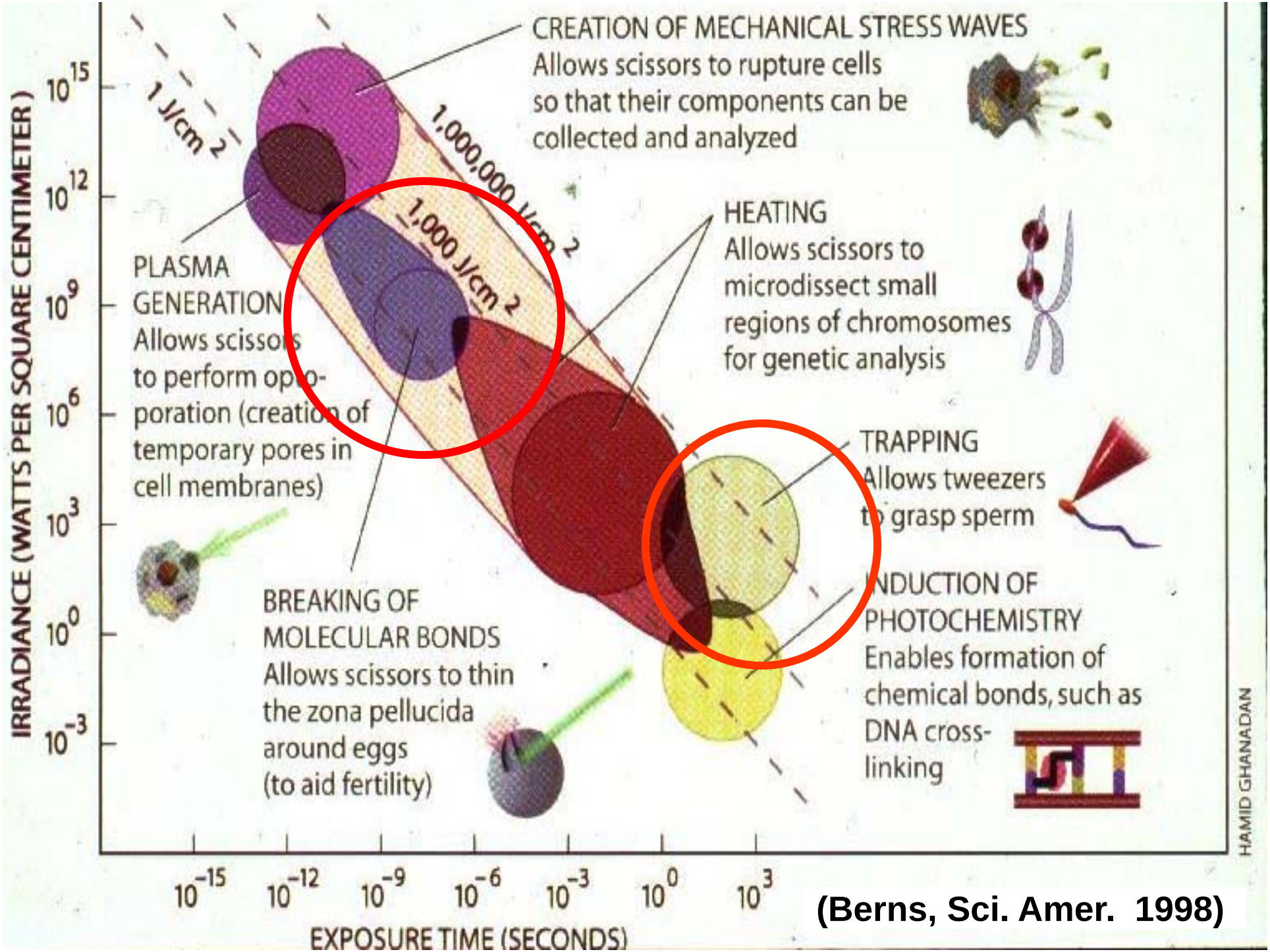


1980's

- **MODE-LOCKED HIGH POWER LASERS**
  - **high irradiances (no dyes needed)**
  - **multiphoton events (2-3 photon absorption)**
  - **microplasmas & shockwaves**

(see papers by Alfred Vogel and Vasan Venugopalan)

- **DIGITAL IMAGE PROCESSING**
  - **cooled ccd's (sensitivity)**
  - **speed (20fps)**



(Berns, Sci. Amer. 1998)

1980's

- **MODE-LOCKED HIGH POWER LASERS**
  - **high irradiances (no dyes needed)**
  - **multiphoton events (2-3 photon absorption)**
  - **microplasmas & shockwaves**

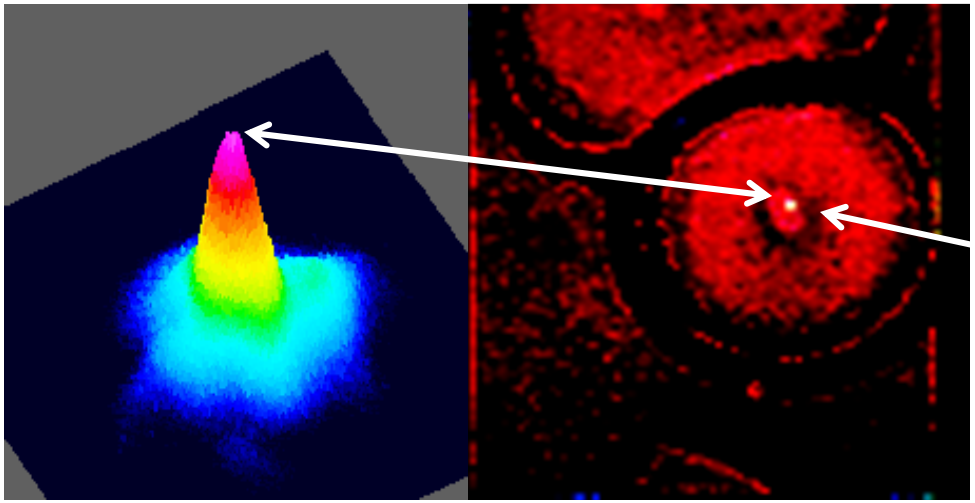
- **DIGITAL IMAGE PROCESSING**
  - **cooled ccd's (sensitivity)**
  - **speed (20fps)**

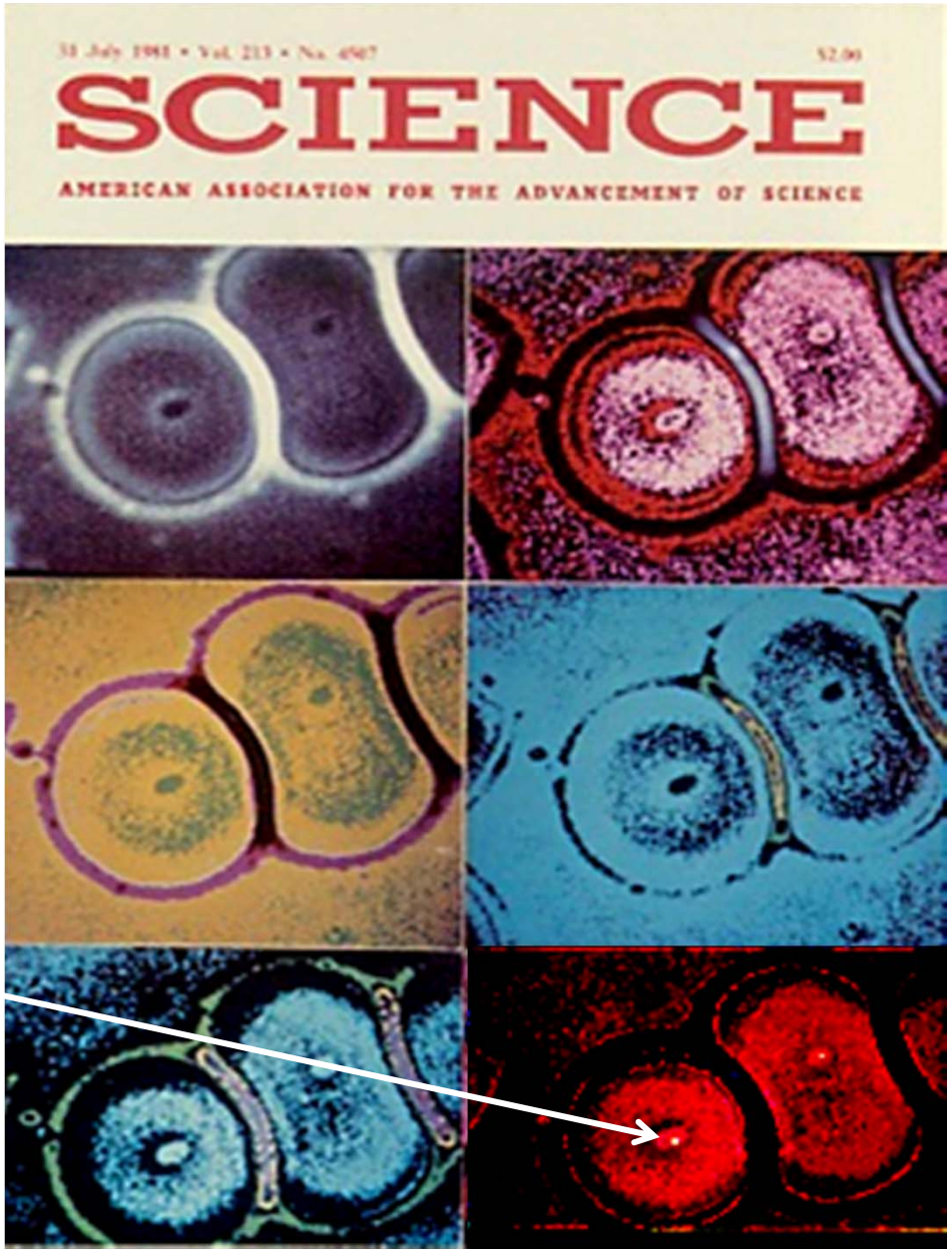
LANDSAT DIGITAL IMAGING APPLIED TO MICROSCOPY

1981

- edge detection
- contrast enhancement
- feature extraction
- “If we can see it we can hit it.”

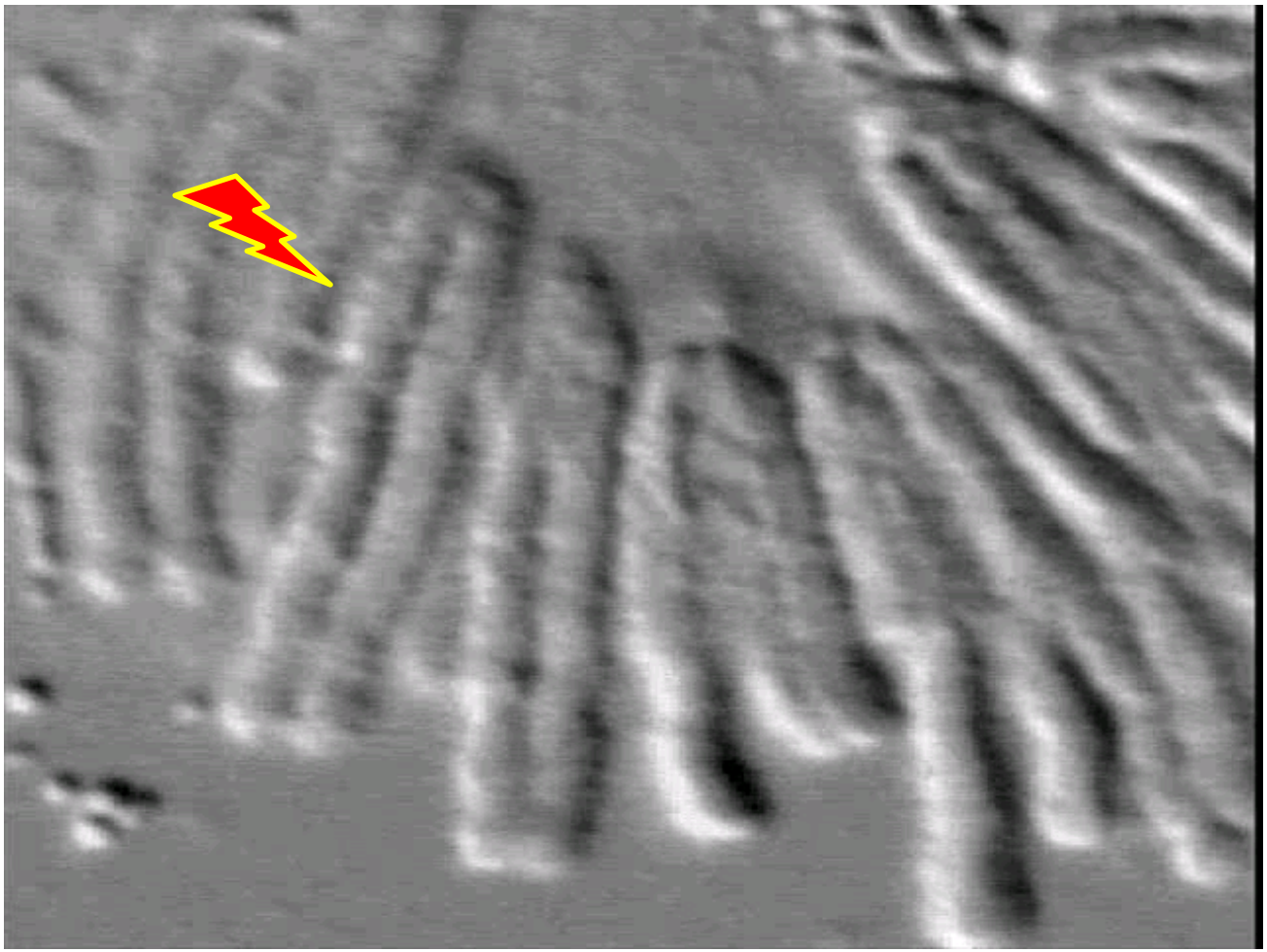
“tip of the Gaussian” = 0.20 μm





Can slice a chromosome like a loaf of bread.

7 ns 532 Nd:YAG: Courtesy of Conly Rieder, SUNY Albany:



4 µm

Can we do genetic surgery ?

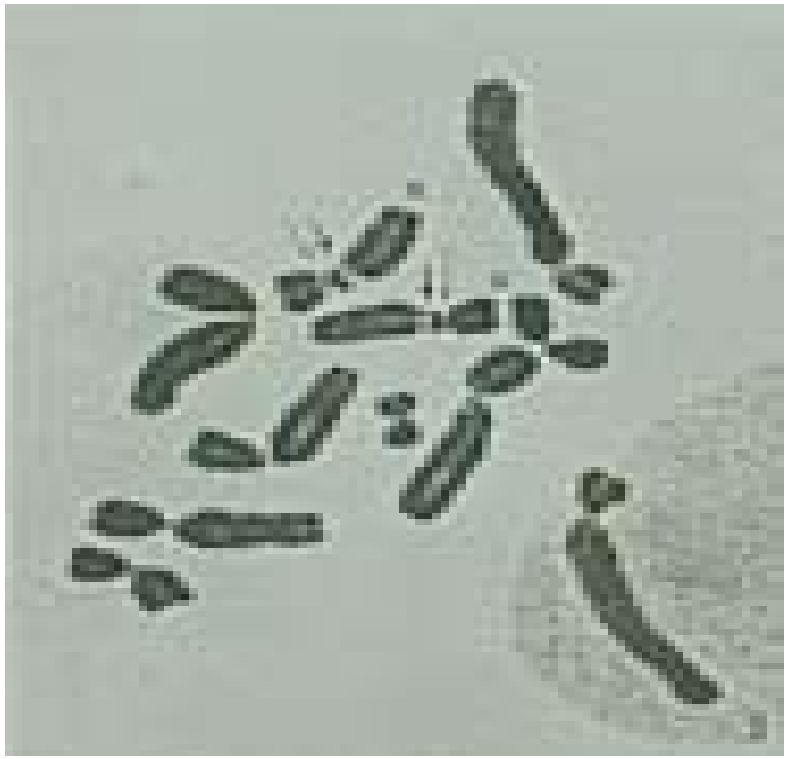
Take genes out ?

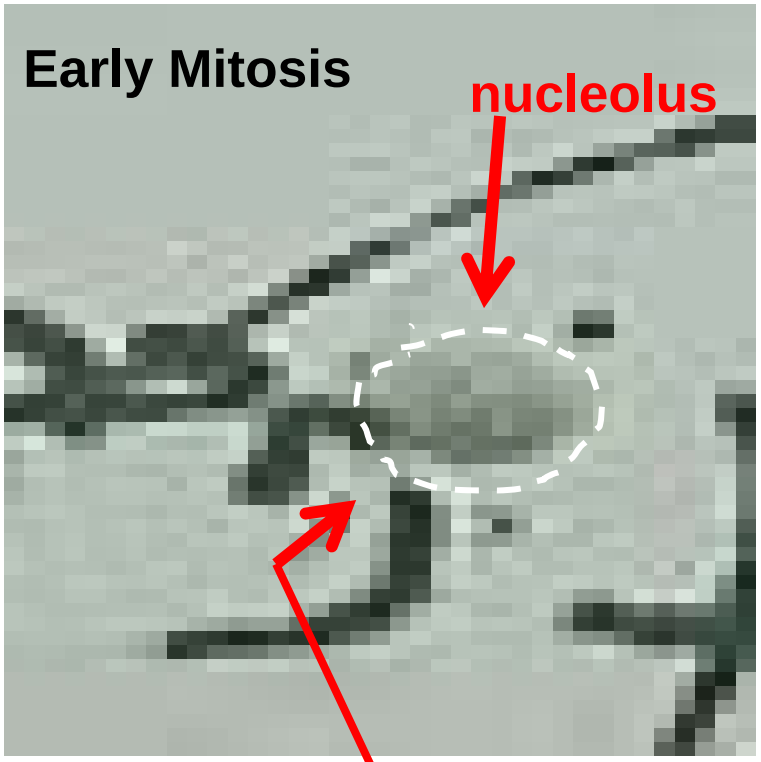
Put genes in ? (won't talk about)

Need the right model system.

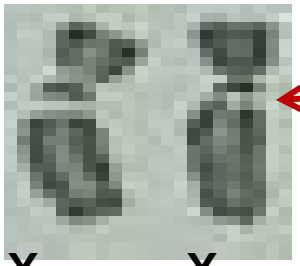
Tasmanian rat kangaroo alias the "long nose potoroo."

Nucleolus genes are on X chromosomes. Their location is visible during mitosis.

