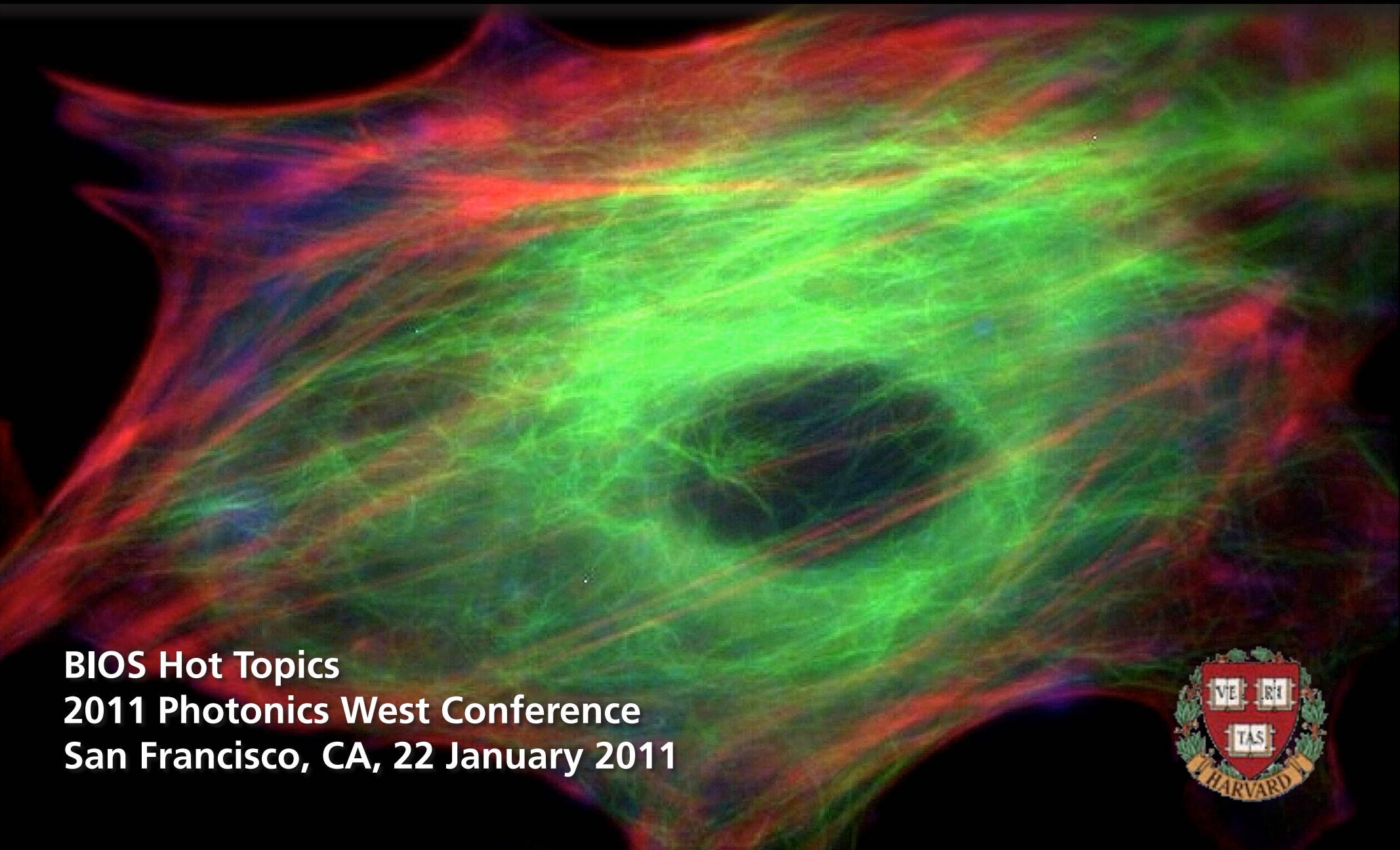
Novel uses of femtosecond laser pulses in biophotonics



BIOS Hot Topics
2011 Photonics West Conference
San Francisco, CA, 22 January 2011

Introduction

short pulse duration $\longrightarrow$ high intensity

Introduction

short pulse duration $\longrightarrow$ high intensity

(even at low energy)

Introduction

high intensity: **disruption**

Introduction

high intensity: **disruption**

low energy: **minimize colateral damage**

Introduction

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Supplementary information accompanies this communication on *Nature*'s website.
Competing financial interests: declared none.

Cell biology

Targeted transfection by femtosecond laser

The challenge for successful delivery of foreign DNA into cells *in vitro*, a key technique in cell and molecular biology with important biomedical implications, is to improve transfection efficiency while leaving the cell's architecture intact. Here we show that a variety of mammalian cells can be directly transfected with DNA without perturbing their structure by first creating a tiny, localized perforation in the membrane using ultrashort (femtosecond), high-intensity, near-infrared laser pulses. Not only does this superior optical technique give high transfection efficiency and cell survival, but it also allows simultaneous evaluation of the integration and expression of the introduced gene.

Previous techniques that have been developed for transfection of cells with DNA[1] include carrier-mediated transfer[2] and transfer by plasma-membrane permeabilization[3], as well as direct transfer[4], but the efficiency of delivery by these methods is low. Moreover, none allows trans-

mediated by intense near-infrared femtosecond laser pulses. Cells were suspended inside a sterile miniaturized cell chamber in 0.5 ml culture medium containing 0.2 μg plasmid DNA vector pEGFP-N1 (4.7 kilobases) encoding enhanced green fluorescent protein (EGFP)[7]. Transmission images of cells were obtained at low power (<5 μW), and the near-infrared laser beam was then focused (under the same microscope) on the edge of the membrane of a target cell, which was exposed to an enhanced mean laser power of 50–100 mW for 16 ms so that transfection could occur. More than 200 cells of each type were targeted in each of 18 replicate experiments; it took 10–15 s to prepare for the transfection of each cell.

We assessed the integration and expression efficiency of the EGFP gene *in situ* by time-lapse two-photon fluorescence imaging[8] at a mean laser power of <1 mW over a period of 72 h, as well as by two-photon fluorescence-lifetime imaging (TPFLIM)[9]. Figure 1 shows that diffraction-limited focusing of intense femtosecond near-infrared laser pulses selectively facilitates transfection of the target cells, but not of the adjacent cells. Expression of EGFP in the transfected cells is also demonstrated by TPFLIM, and the measured fluorescence lifetime of about 2.4 ns is consistent with that reported for cells expressing EGFP[10]. ... type, the transfection ... invariably

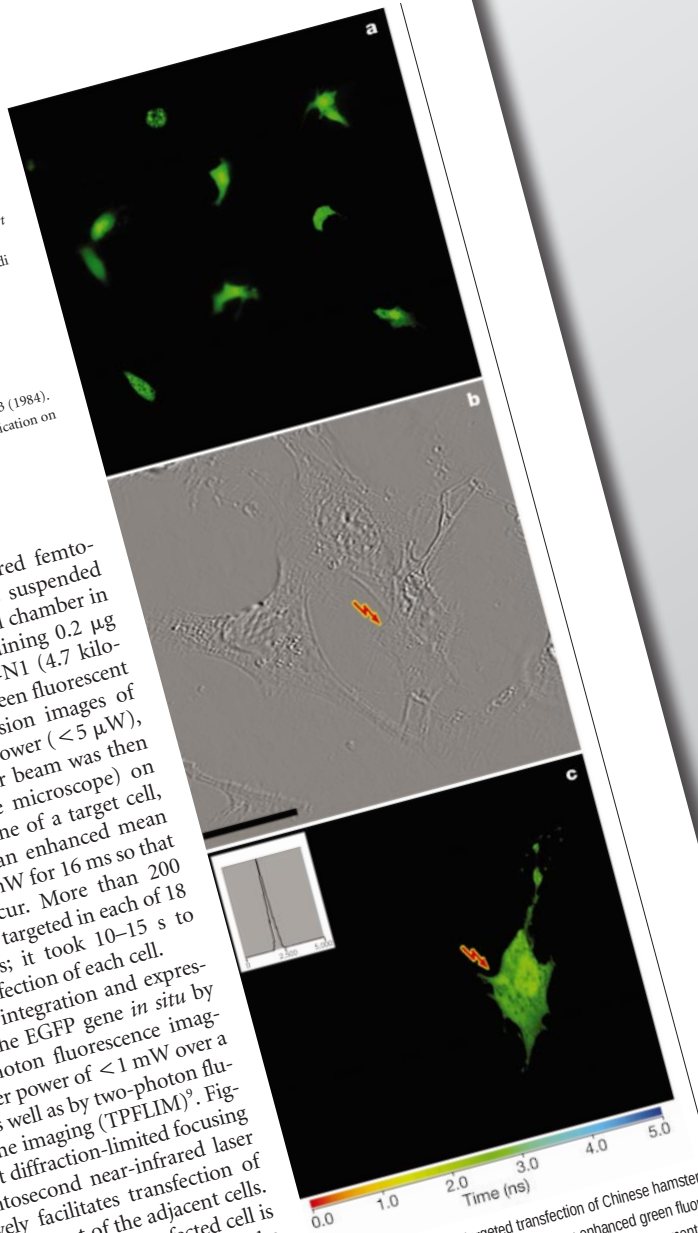
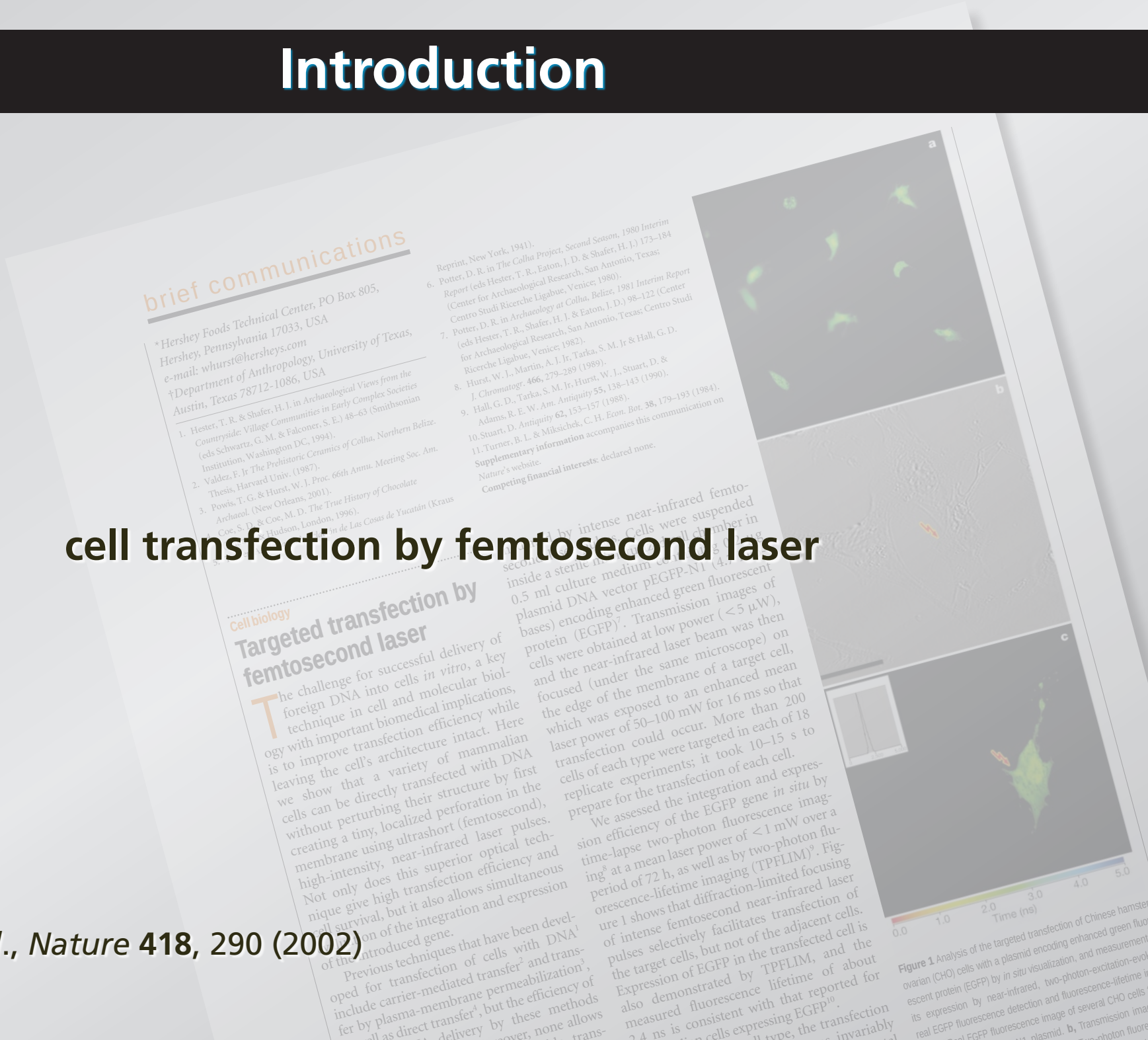