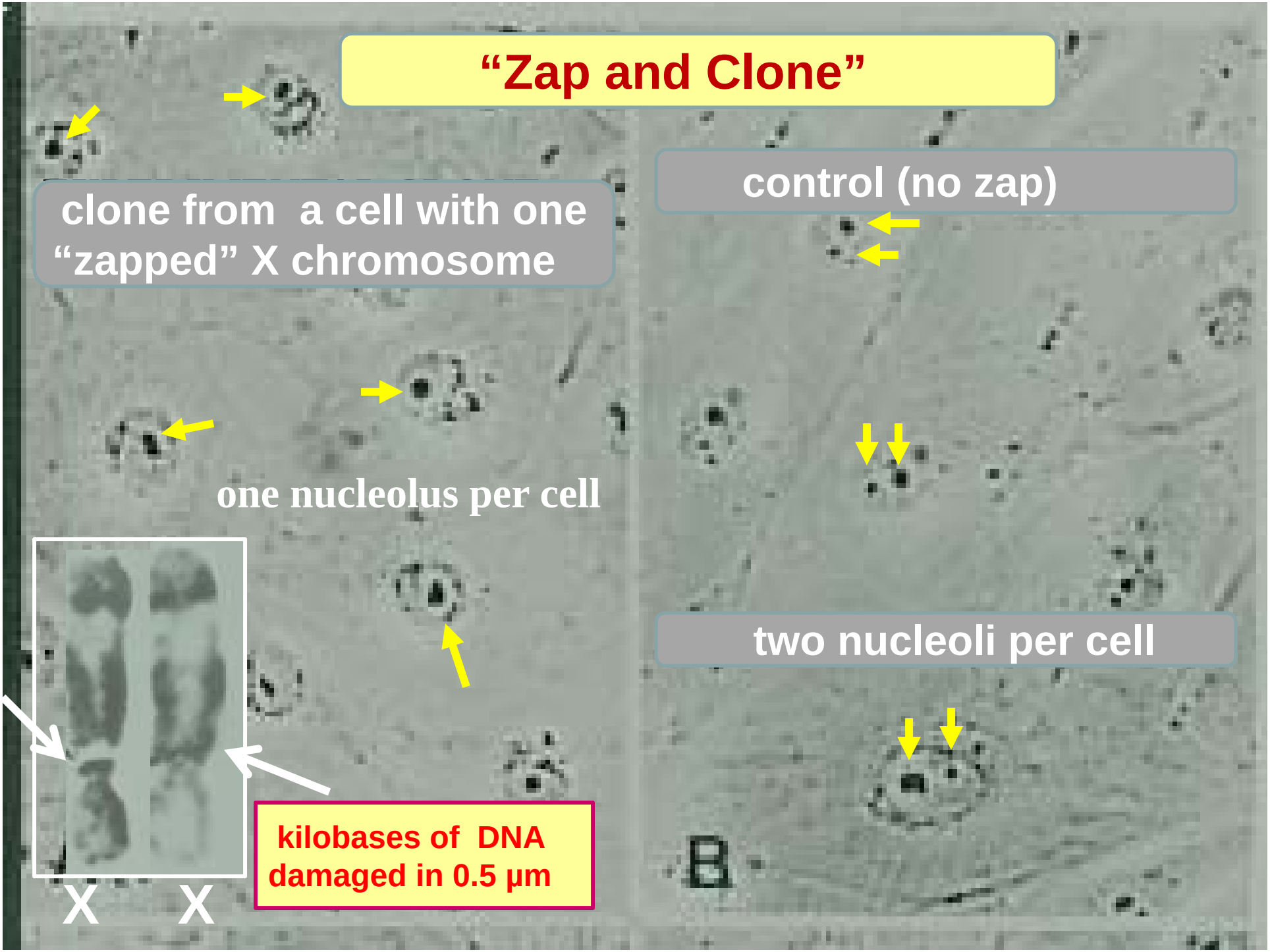




- Only 12 chromosomes
- Cells stay flat



Nucleolar genes


"Zap and Clone"
clone from a cell with one "zapped" X chromosome
control (no zap)
one nucleolus per cell
two nucleoli per cell
X X
kilobases of DNA damaged in 0.5 µm
B

How does the cell repair its DNA?

Does it occur in mitosis?

Now we have the tools to answer those questions

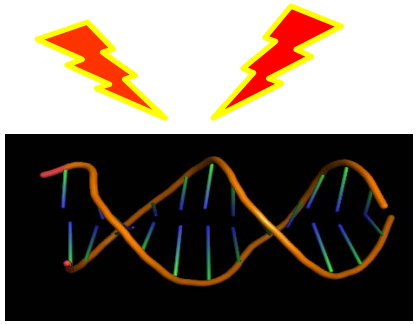
Molecular tools to study DNA repair proteins:

- **Fluorescent antibodies**
- **Green Fluorescent Protein (GFP) in live cells.**
- **FRET ?**

Optical tool produces localized DNA damage:

- **Laser scissors (uv, visible, and NIR)**
  - **2,912 articles on "laser cell microdissection"**

High irradiance: 10^{-9} – 10^{-12} W/cm^2
Short pulses: fs, ps, ns
multiphoton & non-linear physical events



Double Strand Breaks (DSB's)

Damage Recognition Proteins

yH2AX, NBS1, MRE11, Rad 50, Ku and others

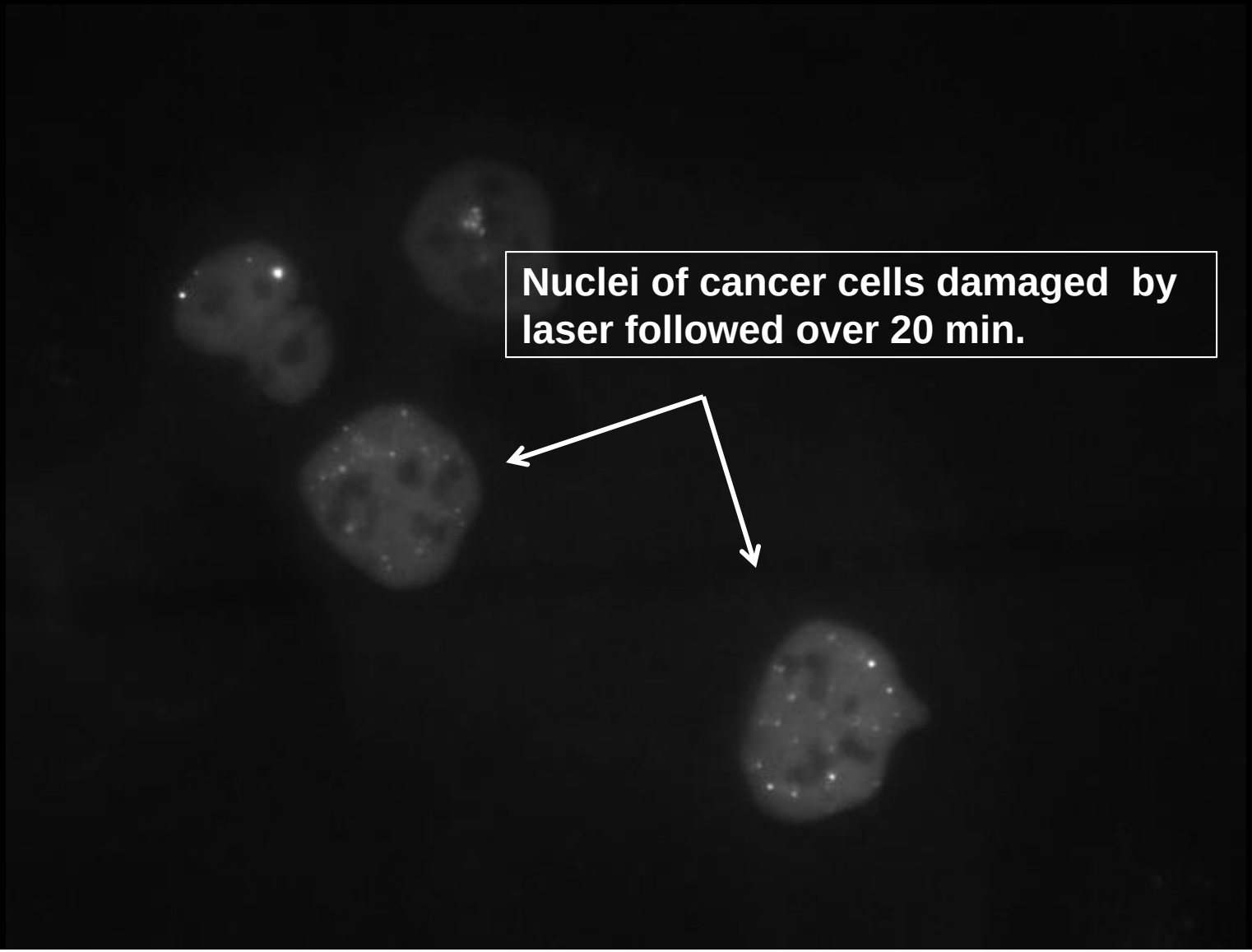
Recruit DNA Repair Proteins

Repair

RAD 51, RAD 52, RPA, XRCC1 & 4, CTIP, RPA
etc……many more

GFP-Nbs1 Live Recruitment

532 nm ns, ps, or 200 fs 700 – 800 nm NIR lasers



Does repair process start in mitosis?

- **The dogma has been that the cell is too busy with the biochemistry of dividing to start repairing damaged DNA in mitosis.**

- **506 PubMed articles: laser microbeam DNA repair:**

  505 on interphase cells

  1 on damage made on mitotic chromosomes

  (Veronica Gomez-Godinez et al. (2010) Nucleic Acids Res. 1-18, doi:10.1093/nar/gkq836)

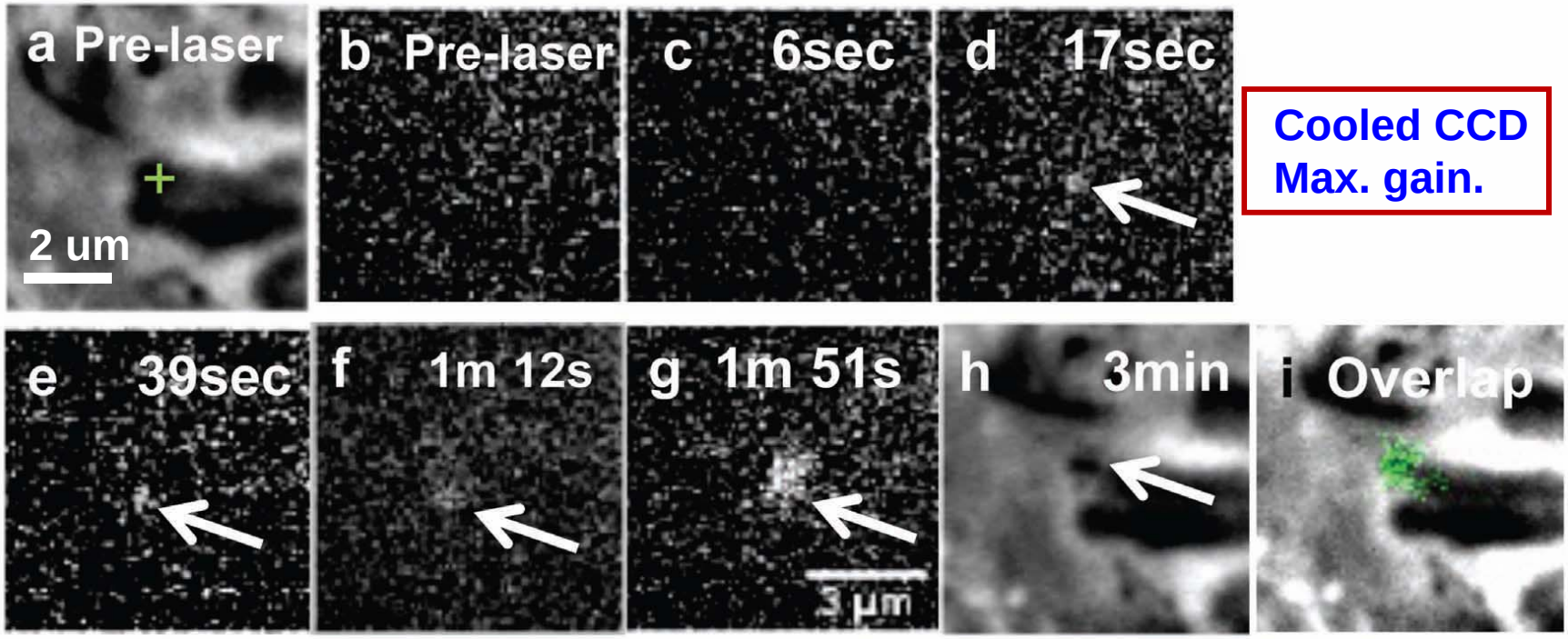
- **We think the cell has to *get right to it*: start the repair process as soon as chromosome damage occurs.**

- **1st Step is to recognize there is damage.**

V.Gomez-Godinez
PhD thesis(2012)

Do DNA damage recognition proteins go to chromosome damage site &, if so, how fast?

Fluorescence of GFP-Nbs1 damage recognition protein



Cooled CCD
Max. gain.

Pretty fast: 17 seconds

Conclude

- **The dogma that DNA repair dos not occur in mitosis is wrong.**
- **Within 17 s, damage is recognized.**
- **Recognition and repair proteins are recruited to the damage sites in mitosis.**
- **Cell has evolved very efficient mechanism to repair DNA**

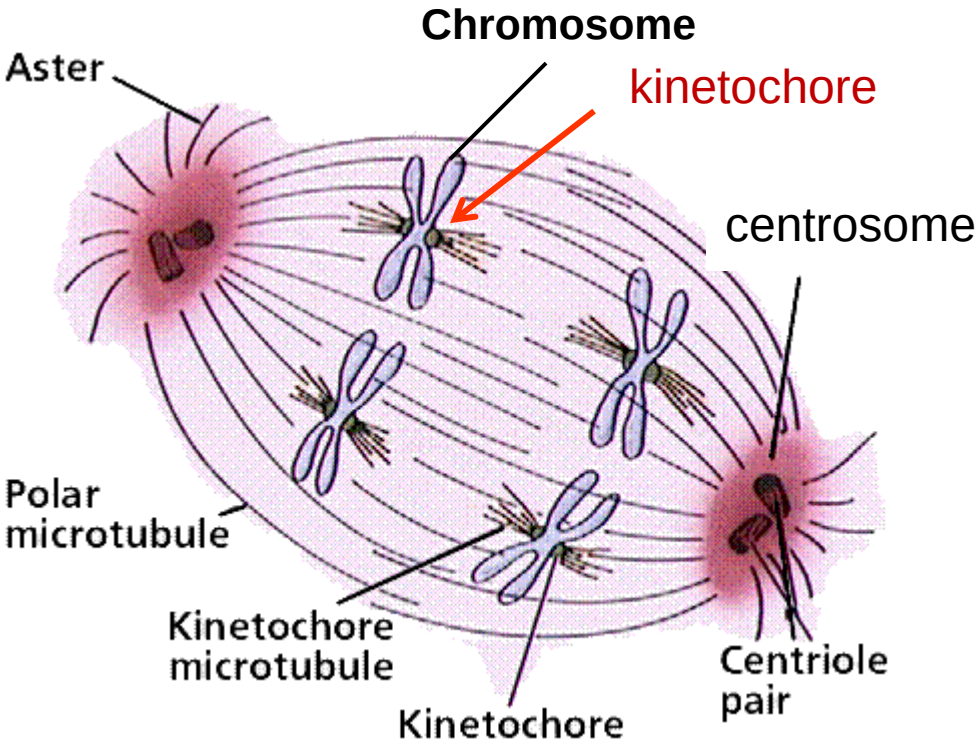
Since we're talking about chromosomes…

Another subject for our optical toolbox is the process of **mitosis**: the orderly separation of chromosomes into daughter cells; ie ***intracellular motility***

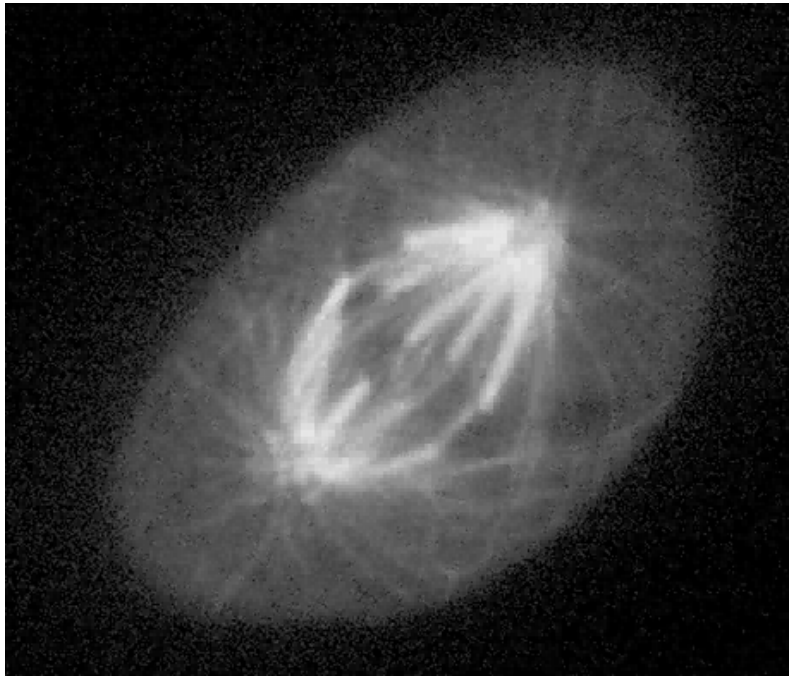
The “Dynamic” Mitotic Spindle

All can be targeted.

1. chromosomes
2. microtubules
3. kinetochores (centromere)
4. the pole (centrosome/centrioles)



GFP-microtubule bundles



@ 10 µm

Courtesy of Alexey Khodjakov: SUNY Albany

How much force do microtubules apply to chromosomes in order to move them?

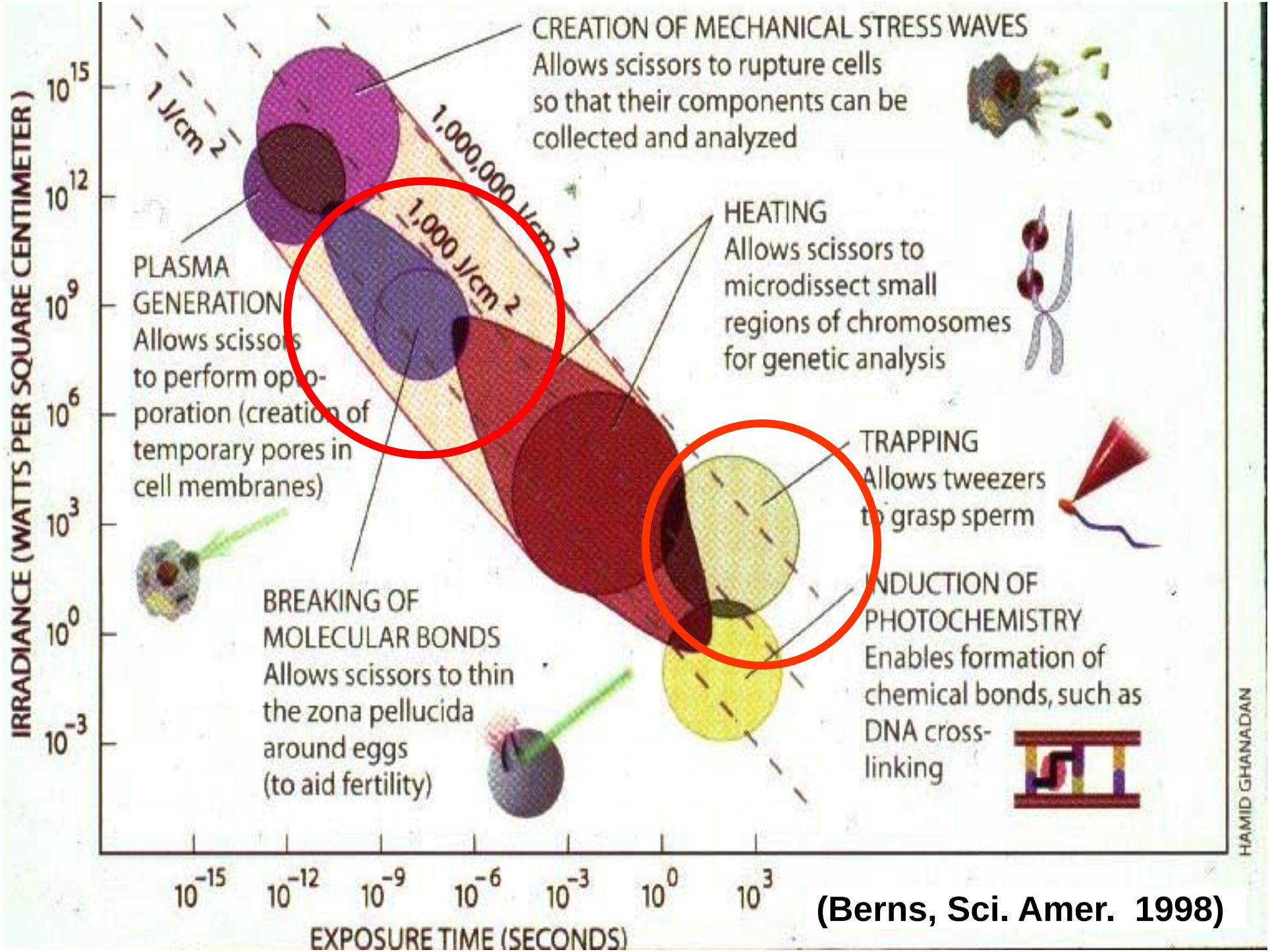
This question has been unresolved since Nicklas (1983) claimed to have measured 700 pN force by sticking needles into chromosomes.

(Nicklas RB. (1983) *J. Cell Biol*. **97**: 542-448.)

Stokes law calculations based on size, velocity, and viscosity: 0.1 - 1 pN.

(Alexander SP and Rieder CL. 1991. *J. Cell Biol*. **113**: 805-815.)

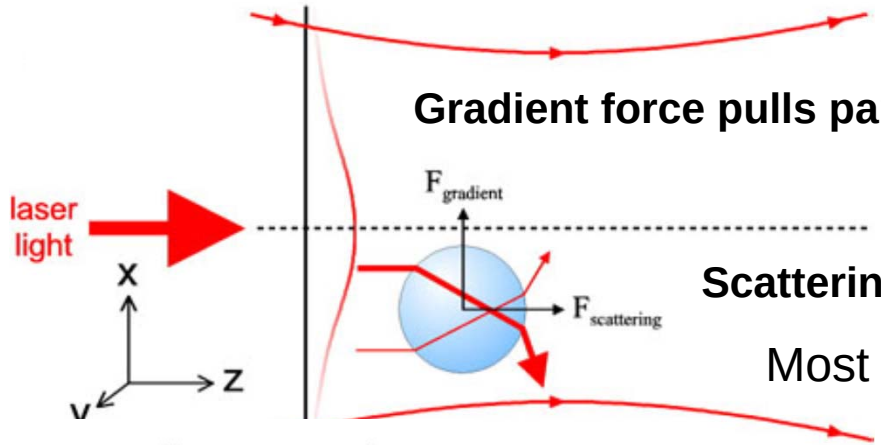
To help answer this questions, we dip into our optical toolbox and use laser tweezers (Arthur Ashkin et al 1987).



(Berns, Sci. Amer. 1998)

Basic principle of an optical trap is momentum transfer from photons to object.

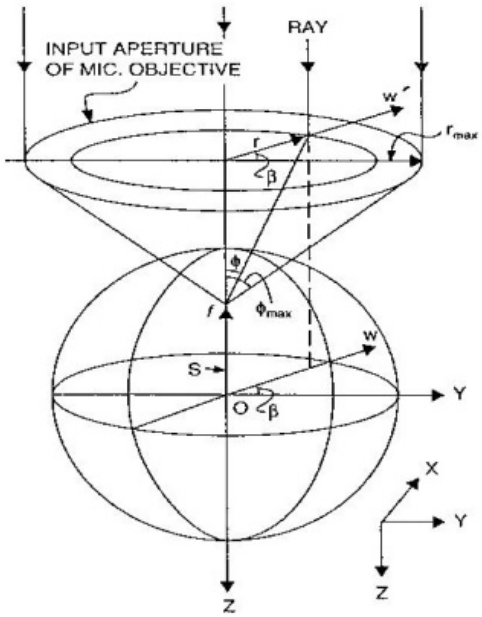
A. Ashkin and J.M.Dziedzic, *Science* 235:1517-20 (1987)

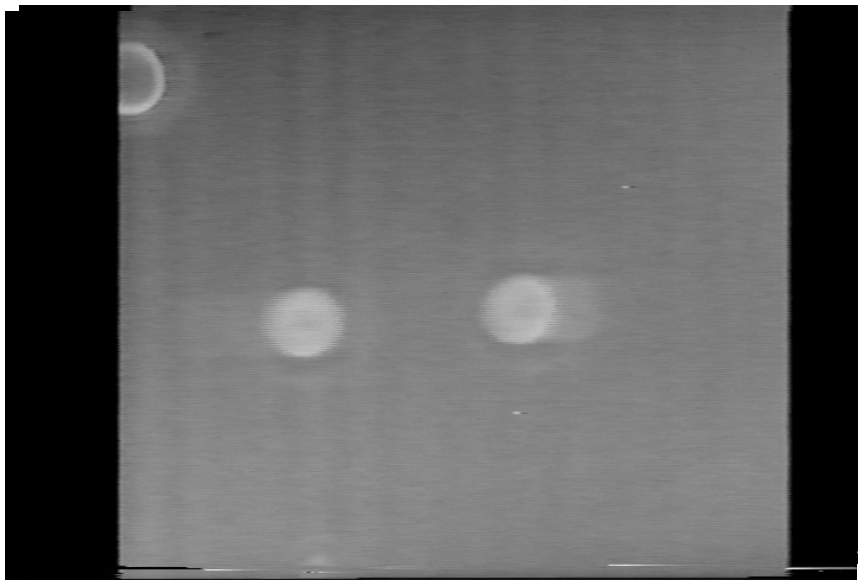


Gradient force pulls particle toward center of the beam

Scattering pushes particle out of the beam

Most biol. expts. F_s is negligible





Equation to determine net force:

$$F_{orce} = \frac{n_1 \cdot P_w}{c} \cdot (Q)$$

n_1 = 1.33 (refractive index of medium)
P = laser power (Watts)
Q = trapping efficiency: % momentum transfer (0.1- 0.13) [recent study: 0.01-0.02]
C = $3x10^8$ m/sec

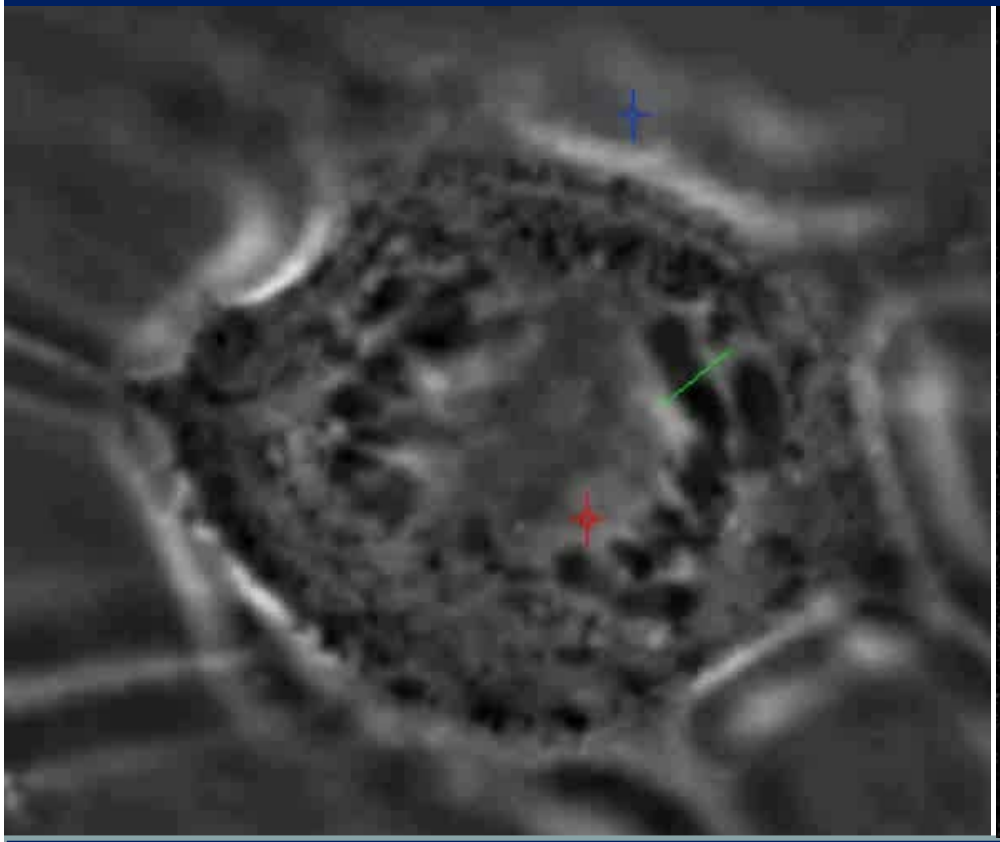
* **Ashkin, A., Methods In Cell Biology, 55:1-27,1998**
* **Koenig et al Cell. Molec. Biol. 501-509, 1996.**

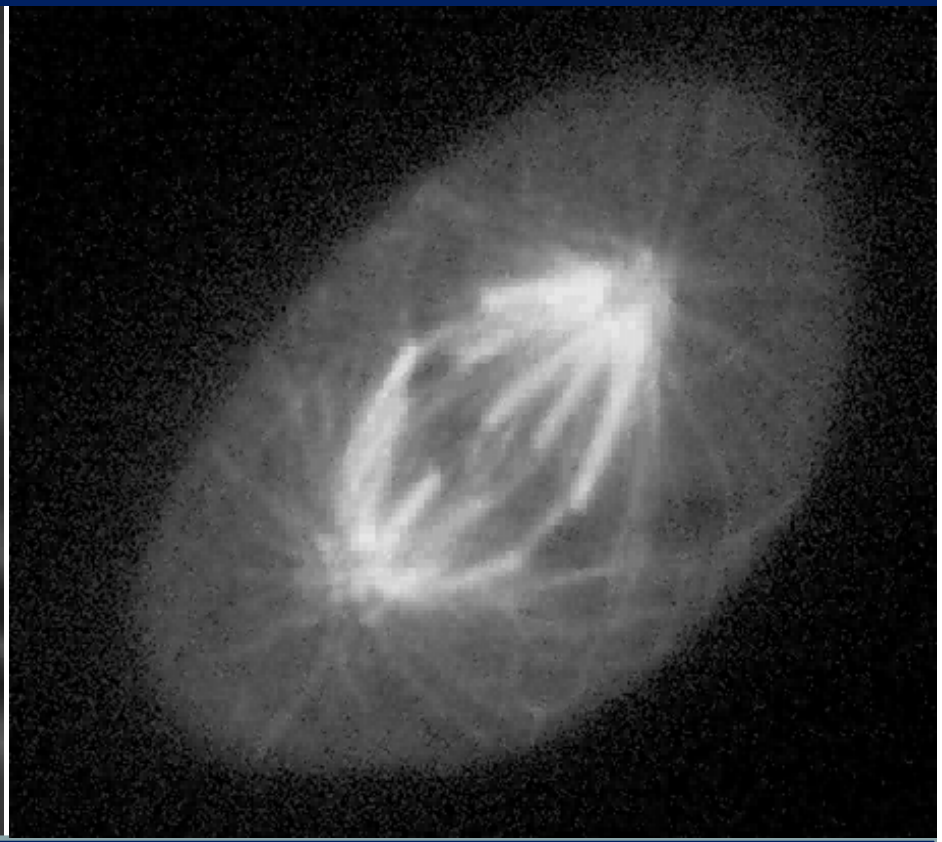
- **Can we measure the forces on a chromosome?**

- **1st can we cut & move a chromosome?**

Use of a laser-induced optical force trap to study chromosome movement on the mitotic spindle. Berns et al., *Proc. Natl. Acad. Sci. USA* 86: 4539-4543, 1989.

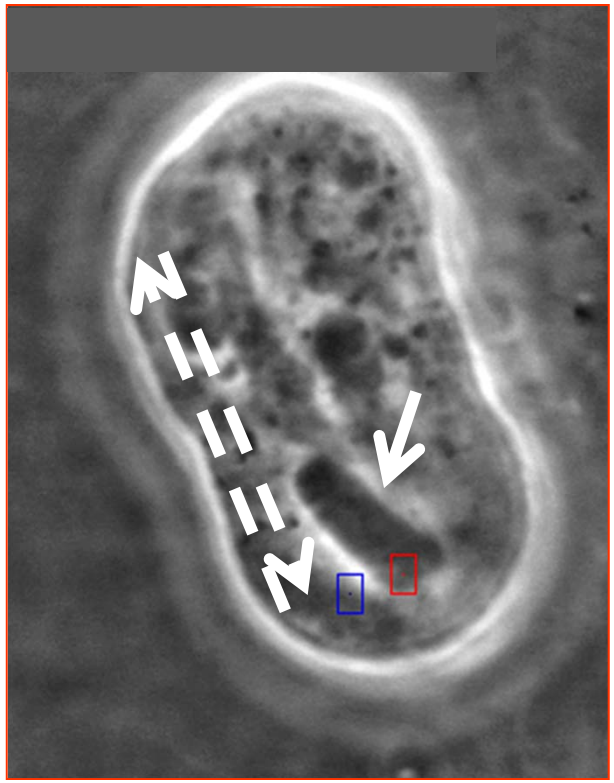
Cut and Trap a Mitotic Chromosome?

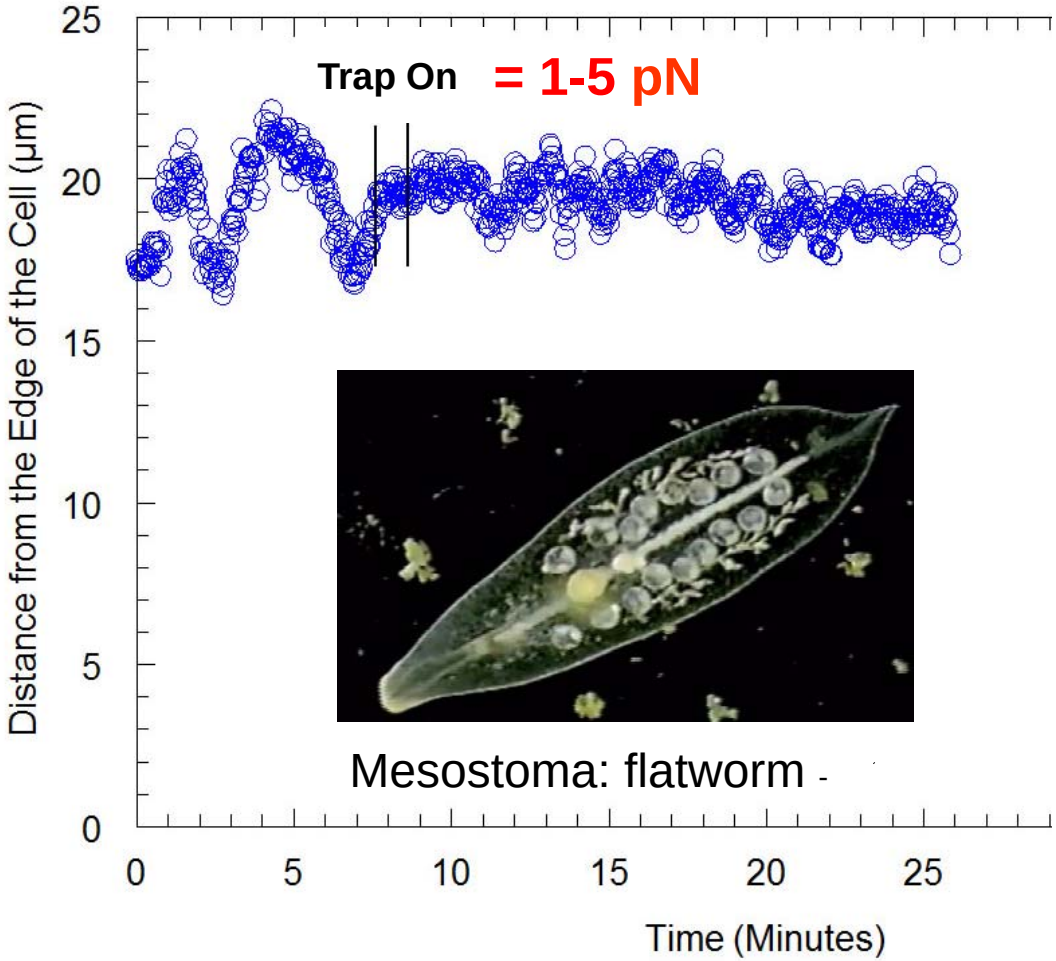




- Cut with fs 800 nm with $3x10^{11}$ W/cm^2 irradiance in focal spot.
- Trap: 1064 nm, 100-300mW in spot = 45 - 130 pN. (Q = 0.1)
- But this doesn't measure how much force microtubules apply.
- Will show lay-out of the laser systems in the second talk.