Figure 1 Analysis of the targeted transfection of Chinese hamster ovarian (CHO) cells with a plasmid encoding enhanced green fluorescent protein (EGFP) by *in situ* visualization, and measurement of its expression by near-infrared, two-photon-excitation-evoked real EGFP fluorescence detection and fluorescence-lifetime ... EGFP fluorescence image of several CHO cells ... plasmid. **b,** Transmission ... two-photon fluores...

Introduction

cell transfection by femtosecond laser

Tirlapur, *et al.*, *Nature* **418**, 290 (2002)



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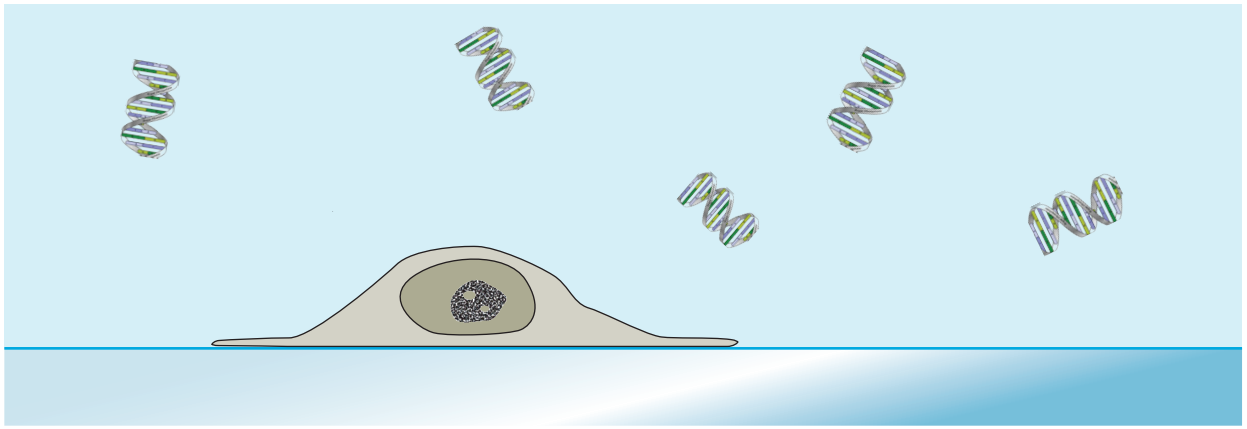

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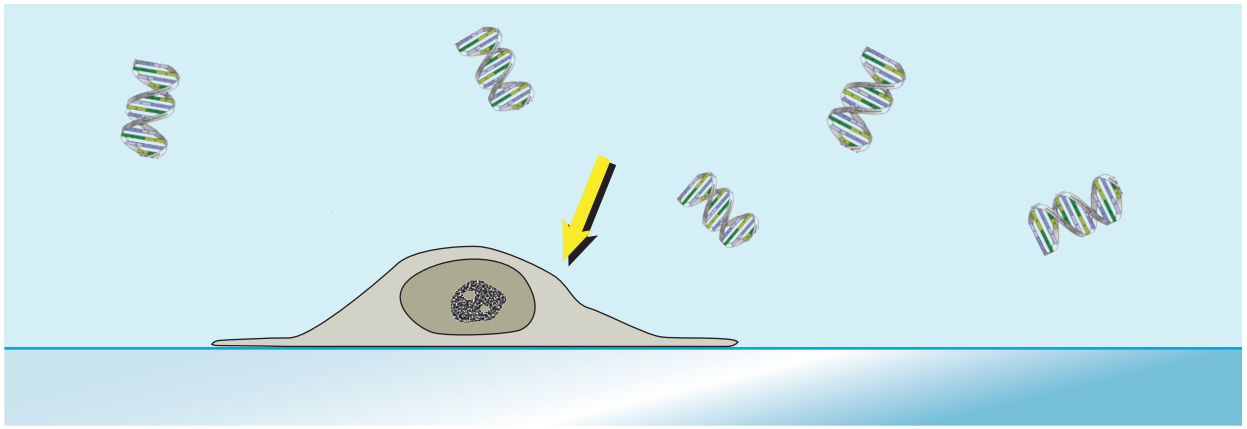

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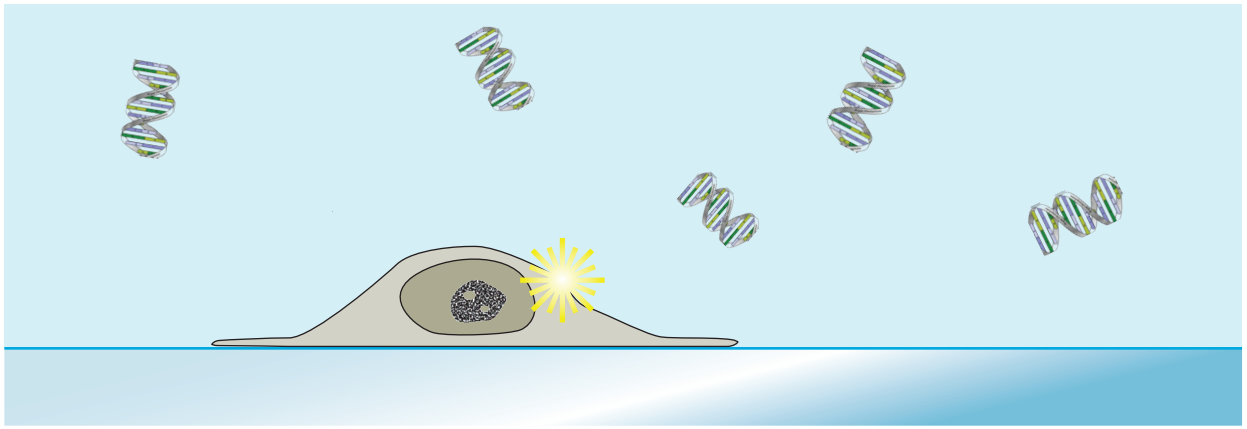

Tirlapur, *et al.*, *Nature* **418**, 290 (2002)

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L
Naked DNA	VL	L	H	L
Polymer/lipid	M	M	H	H
Viral transfection	M	H	H	H
Electroporation	H	H	H	L

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Viral transfection	M	H	H	H
Electroporation	H	H	H	L
Laser poration	VL	H	VL	L

Cell transfection

	Toxicity	Efficiency	Throughput	Specificity
Goal	VL	H	H	L
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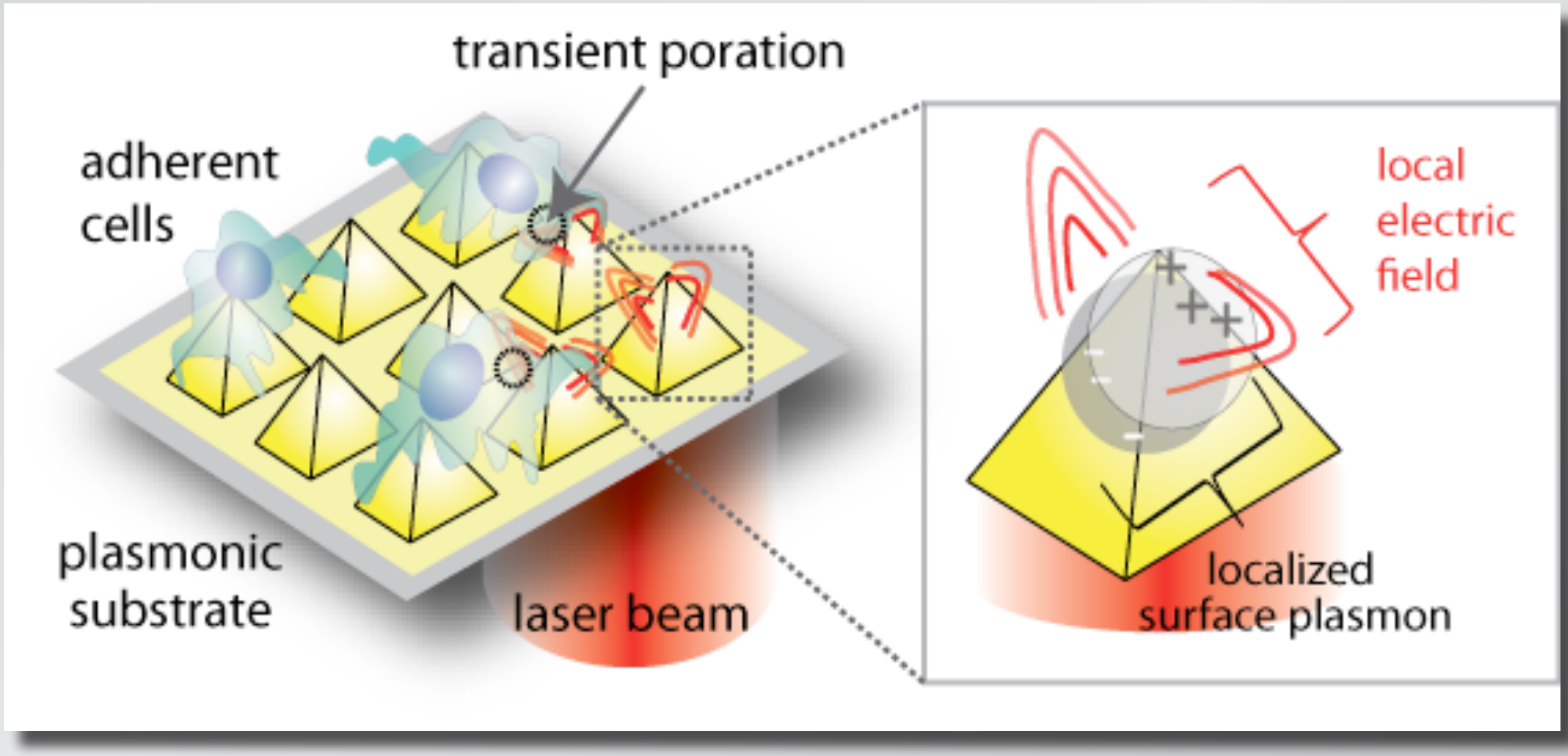
Electroporation	H	H	H	L
Laser poration	VL	H	VL	L