Enter a flatworm: gamete chromosome oscillate (are pulled) between the poles before cell divides.





Art Forer and Jessica Ferraro, brought their samples from York Univ., Toronto and did experiments in February.

How much force do microtubules apply to chromosomes?

Less than 700 pN force suggested by Nicklas.

(Nicklas RB. (1983) *J. Cell Biol*. 97: 542-448.)

A bit more than Stokes law calculations: 0.1 - 1 pN.

(Alexander SP and Rieder CL. 1991. *J. Cell Biol*. 113: 805-815.)

BEADS ON CELLS
FRET

GFP → FRET BIOSENSORS

(Fluorescence Resonance Energy Transfer)

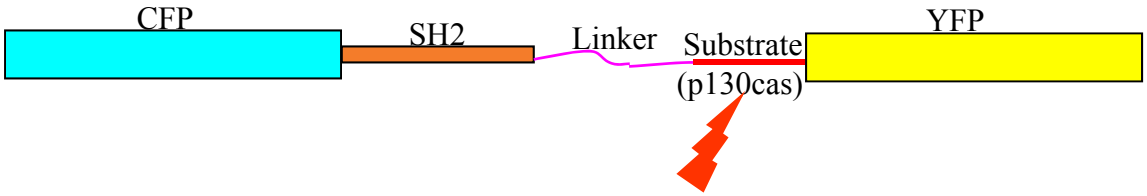
↓

Intracellular signaling

Wang, Y, E. L. Botvinick, Y. Zhao, M. W. Berns, S. Usami, R. Y. Tsien and S. Chien. Visualizing the mechanical activation of Src. (2005) *Nature* 434, 1040-1045.

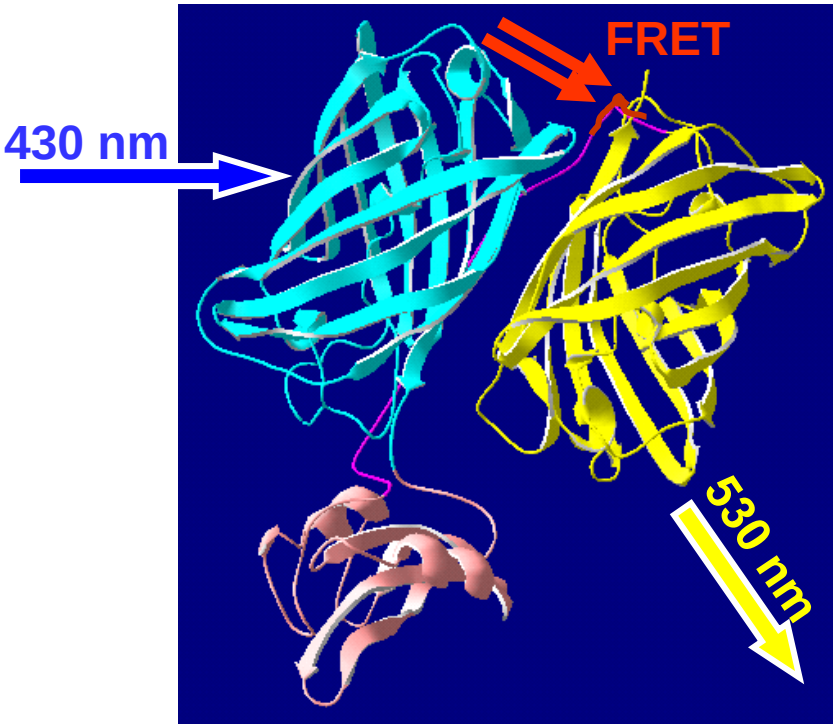
Src kinase biosensor: encoded in cell's DNA

look for: change in FRET efficiency in presence of Src



Detects the Src kinase

Strong FRET



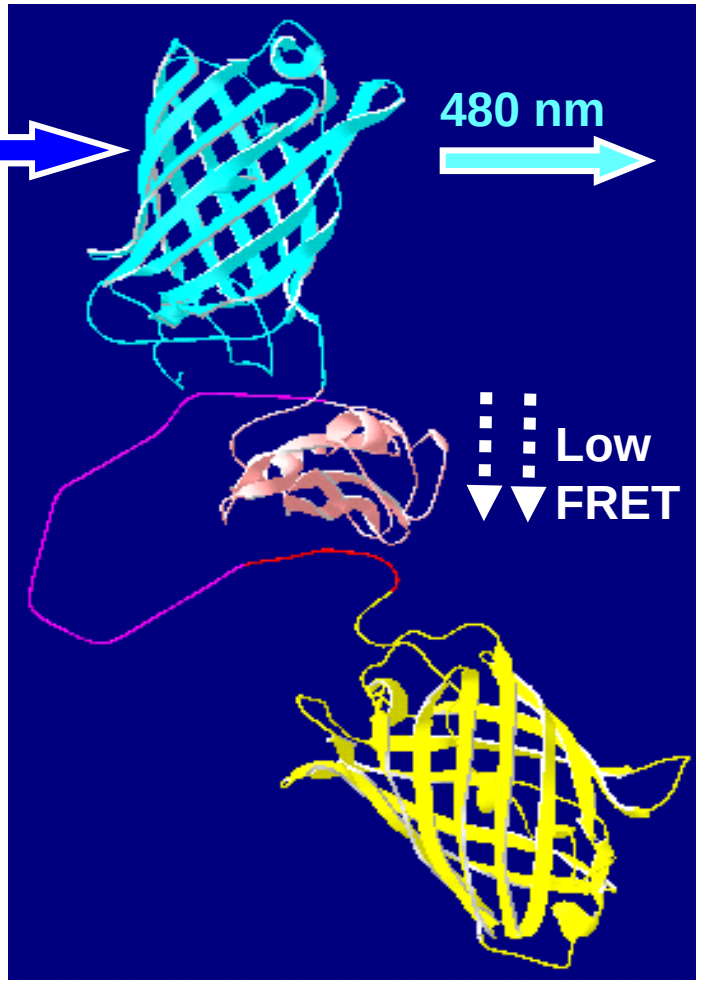
Src kinase catalyzes phosphorylation of the biosensor

Phosphorylation ⇀

↽ Dephosphorylation

No Src kinase
get strong FRET

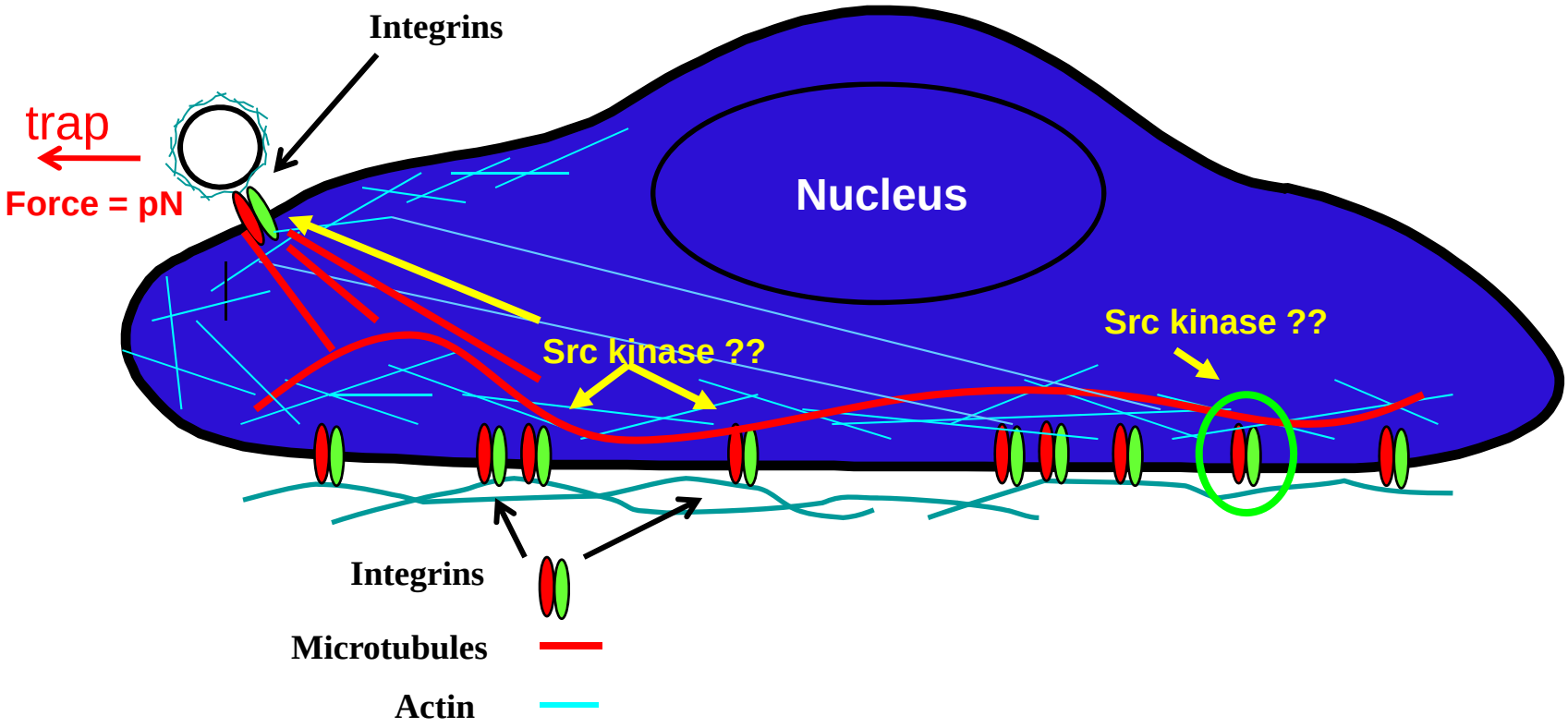
Weak FRET

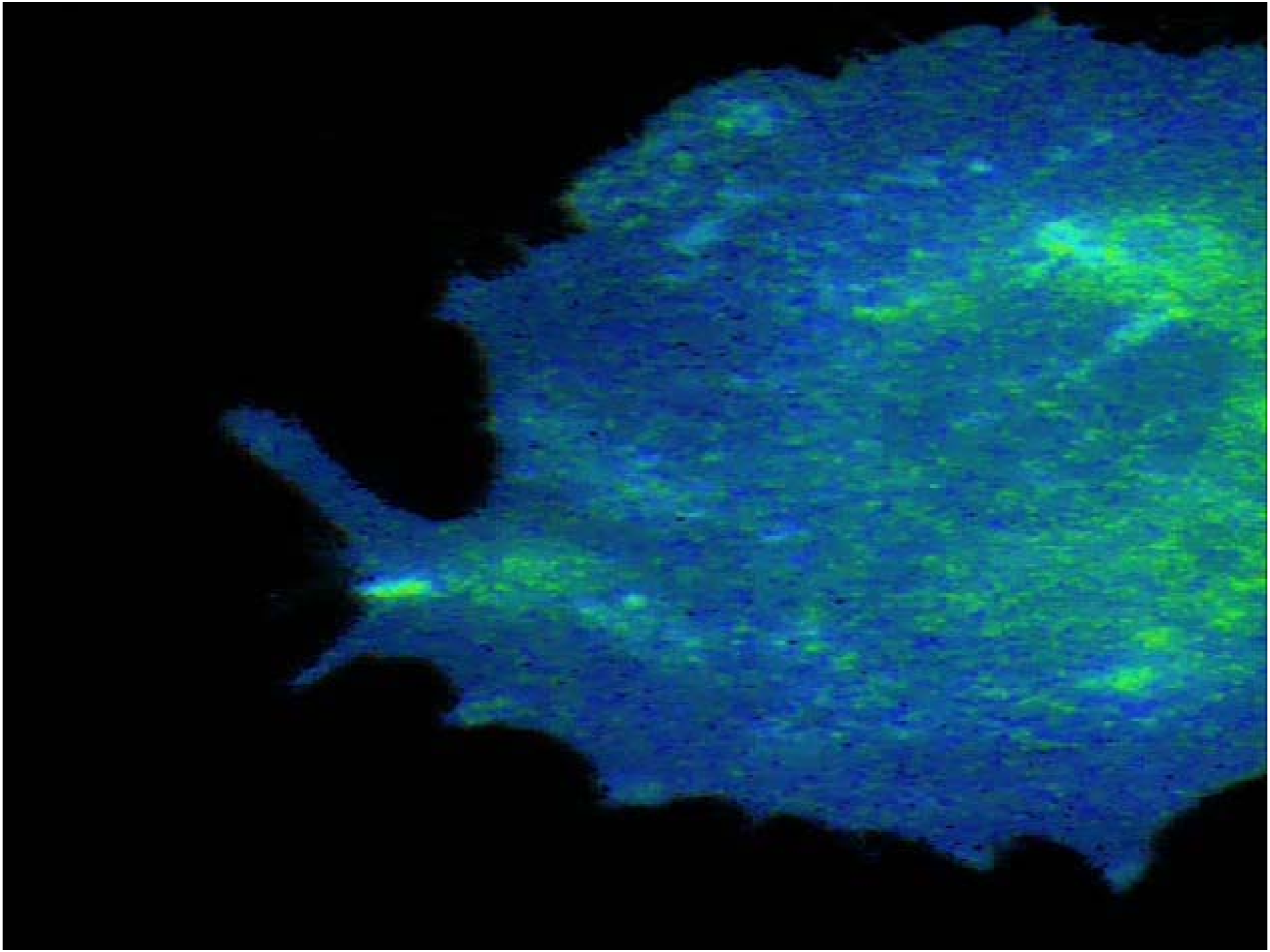


Hypothesis: Src kinase mediates mechanotransduction

Test:

- Attach bead to surface of cell and apply trapping force
- Does Src kinase activity change in response to force ?
- Need an intracellular “biosensor” for Src kinase





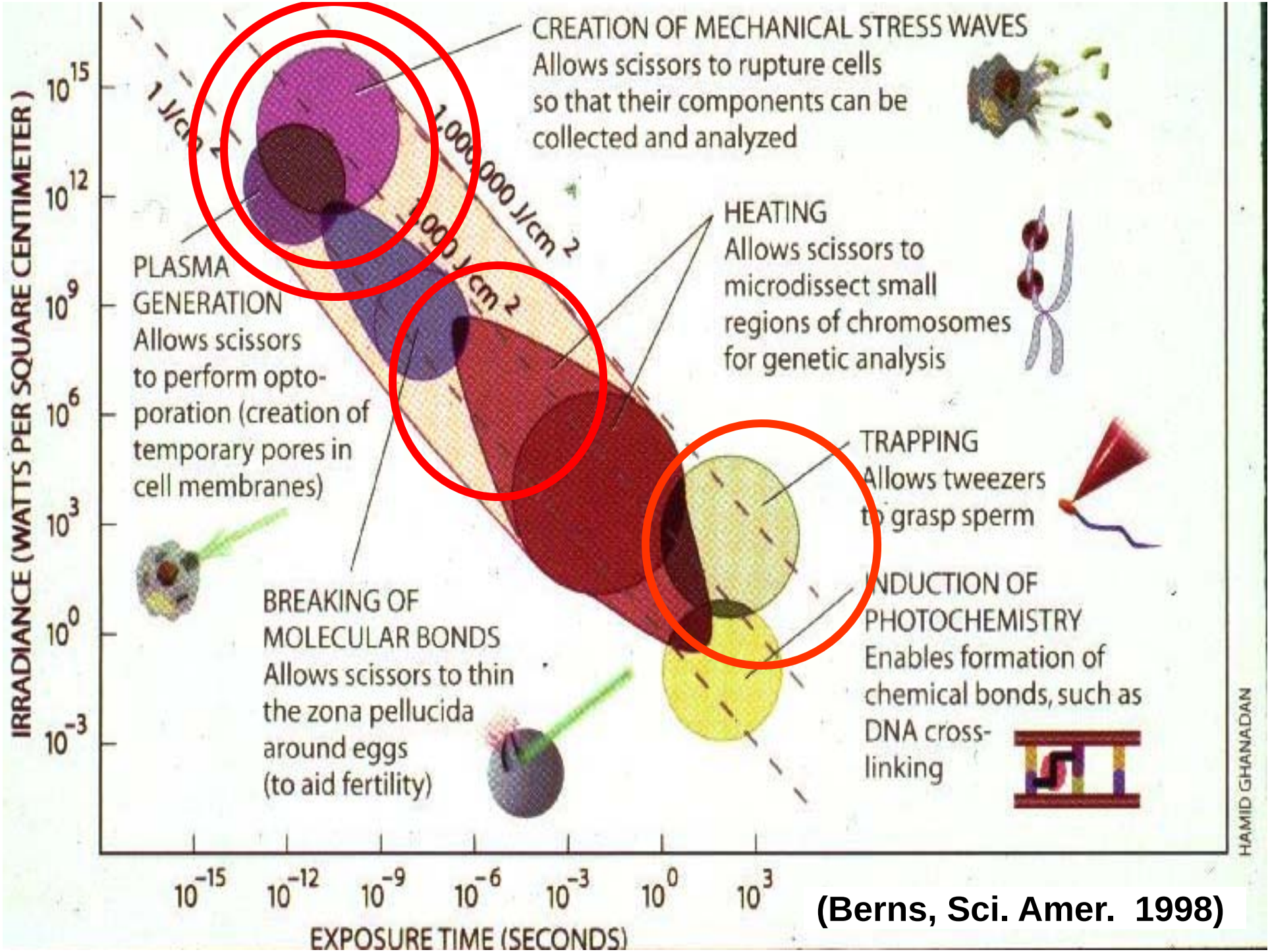
Other FRET Biosensors

Vinculin Tension Sensor (VinTS)

- FRET sensor of tension-induced strain; detects pN (Grashoff & Schwartz et al., *Nature,* 2010).