Cell transfection

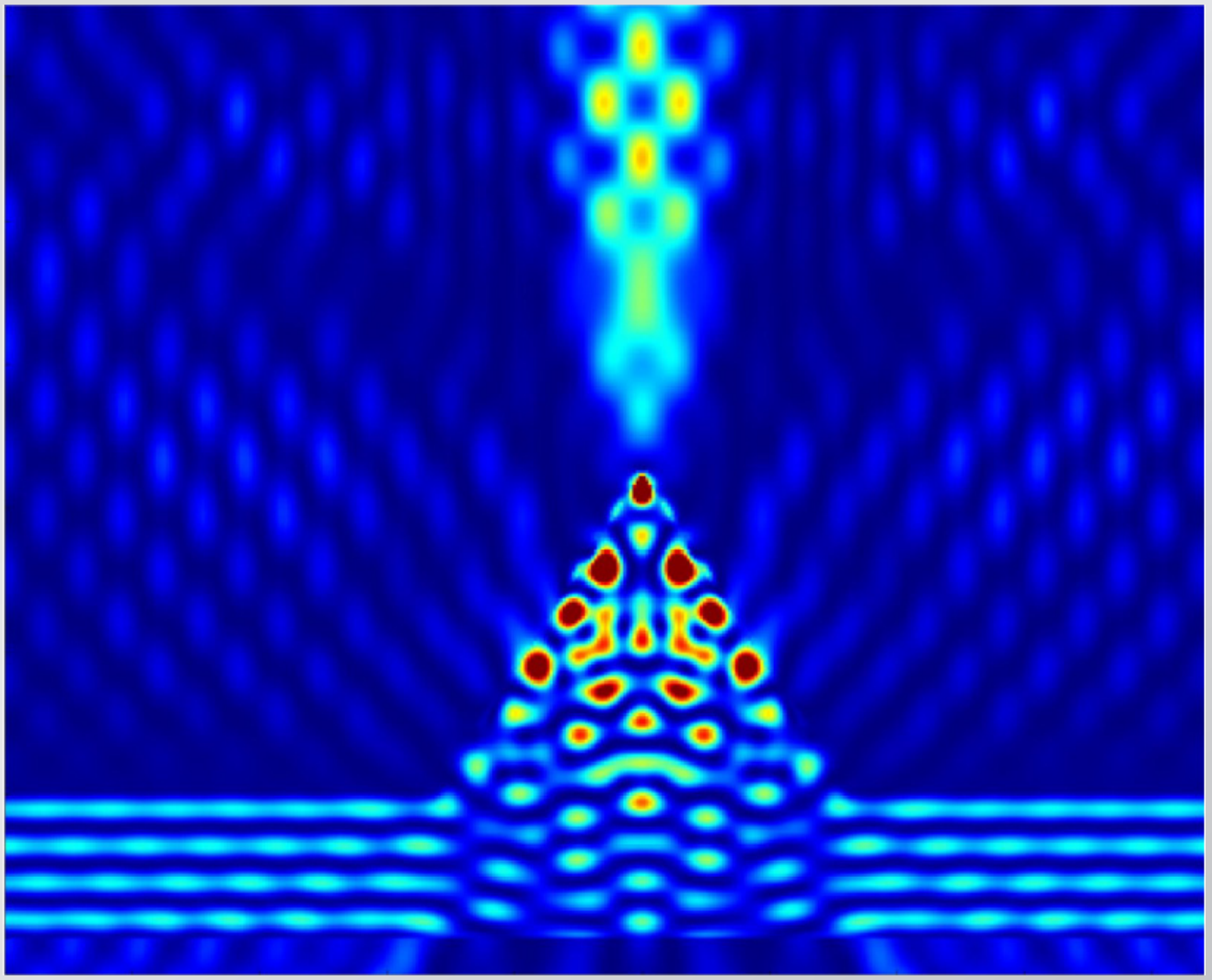
use structured plasmonic substrate

Cell transfection

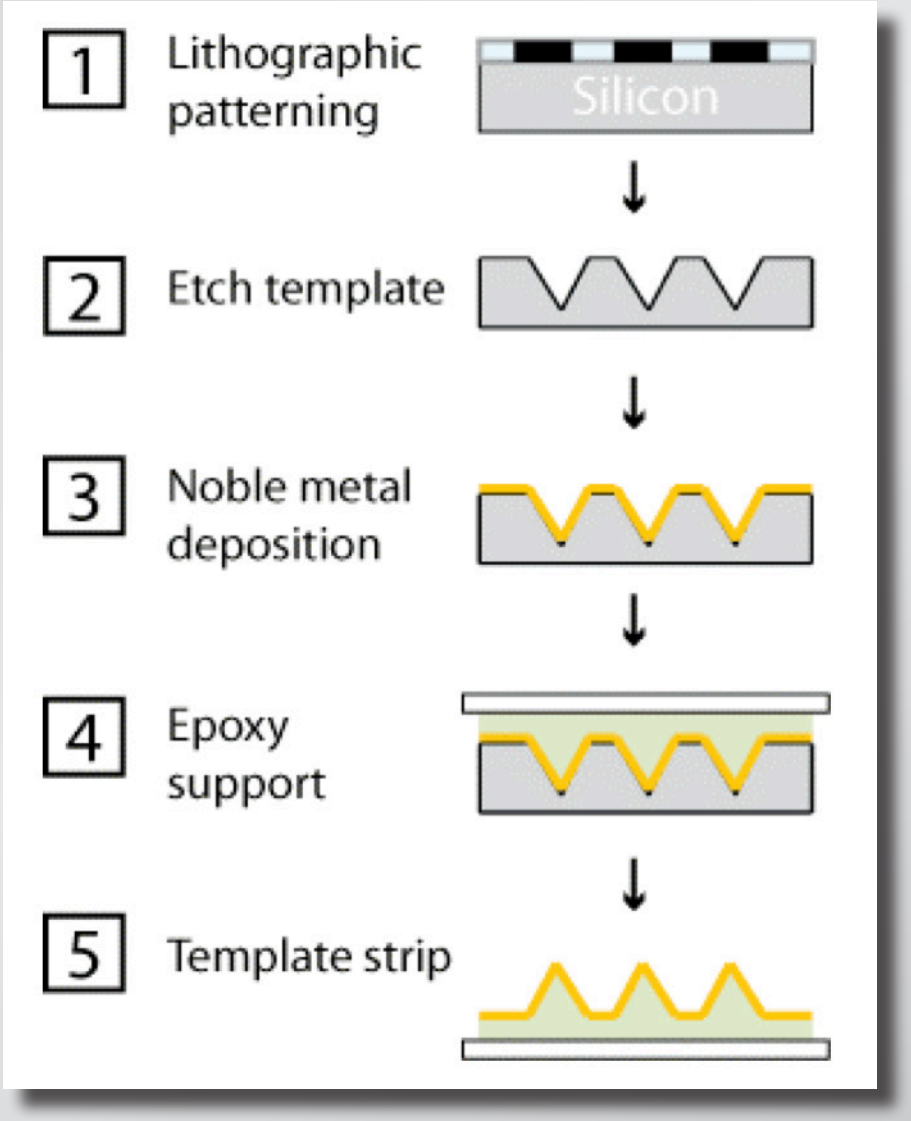


Cell transfection

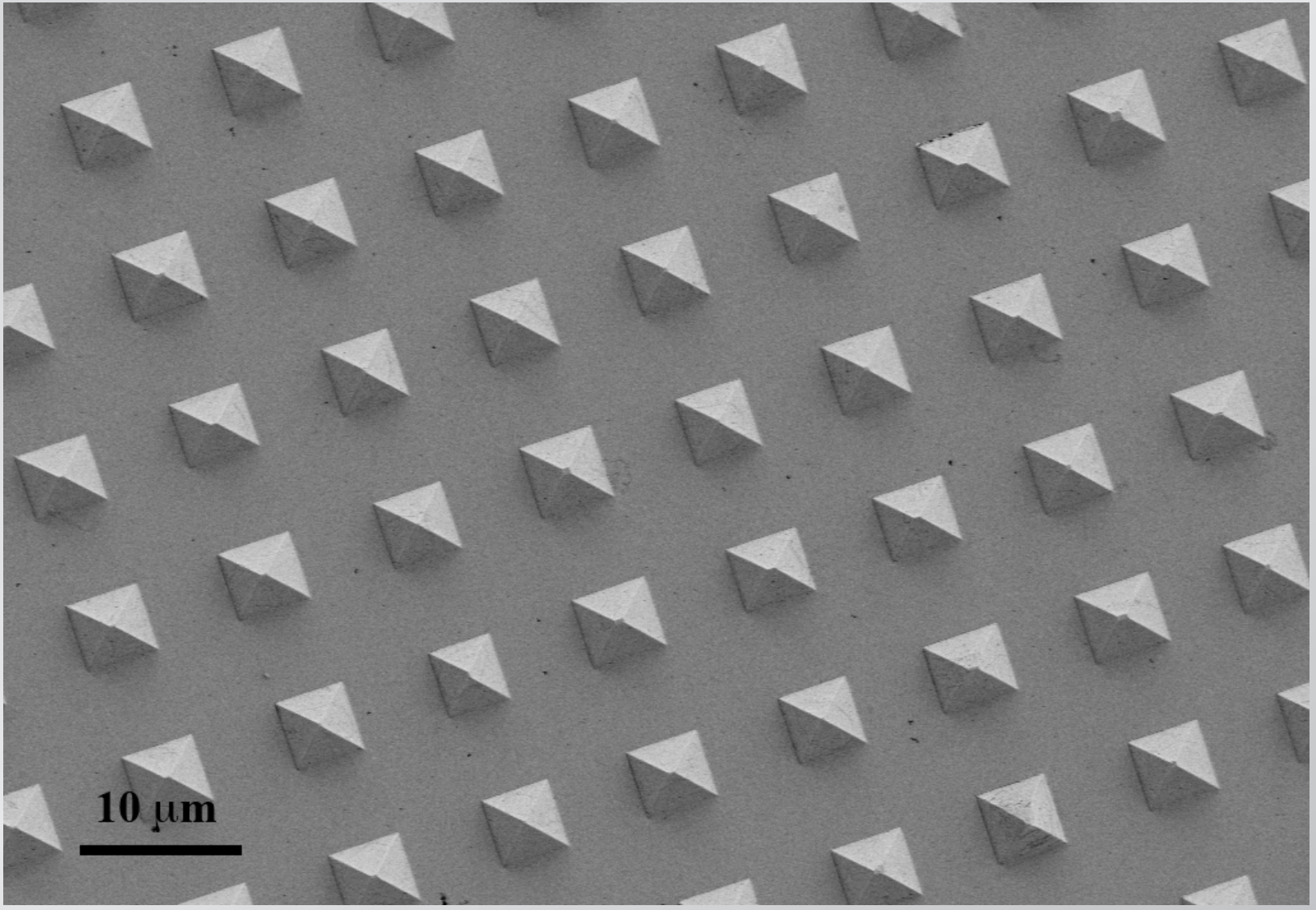
field enhancement at tip



Cell transfection

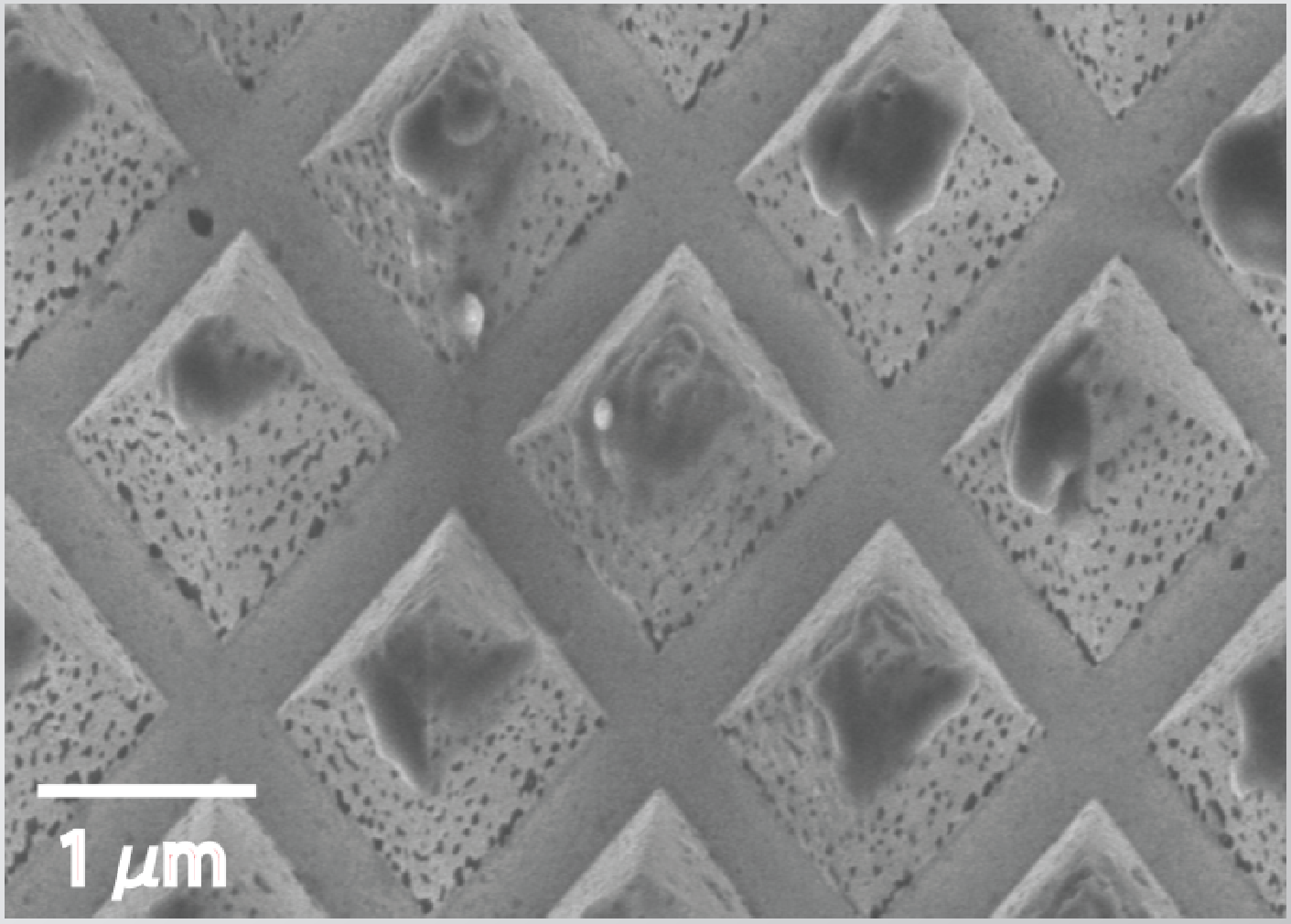


Cell transfection



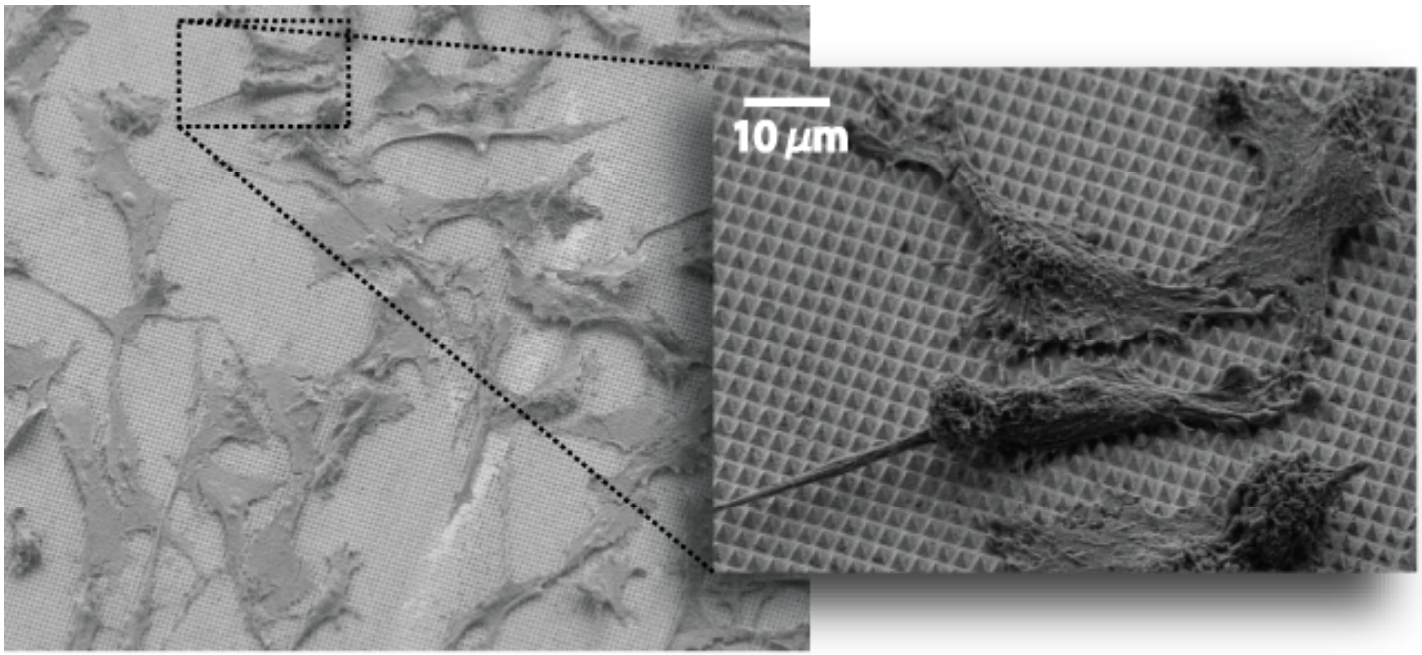
Cell transfection