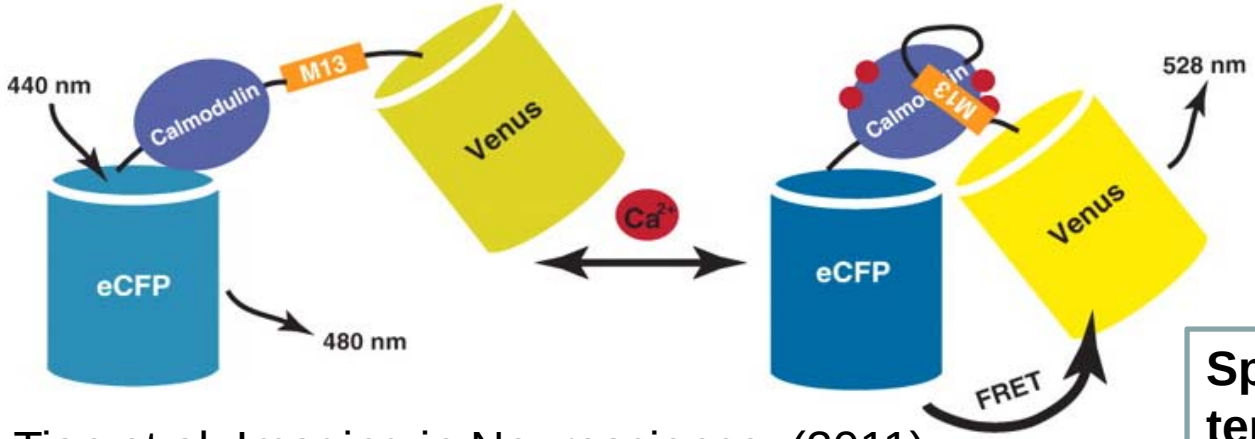
- VinTS with laser fs nanosurgery to determine how single stress fibers distribute their tensile loads across vinculin molecules within focal adhesions. (Chang &Kumar , *J. Cell Sci*. 2013)

- **Calcium Biosensor (D3CPV)**
  Sudden shear stress caused by laser induced shockwaves allow study of subsequent mechanotransduction.
  (2014 Veronica Gomez et al: *Micros. Res. Tech)*



(Berns, Sci. Amer. 1998)

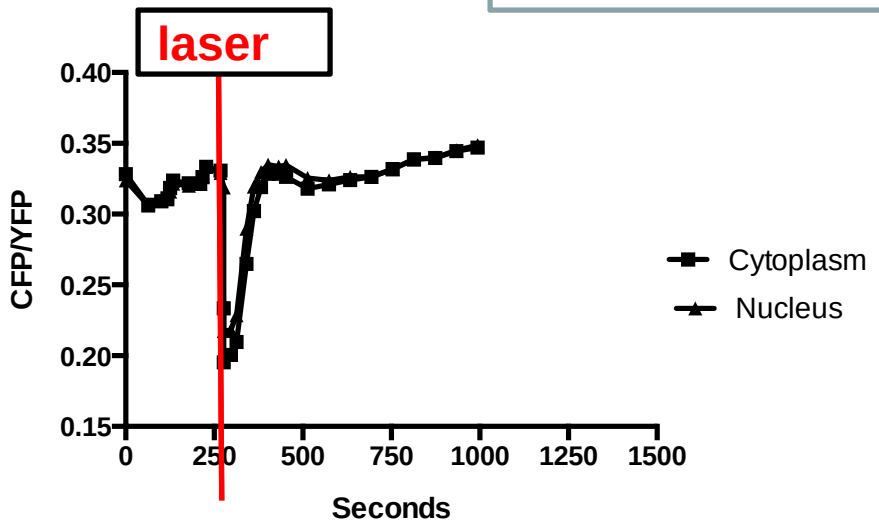
D3CPV Calcium Biosensor



L., Tian et al. Imaging in Neuroscience. (2011)

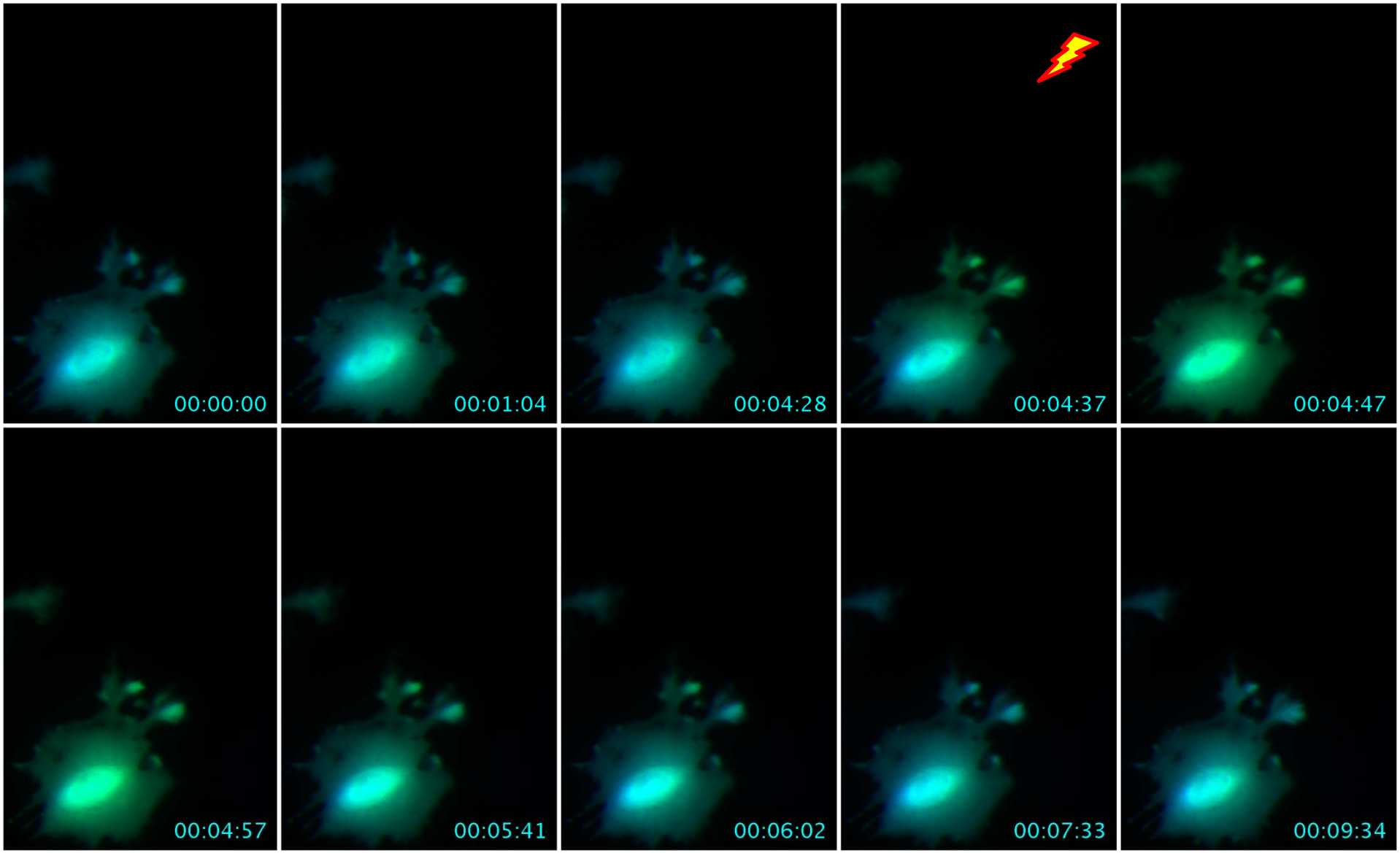
Spatial & temporal detector of calcium

Single cell response to laser-induced shock wave



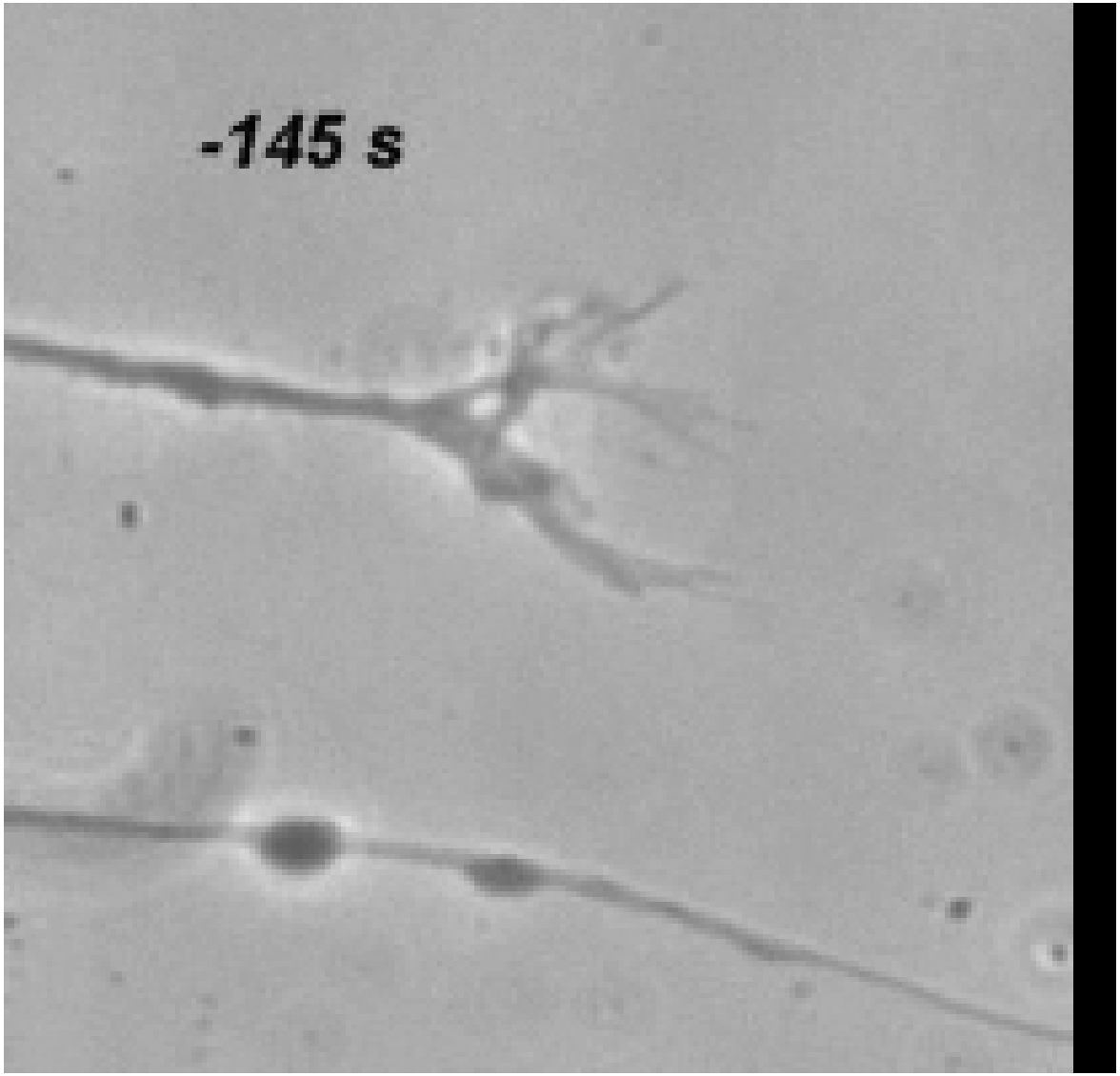
Cells in Ca^{+} free medium I

2014: Veronica Gomez et al



1. The cells survive
2. Shockwave effect is transitory
3. Now we have a model to study shockwave effects on single cells (neurons)

Brief detour to neuro-photonics

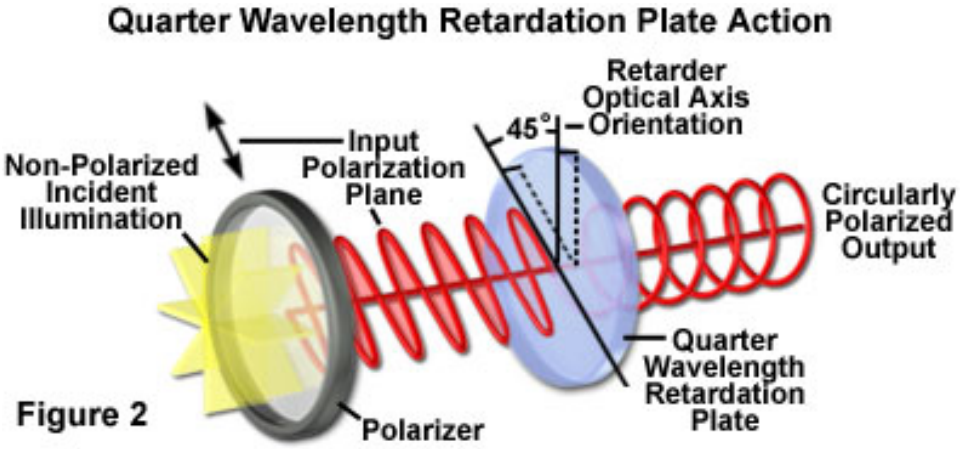


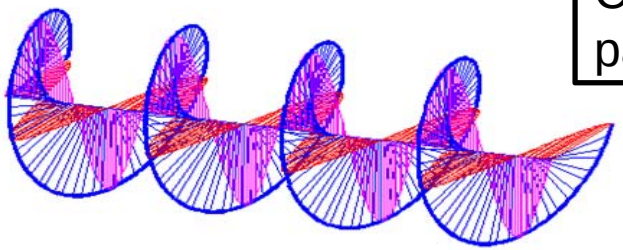
Two axons from retinal ganglion cells from goldfish. One damaged by high power laser pulse. The other responds to aid in healing

Similar responses have been found in neurons from rat hippocampus and neurons from pluripotent stem cells.

The challenge is to dissect out the molecular signaling that is occurring on the cell surface and inside the cell. This is where FRET could be a very valuable tool.

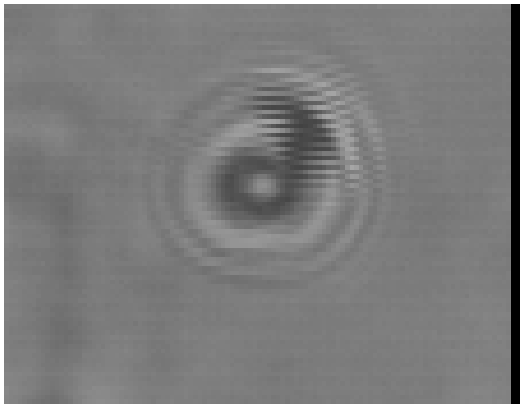
Use circular polarized light to rotate a anisotropic particle near single neurons.

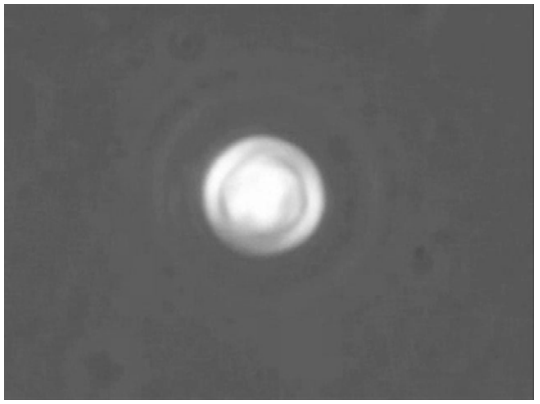




Crystalline anisotropic particle

calcite

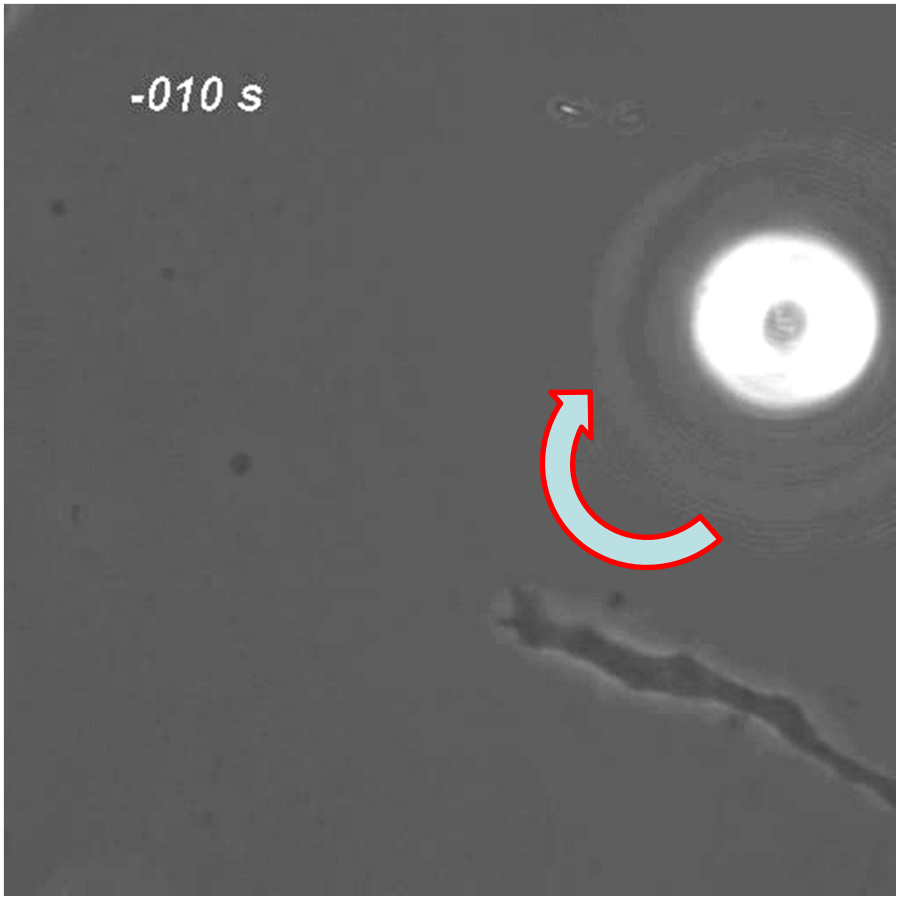




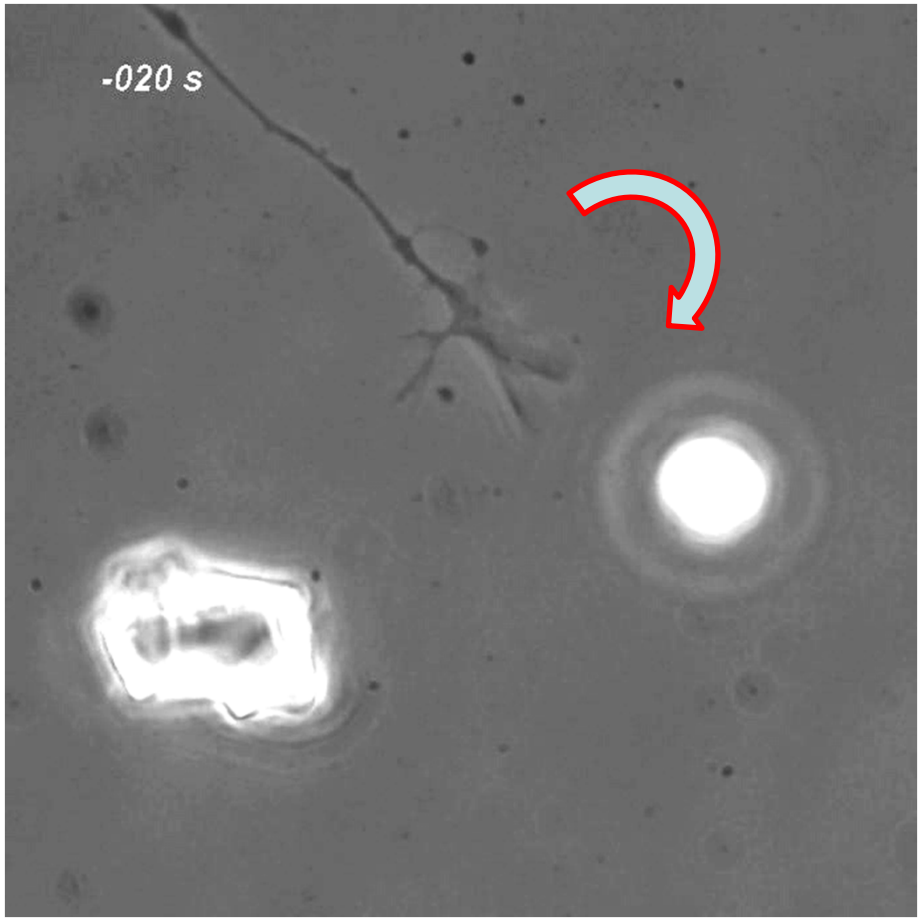
vaterite

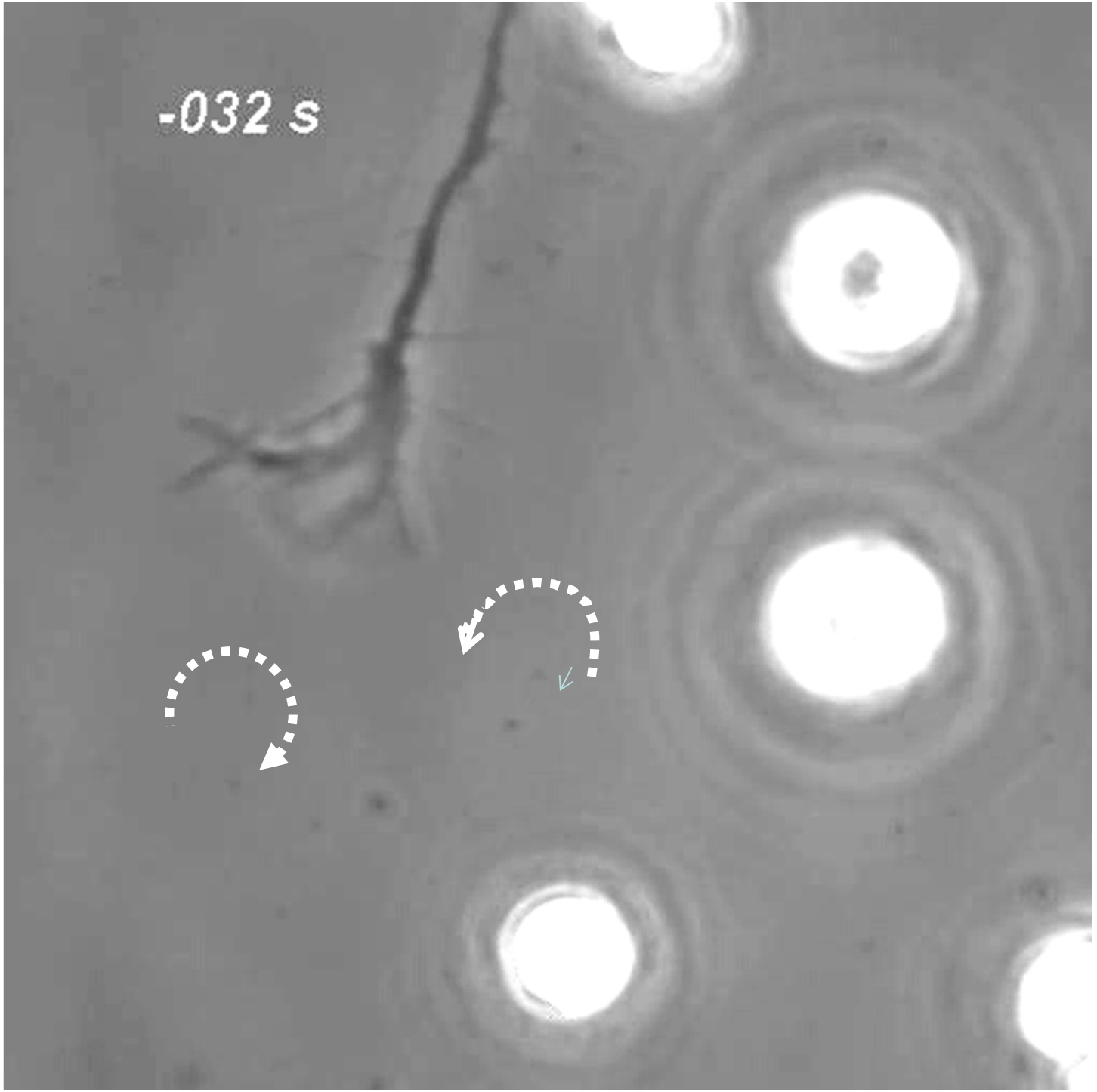
Halina Rubinsztein-Dunlop and Timo A. Nieminen, Univ. Queensland, Brisbane, Aus.

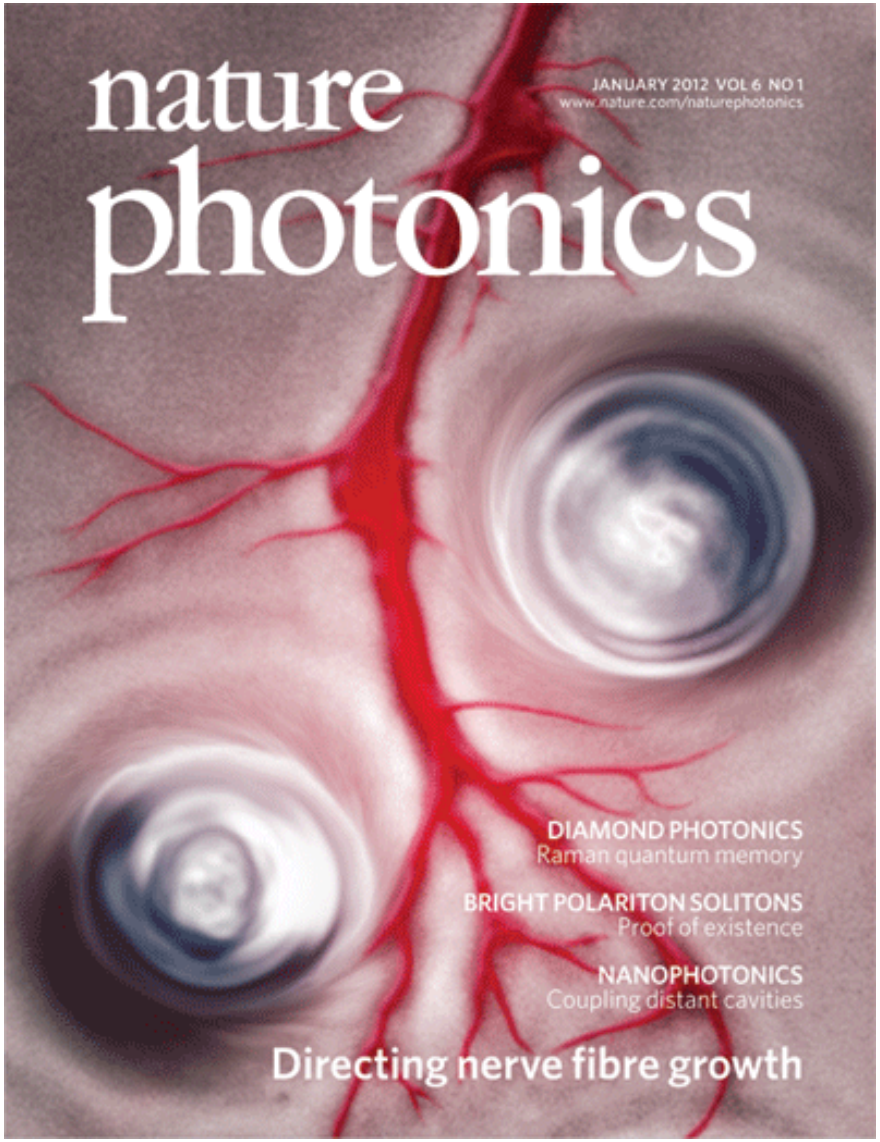
Antegrade rotation



Retrogade rotation




-032 s

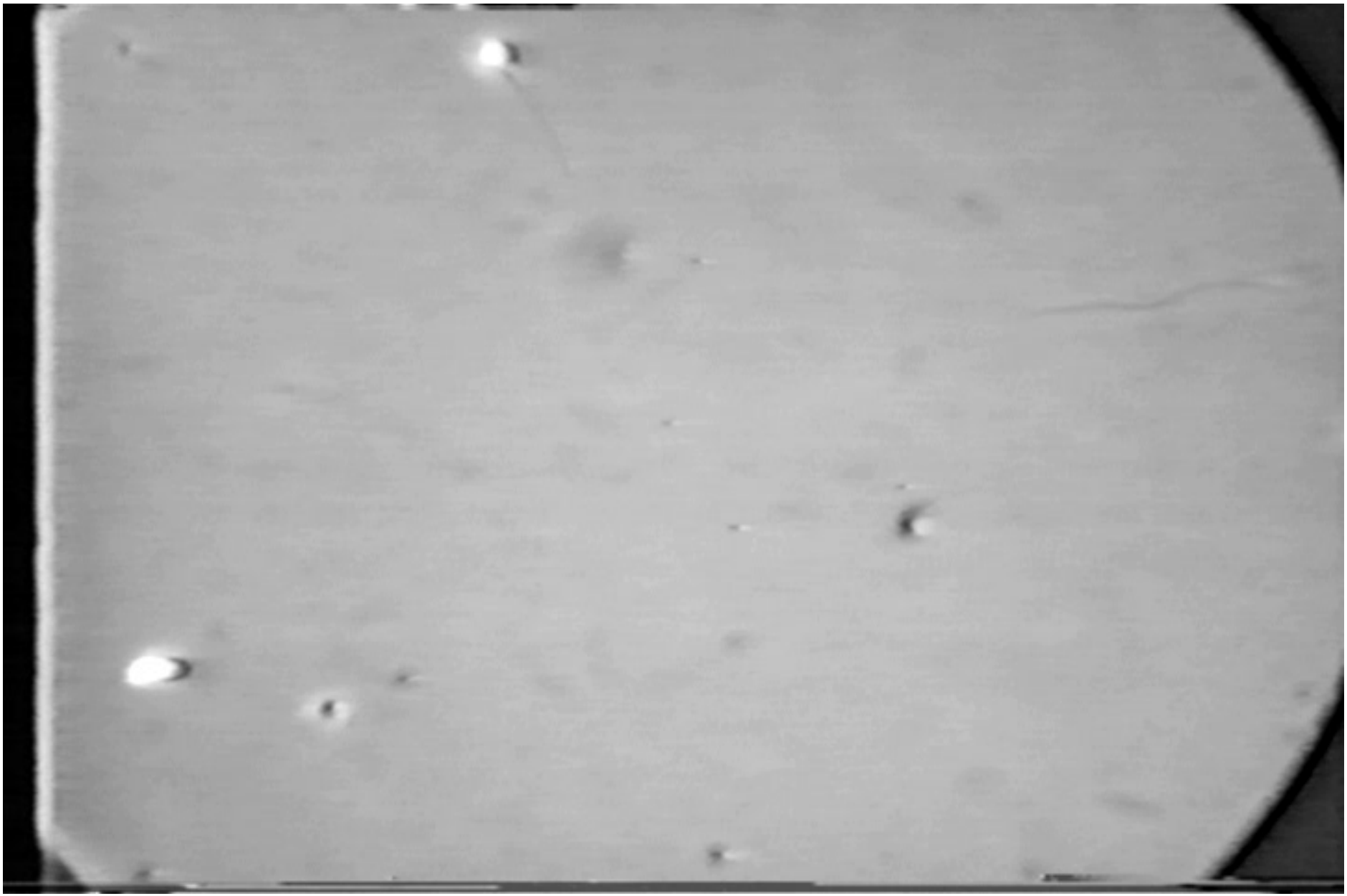


Wu, T., T. A. Nieminen, S. Mohanty, J. Miotke, R. L. Meyer, H. Rubinsztein-Dunlop and M. W. Berns. *A photon-driven micrometer can direct nerve fibre growth*. January 2012

Can we trap really fast motile cells?

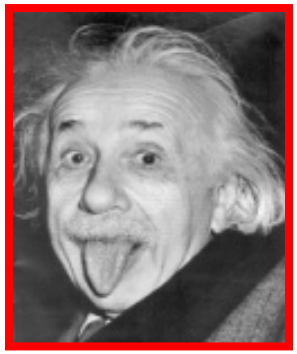
SPERM

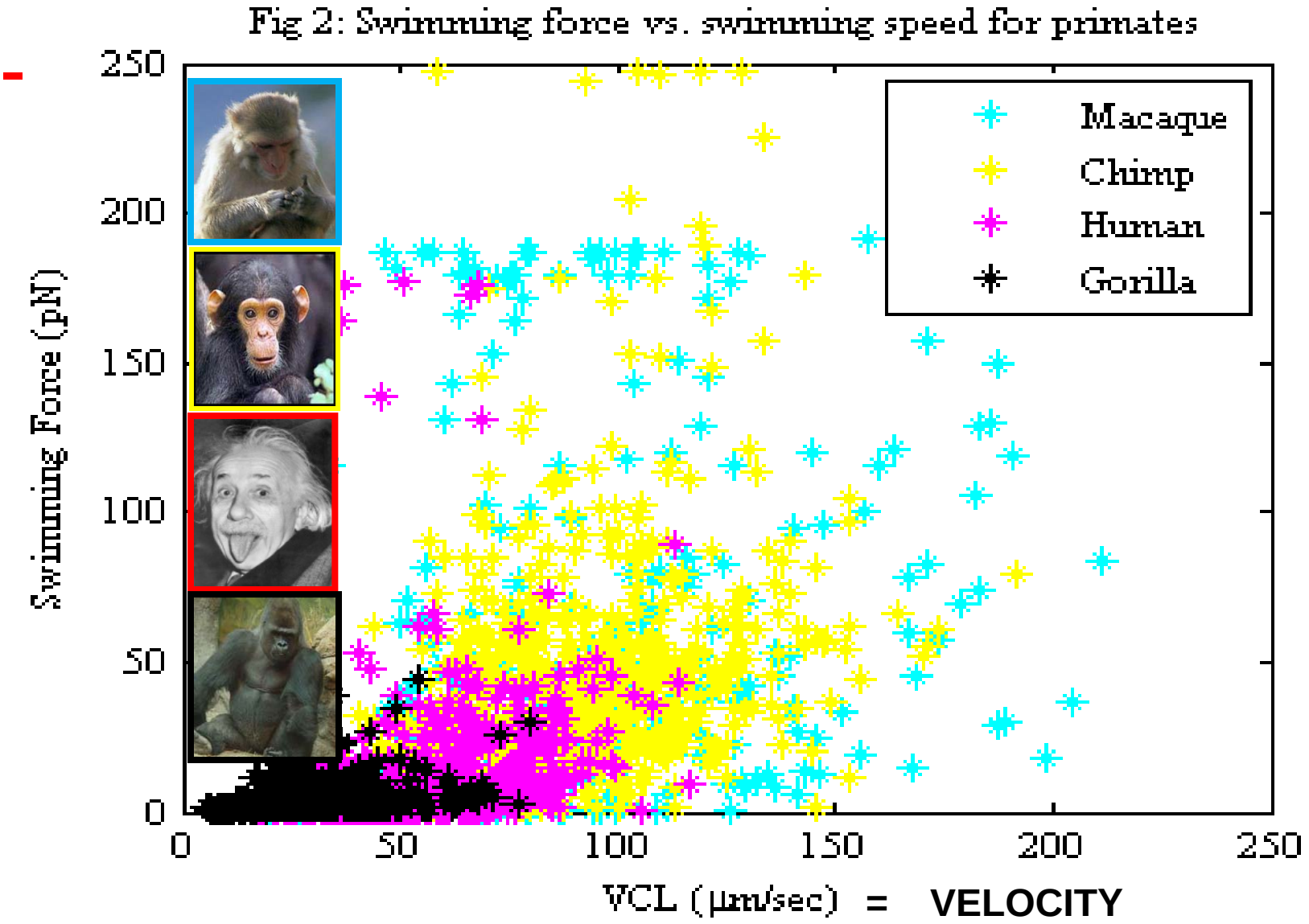
Tadir, Y., W. H. Wright, O. Vafa, T. Ord, R. H. Asch and M. W. Berns. Force generated by human sperm correlated to velocity and determined using a laser generated optical trap. Fertil. Steril. 53: 944-947, **1990.**



Evolutionary-anthropological question: is there sperm competition?

Hypothesis: Sperm from species where many males mate with one female (chimpanzees) swim faster and with more force than sperm from species where only one male mates with the females (gorillas).