two-photon polymerization enhancement



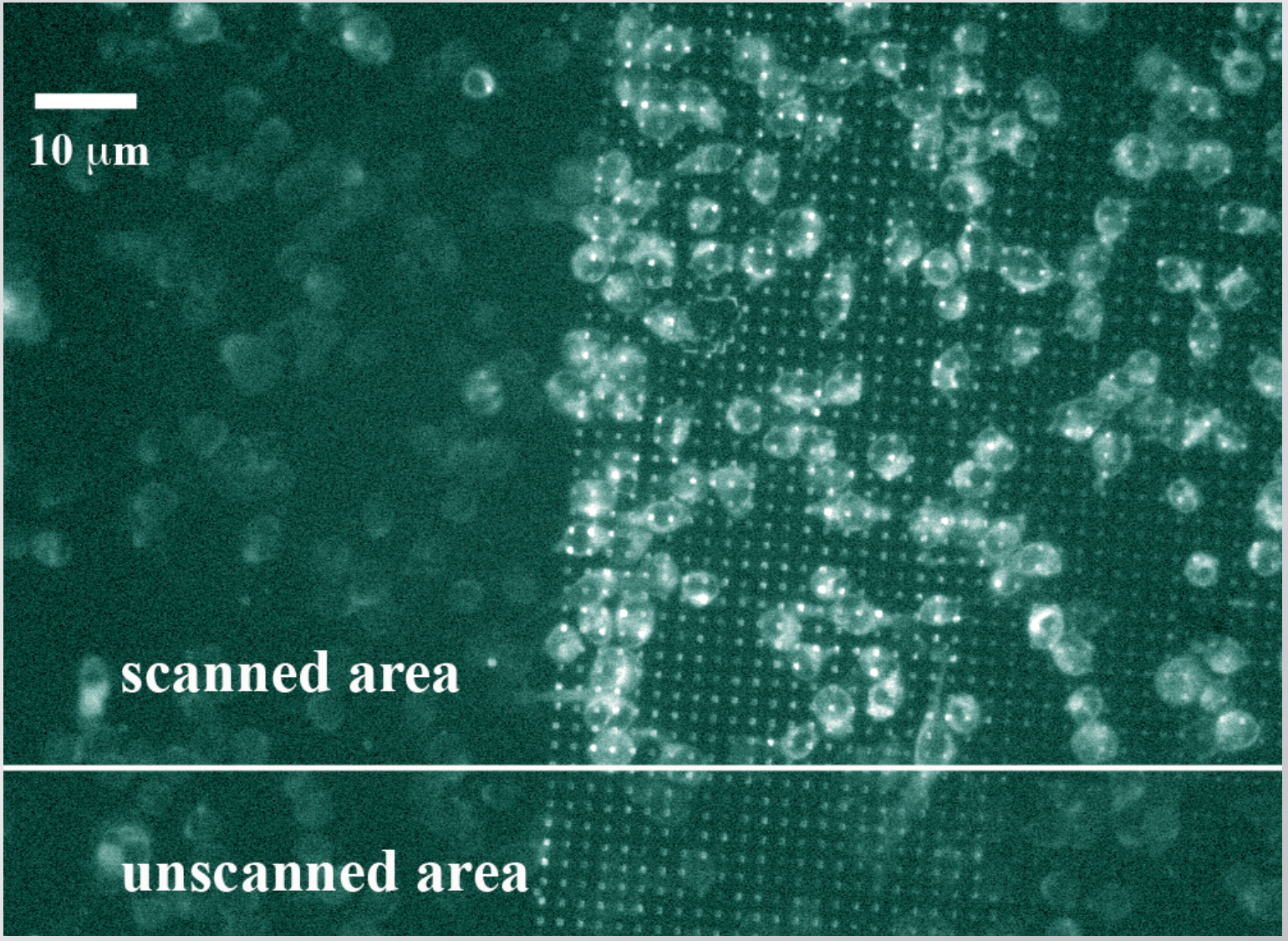
Cell transfection

attachment of TE cells on pyramid arrays



Cell transfection

only exposed cells on pyramids take up dye



Cell transfection

full details:

Paper 7911-17 tomorrow @ 3:50 pm

Subcellular surgery

can we probe the dynamics of the cytoskeleton?

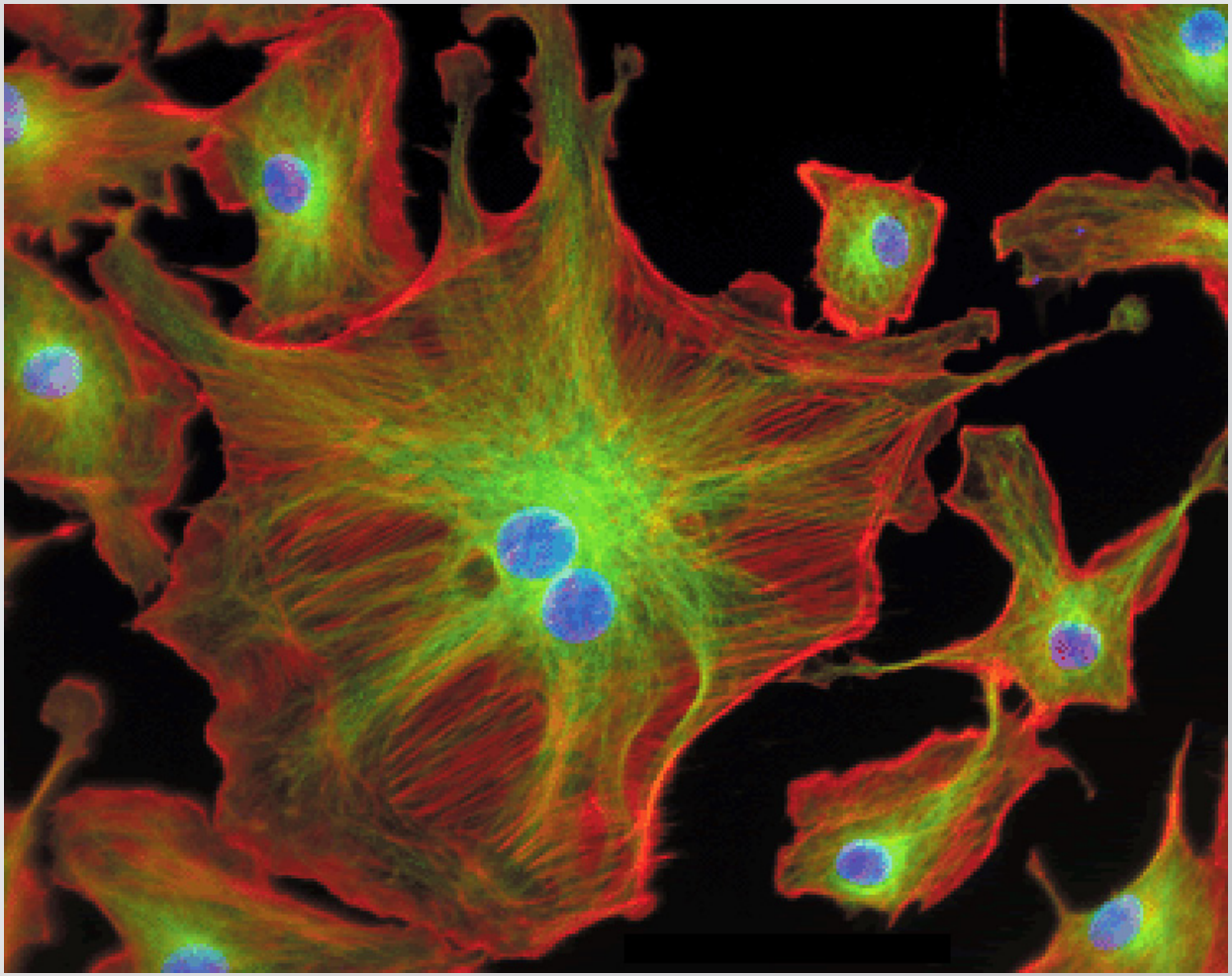
Subcellular surgery

Requirements:

- submicrometer precision (in bulk)
- no damage to neighboring structures
- independent of structure/organelle type

Subcellular surgery

cytoskeleton



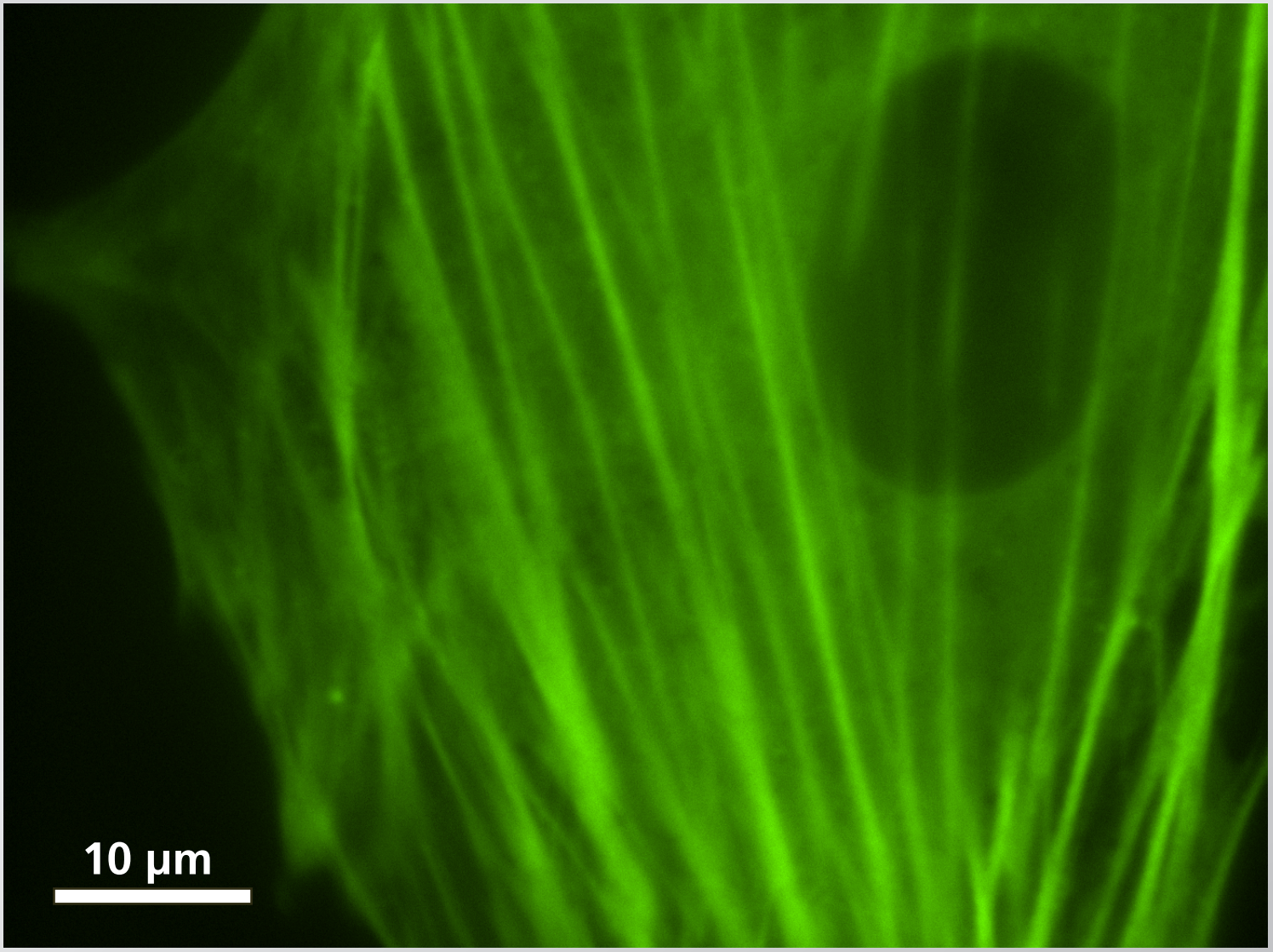
Subcellular surgery

Cytoskeleton

- gives a cell its shape
- provides a scaffold for organelles
- responsible cell motion and attachment
- facilitates intracellular transport and signaling
- required for cell division