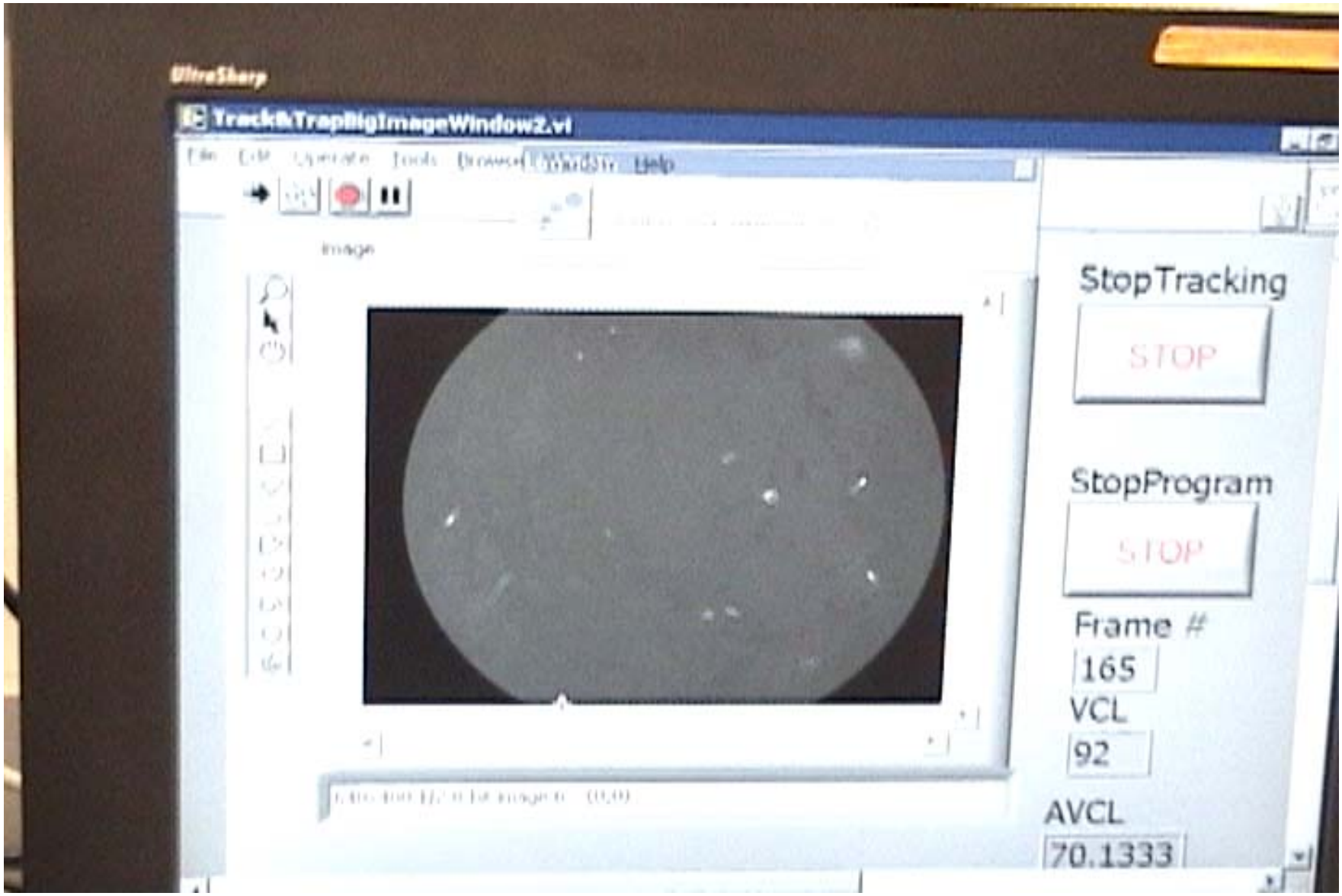


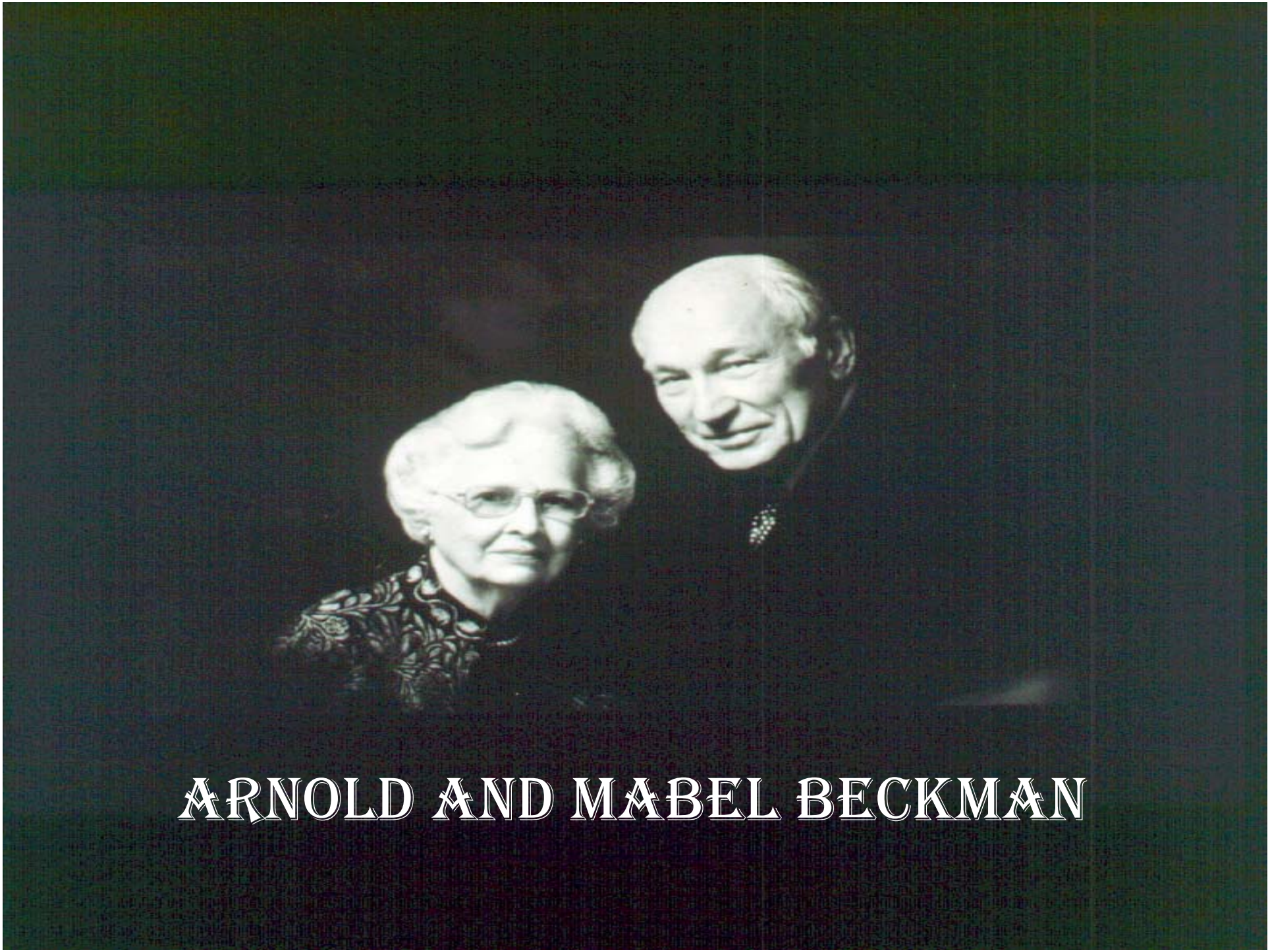


Nascimento et al, *J. R. Soc. Interface (2008) 5, 297–302.*

The “Aussie” connection

Halina Rubinsztein-Dunlop, U. Queensland





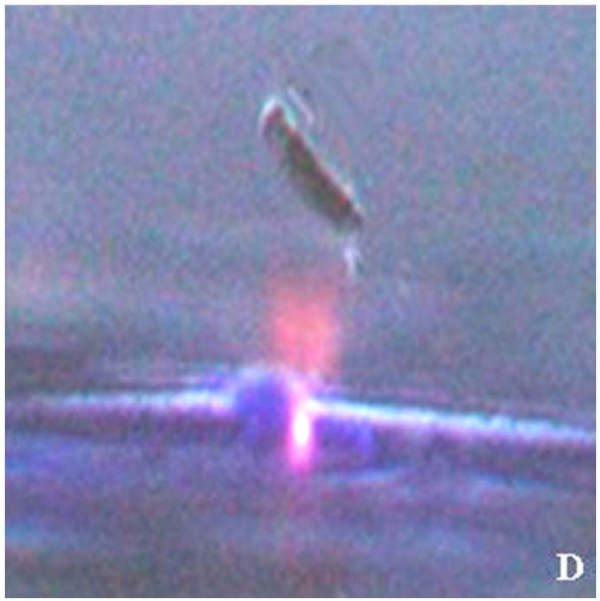
ARNOLD AND MABEL BECKMAN

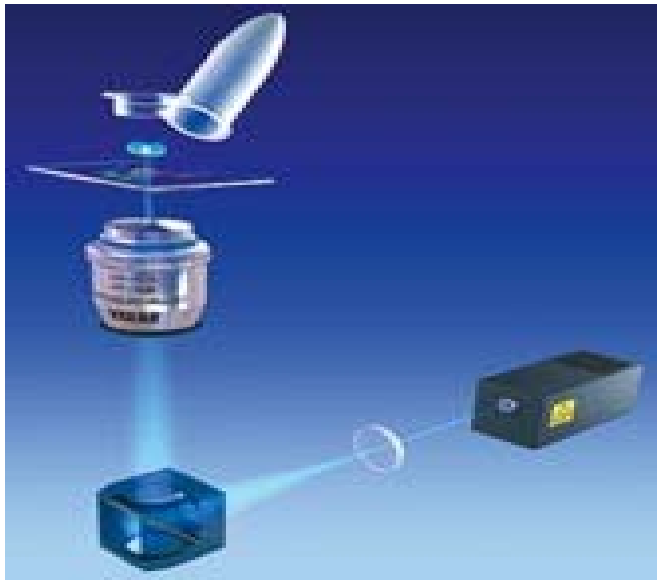
3. Laser Capture: Plasma Mediated

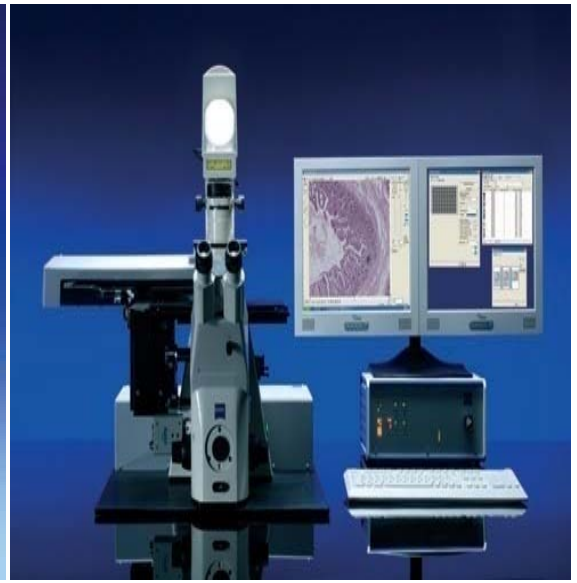
From K. Schuetze et. al, Meth. Cell Biol., 82:650-670 (2007)

www.palm-microlaser.com

Sample within a cell nucleus
Extract and PCR DNA
Cancer genotyping
Drug discovery
Live or dead cells



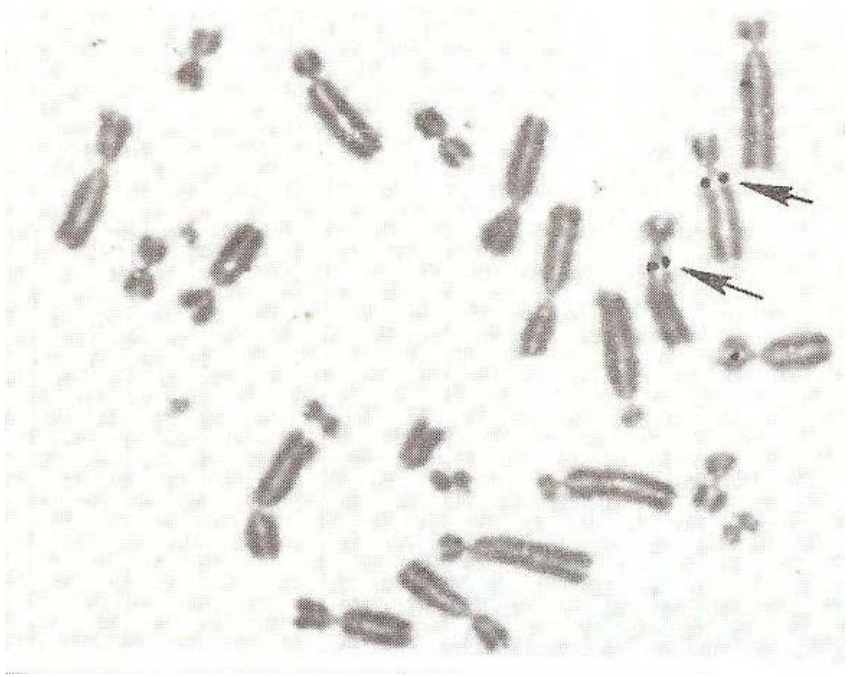


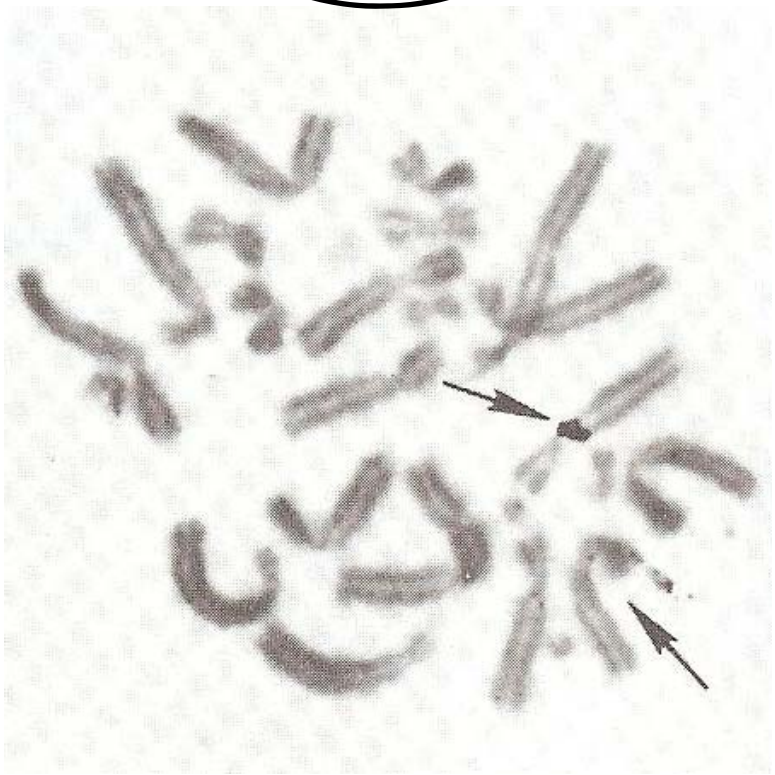


Clonal cell from a cell that had one block of ribosomal genes removed

normal

minus cell





In Germany, Karl Otto Greulich's lab in Heidelberg was dissecting chromosomes and PCR cloning genes @ 1986 using excimer pumped dye laser.

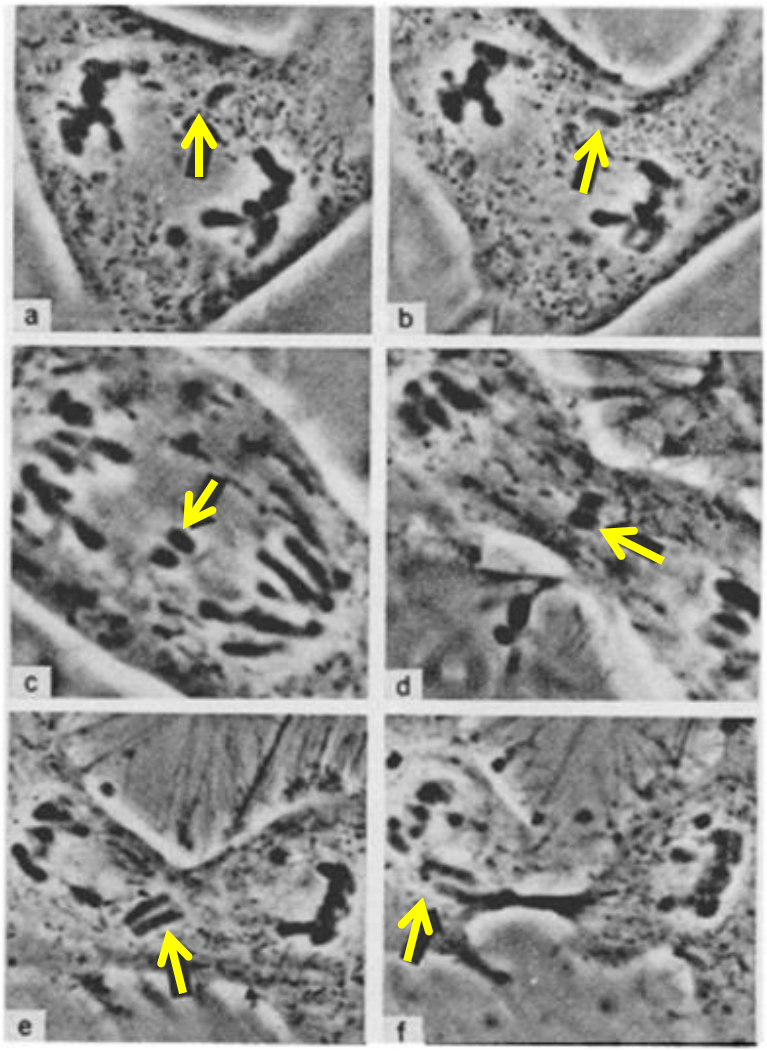
DNA REPAIR IS A "HOT" FIELD

Many labs use different laser microbeams to create DNA damage and then study repair BUT most study repair in interphase – what about MITOSIS, when the DNA/chromosomes are most condensed?

[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]
ns N2 UVA/ BrDU	ns N2 UVA/BrDU	ns N2 UVA/ BrdU or IdU	ns N2-dye UVA/Hoechst	ns N2 UVA	ns N2-dye UVA	ns Nd:YAG green	ns Nd:YAG green	ps Nd:YVO_4 green	fs Ti: sapphire NIR	fs Ti: sapphire NIR
337 nm	337 nm	337 nm	390 nm	337 nm	365 nm	532 nm	532 nm	532 nm	800 nm	800 nm
4 ns	4 ns	3 ns	4 ns	4 ns	4 ns	6 ns	7 ns	12 ps	200 fs	200 fs
0.04 μJ	0.008 μJ[a]	0.15 μJ[b]	0.25 μJ	0.27 μJ	0.20 μJ	0.32 μJ	0.4 μJ	0.19 nJ[c]	0.13 nJ	0.72 nJ
6 Hz	6 Hz	30 Hz	~10 Hz	6 Hz	10 Hz	10 Hz	10 Hz	76 MHz, 30	76 MHz	76 MHz, 1

Kong et al. (2009), Comparative analysis of different laser systems to study cellular responses to DNA damage in mammalian cells; *Nucleic Acids Research*, April pg. 1–14 (on-line).

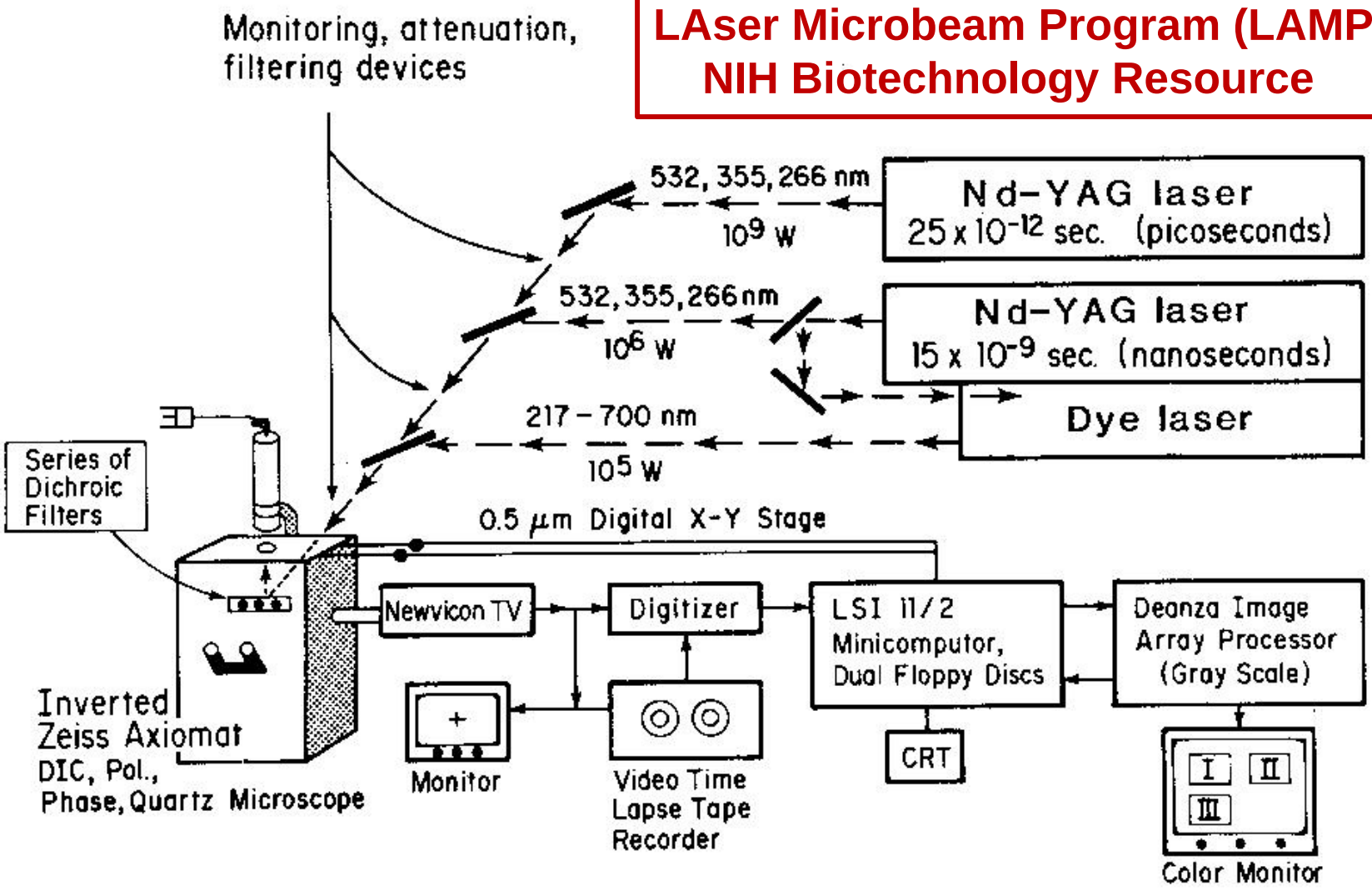
Remove whole or partial chromosomes arms



Short pulse (ns, ps) & tunable (217 – 700nm)

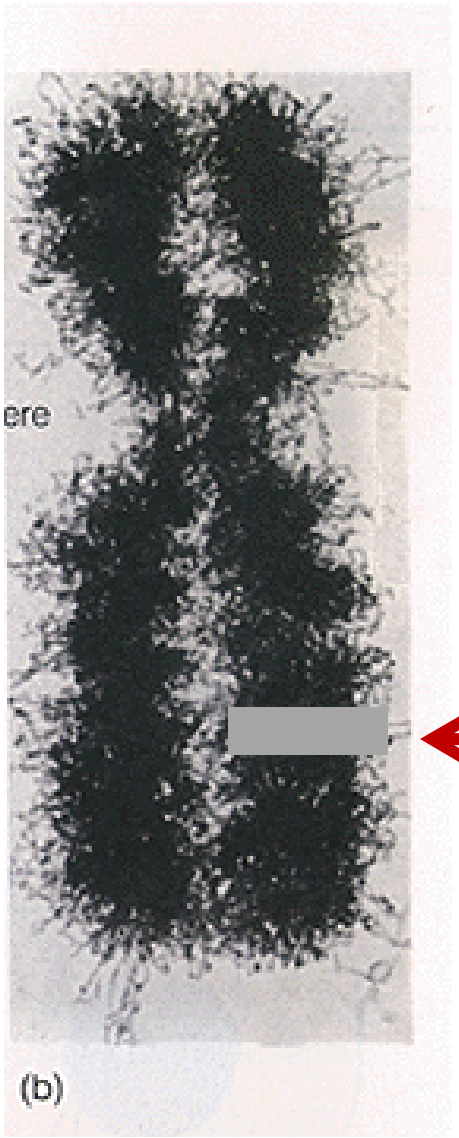
+ digital image processing

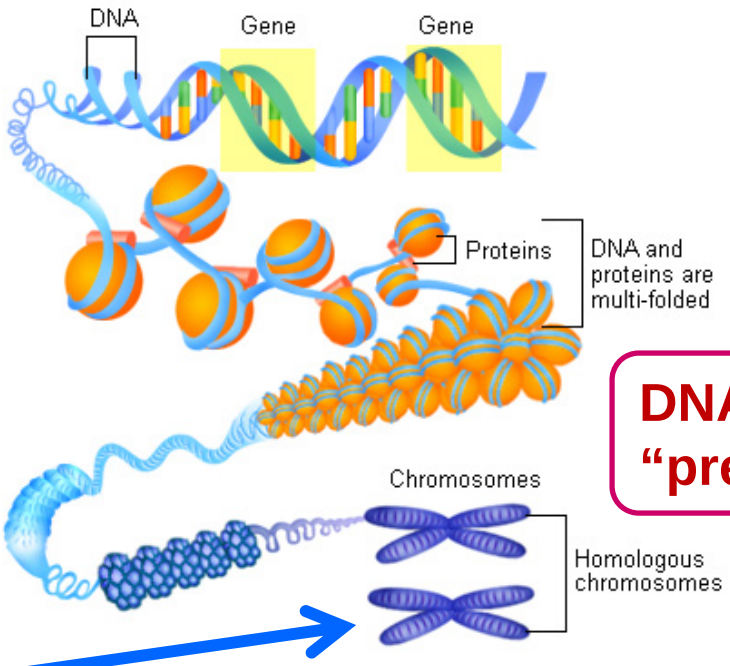
LAser Microbeam Program (LAMP)
NIH Biotechnology Resource



SCIENCE, VOL. 213, 31 JULY 1981

It’s an awful lot of DNA we’ve damaged!





DNA Packaging “pretty intense”

0.5 um is a lot of damaged DNA (kilobases)

Recognition & Repair Proteins Are In Mitosis

(γH2AX is a DBS marker)

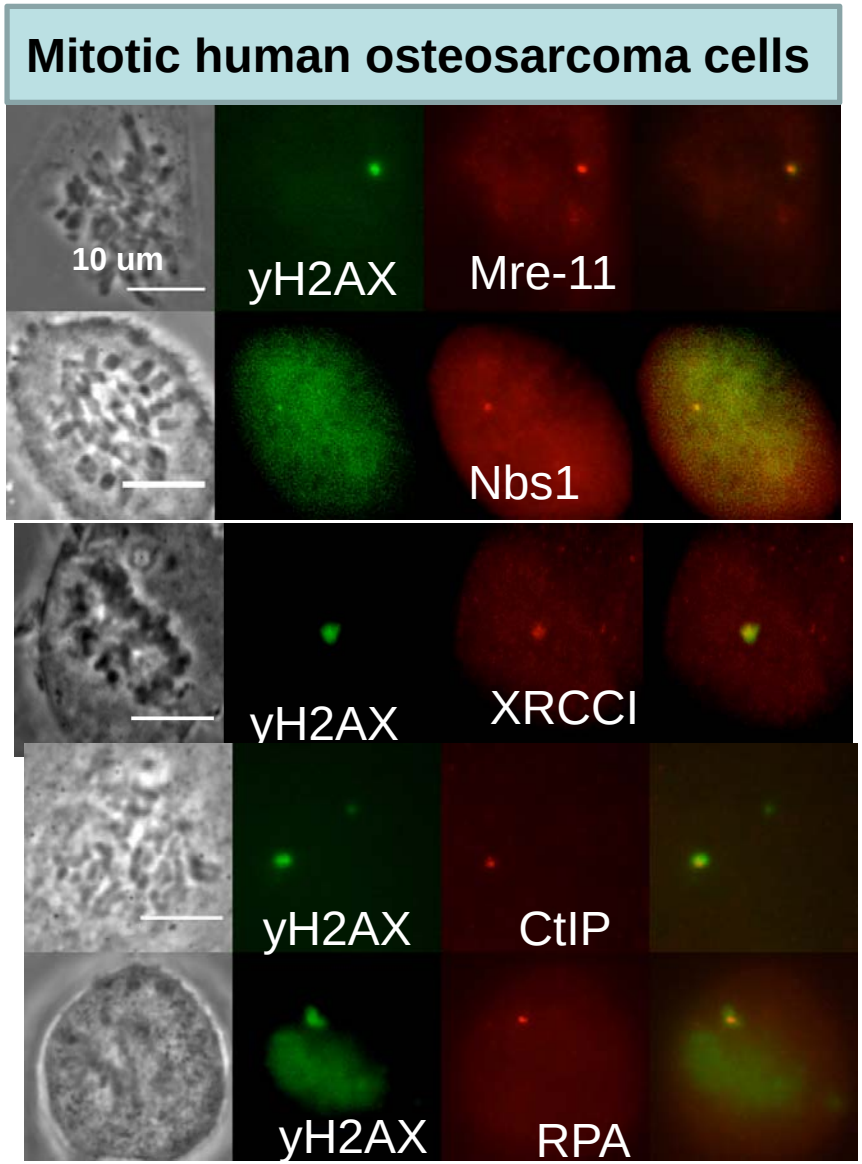
Recognition

γH2AX, NBS1, MRE11, Rad 50, Ku and others

Repair

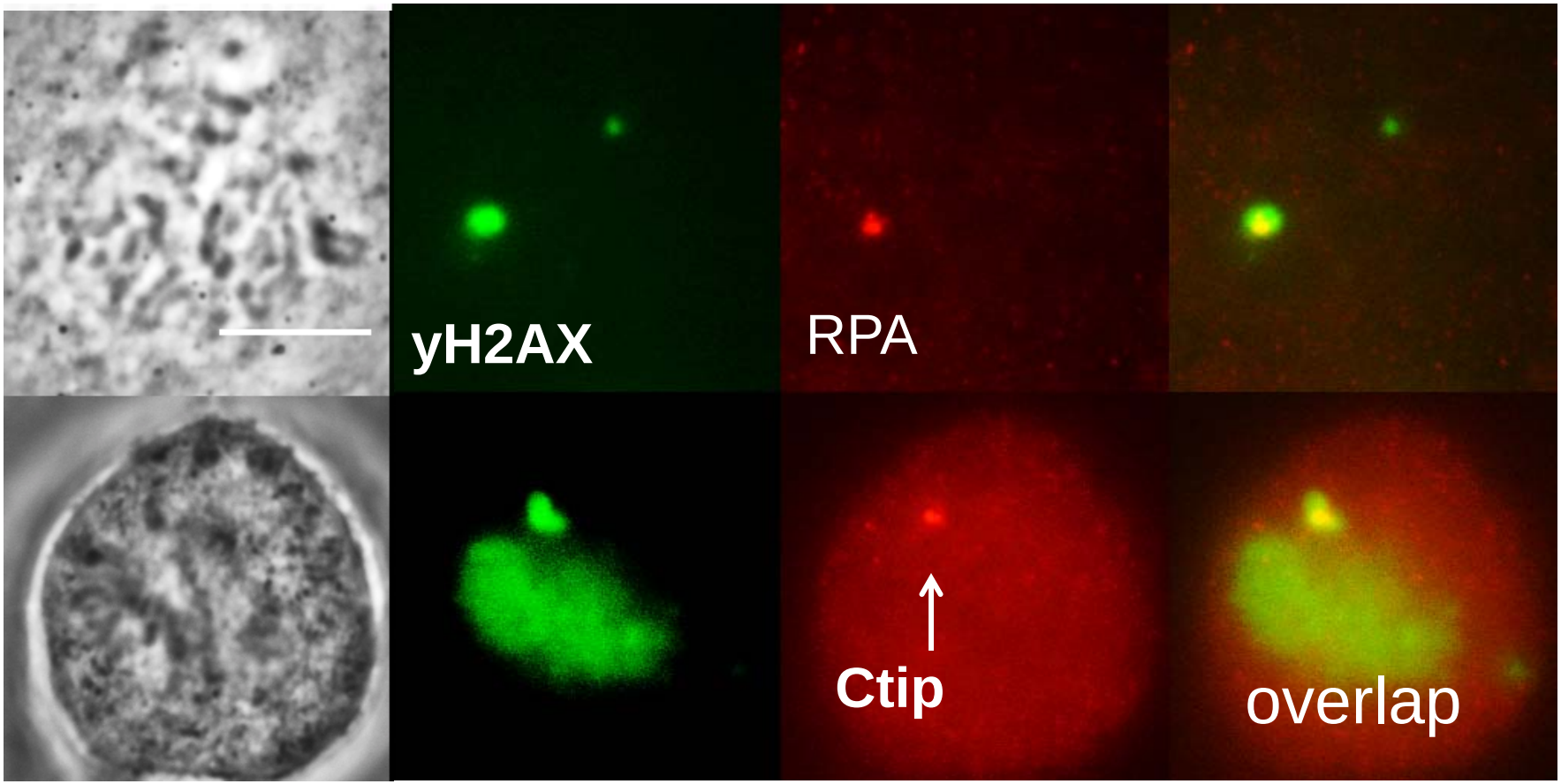
RAD 51, RAD 52, RPA, XRCC1 & 4, CTIP, etc……many more

V.Gomez-Godinez PhD thesis(2012)



More DNA Repair Proteins

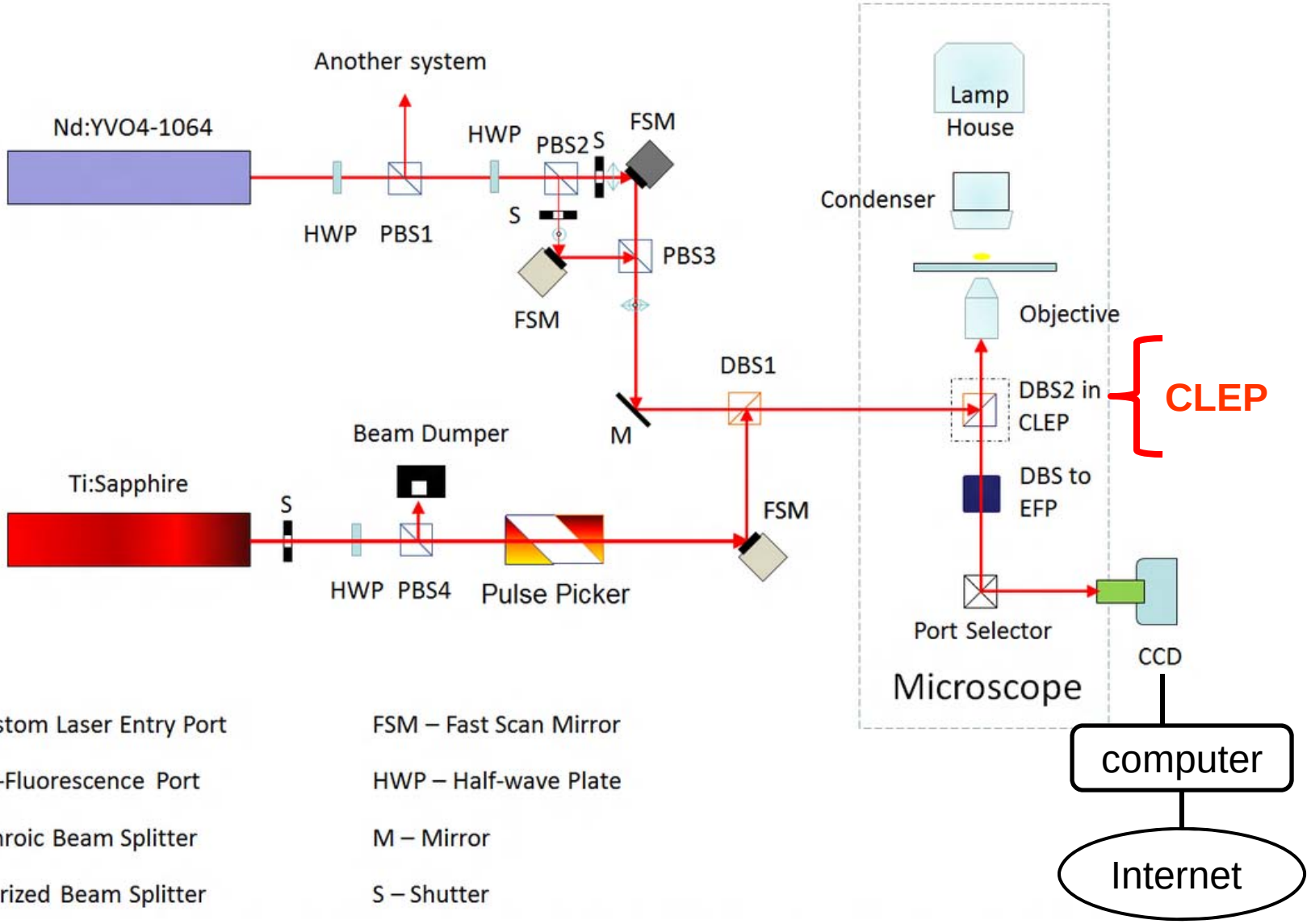
Mitotic Cancer Cells



V.Gomez-Godinez PhD thesis(2012)

RoboLase IV ("Internet Accessible")

(L. Z. Shi , Q. Zhu , S. Parsa, M. S. Harsono, N. Hyun. Botvinick, E.B)



CLEP – Custom Laser Entry Port

EFP – Epic-Fluorescence Port

DBS – Dichroic Beam Splitter

PBS – Polarized Beam Splitter

FSM – Fast Scan Mirror

HWP – Half-wave Plate

M – Mirror

S – Shutter

Berns, M. W., W. H. Wright, B. J. Tromberg, G. A. Profeta, J. J. Andrews and R. J. Walter. Use of a laser-induced optical force trap to study chromosome movement on the mitotic spindle. Proc. Natl. Acad. Sci. USA 86: 4539-4543, 1989.