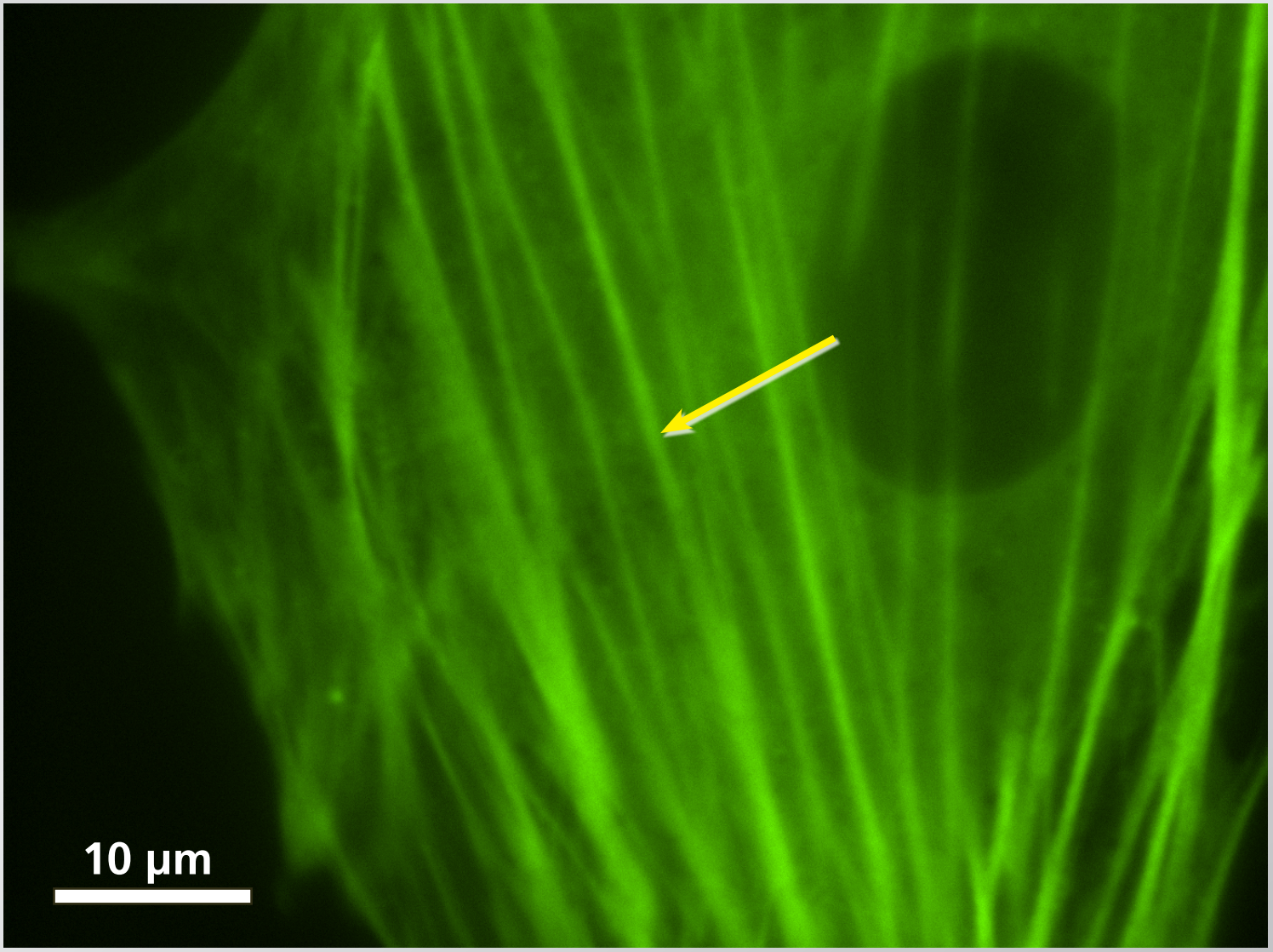
Subcellular surgery

YFP-labeled actin fiber network of a live cell



Subcellular surgery

cut a single fiber bundle



Subcellular surgery

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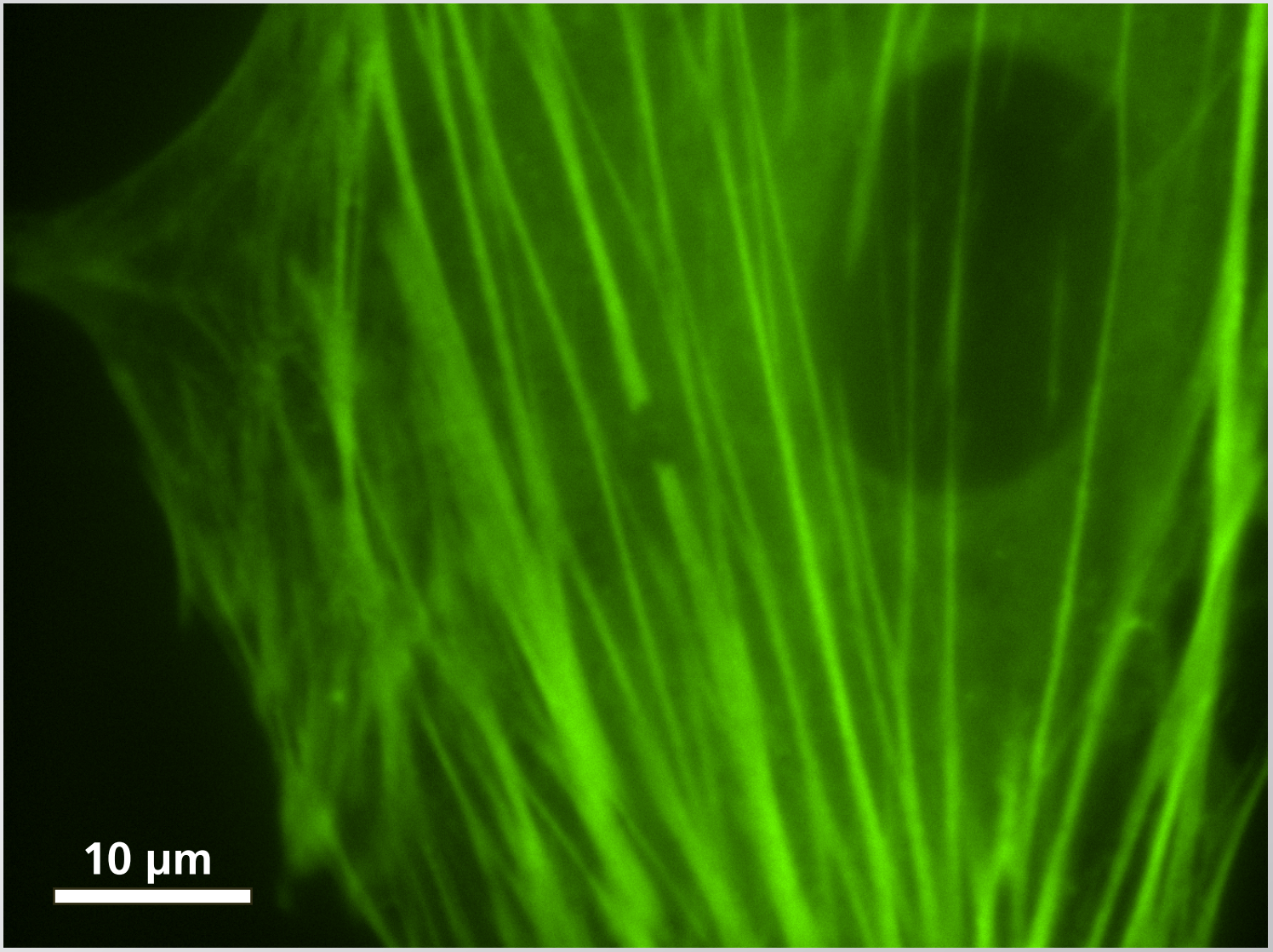
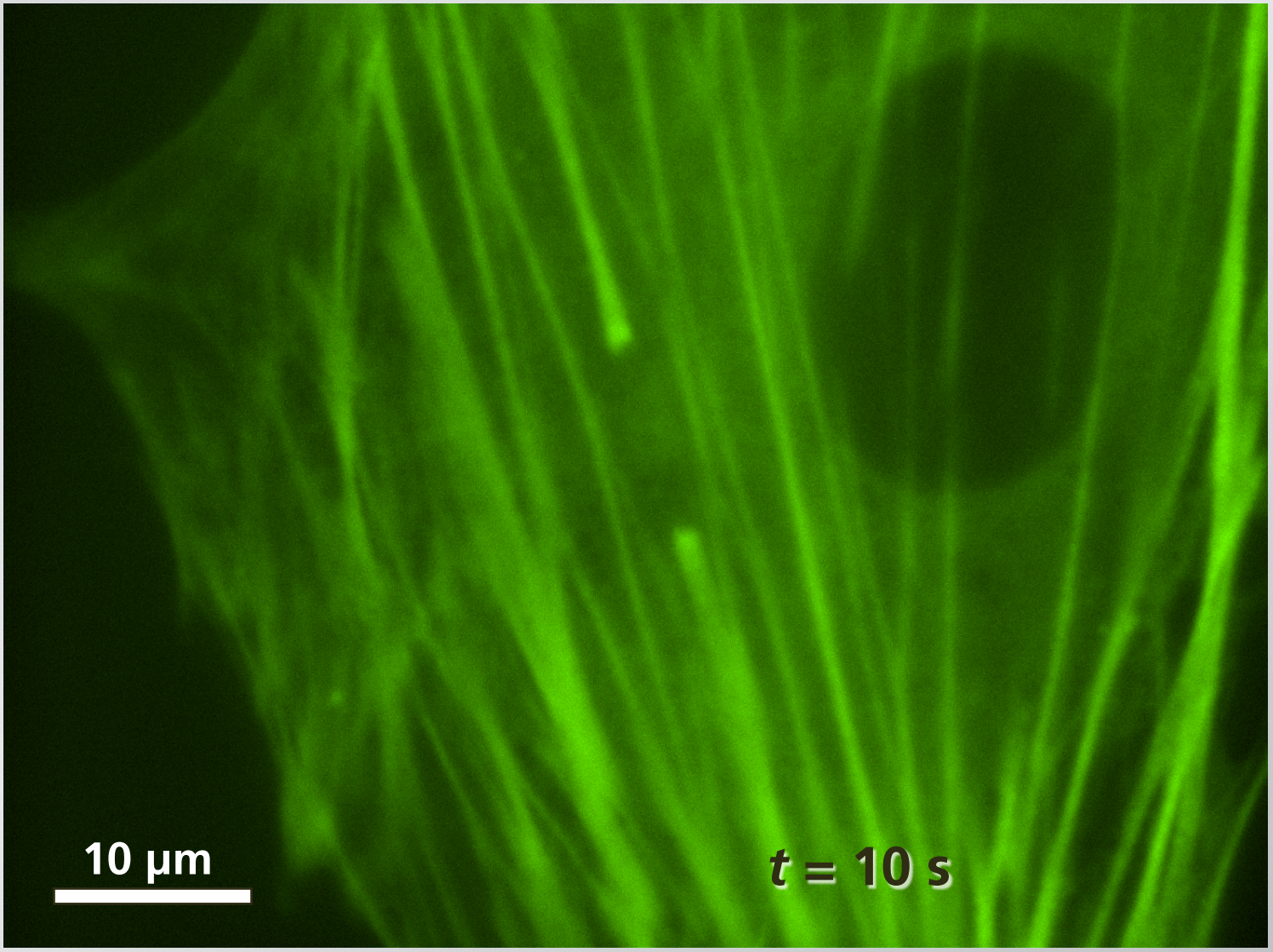
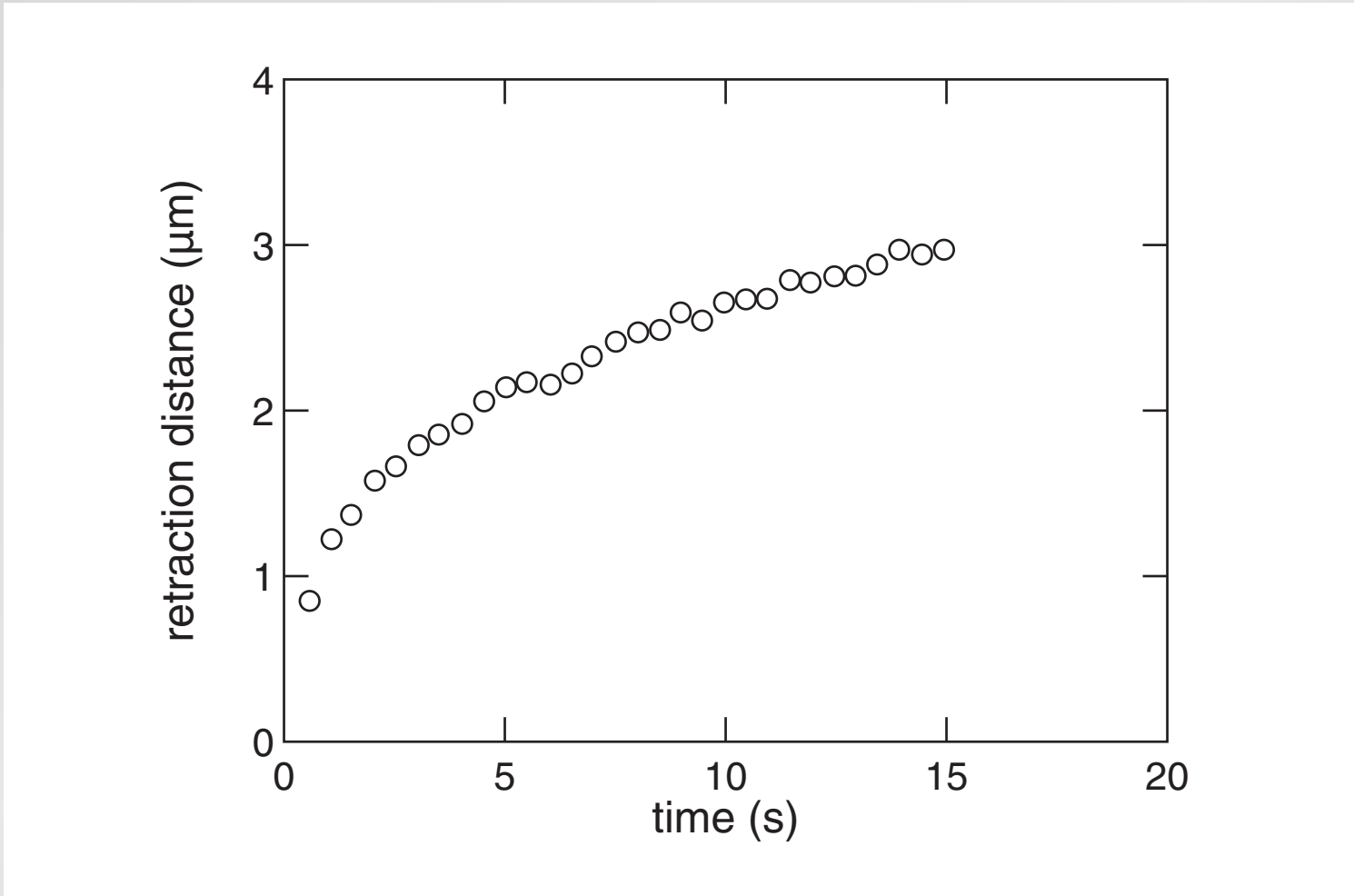


Subcellular surgery

gap widens with time

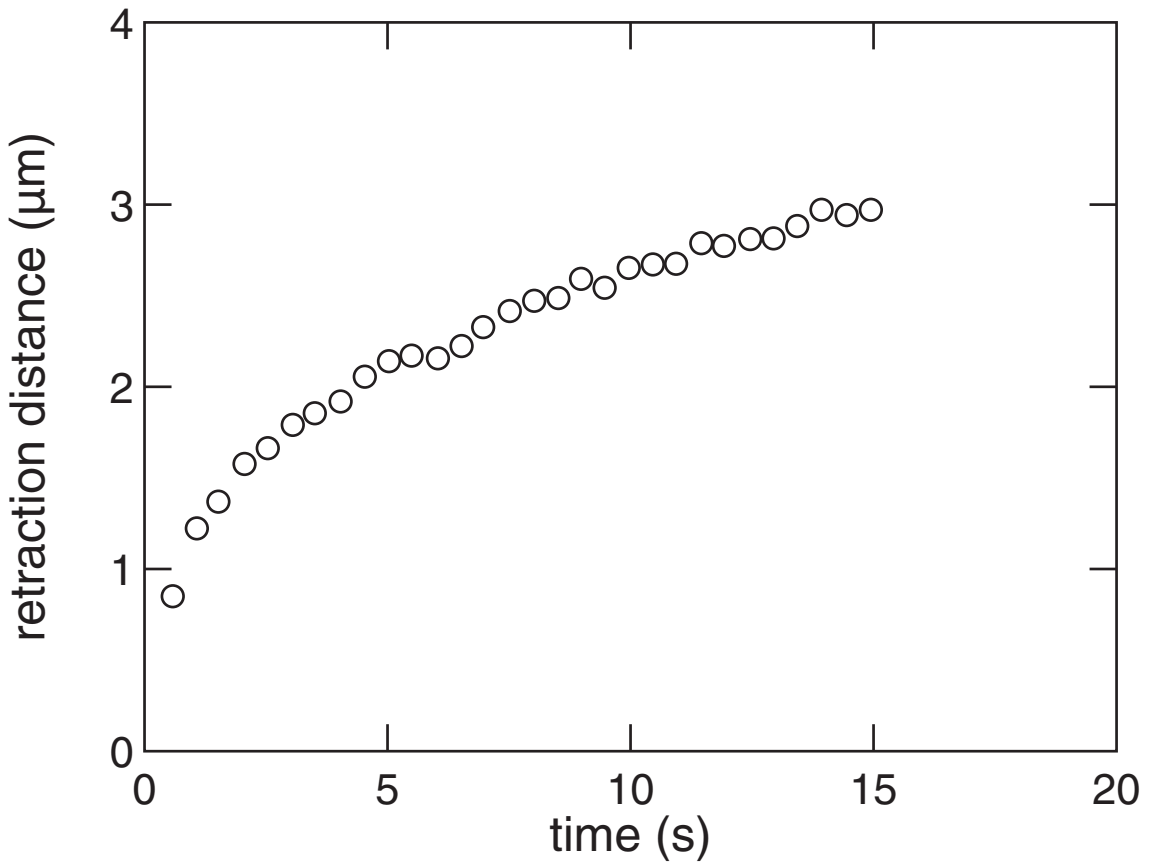


Subcellular surgery



Subcellular surgery

overdamped spring: $\Delta L = L_{\infty}(1 - e^{-t/\tau}) + L_{o}$



Subcellular surgery

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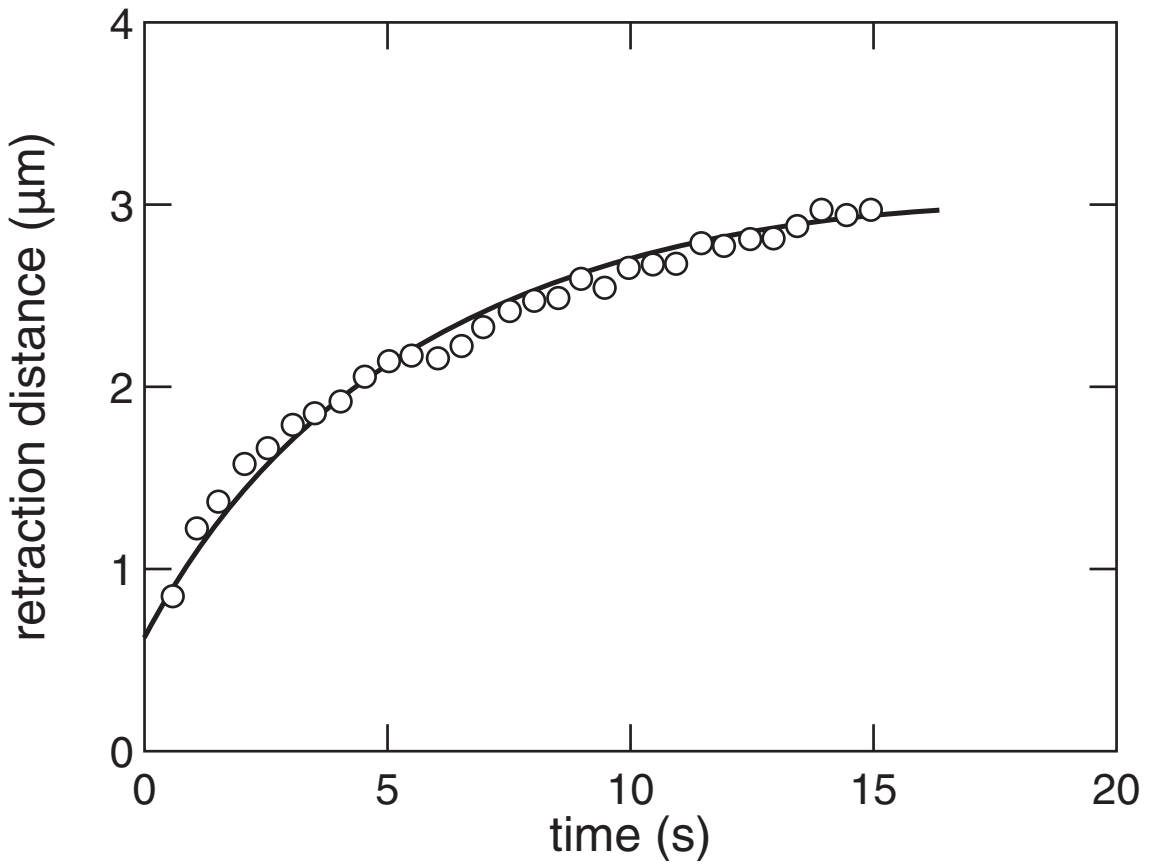
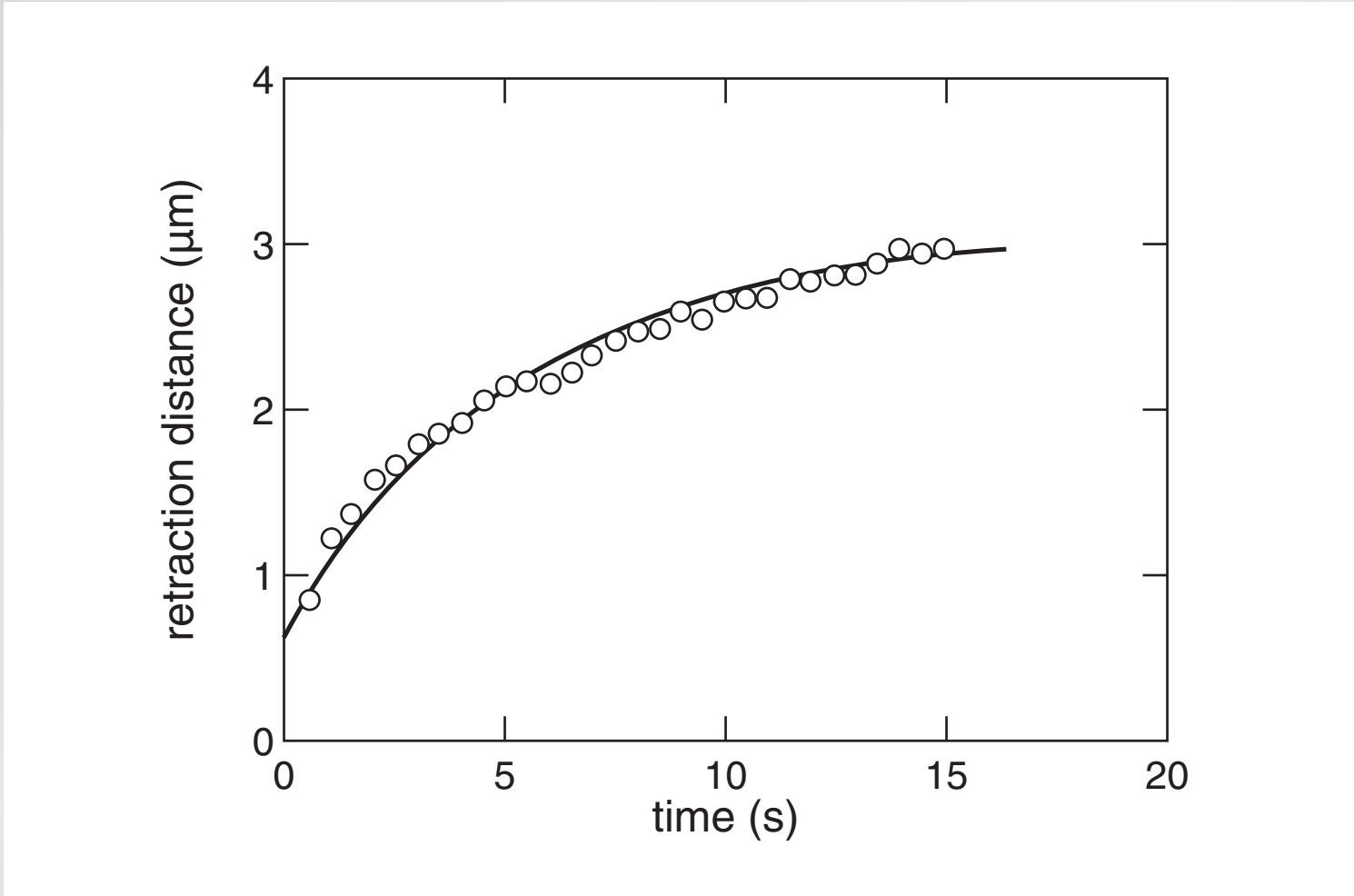


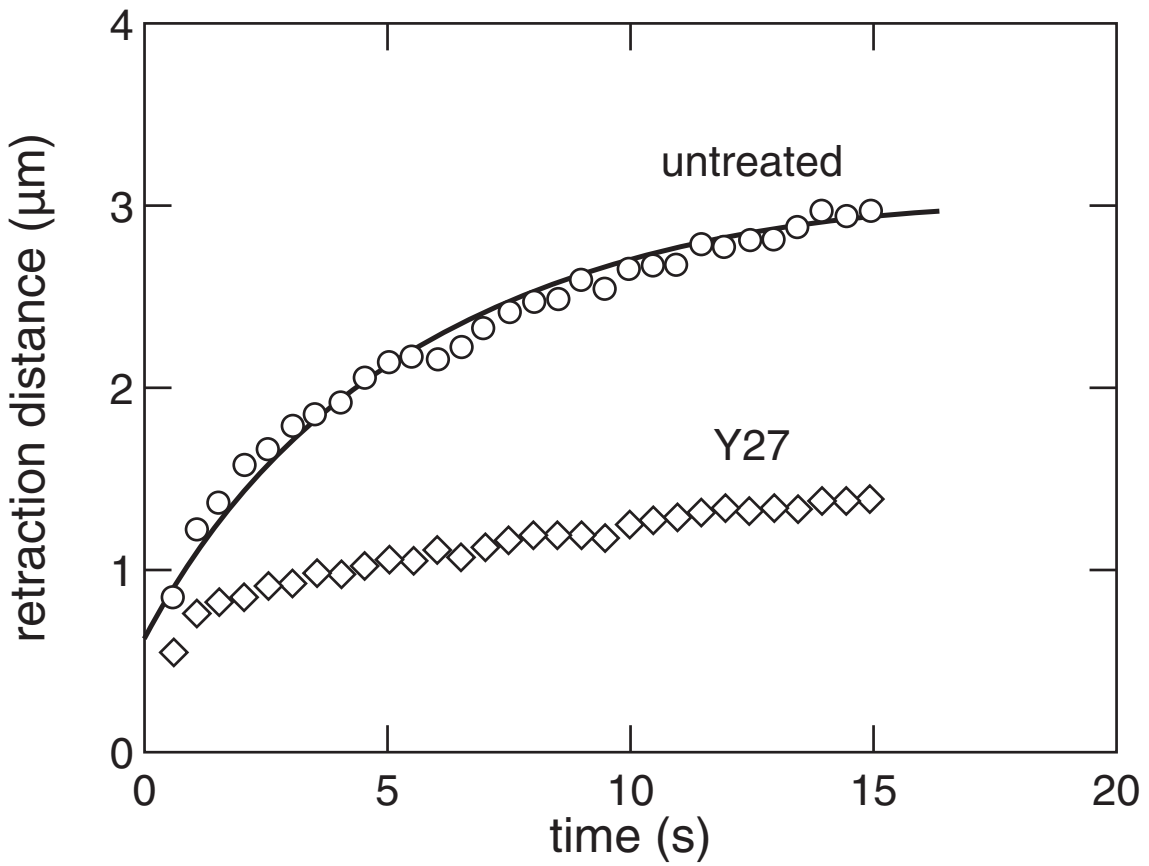
Subcellular surgery

tension in actin filaments is generated by myosin motors



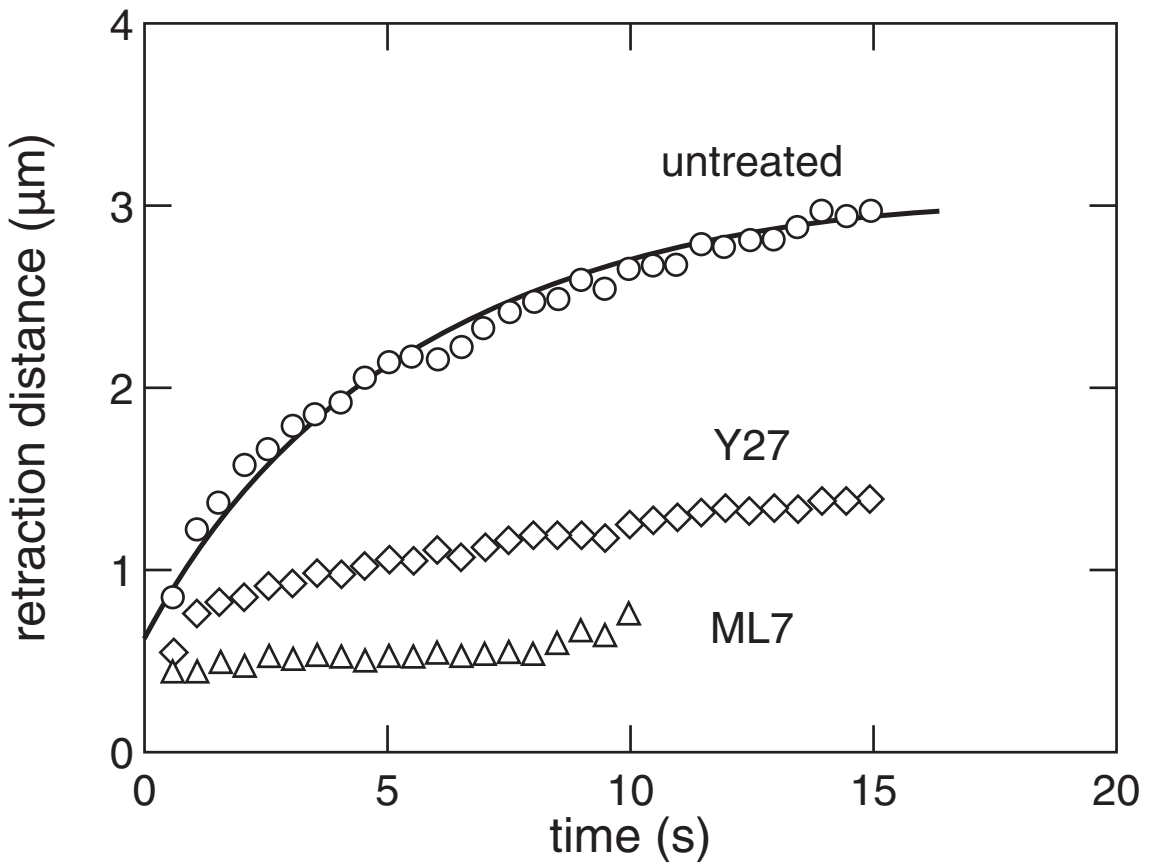
Subcellular surgery

Y27: inhibits some myosin activity

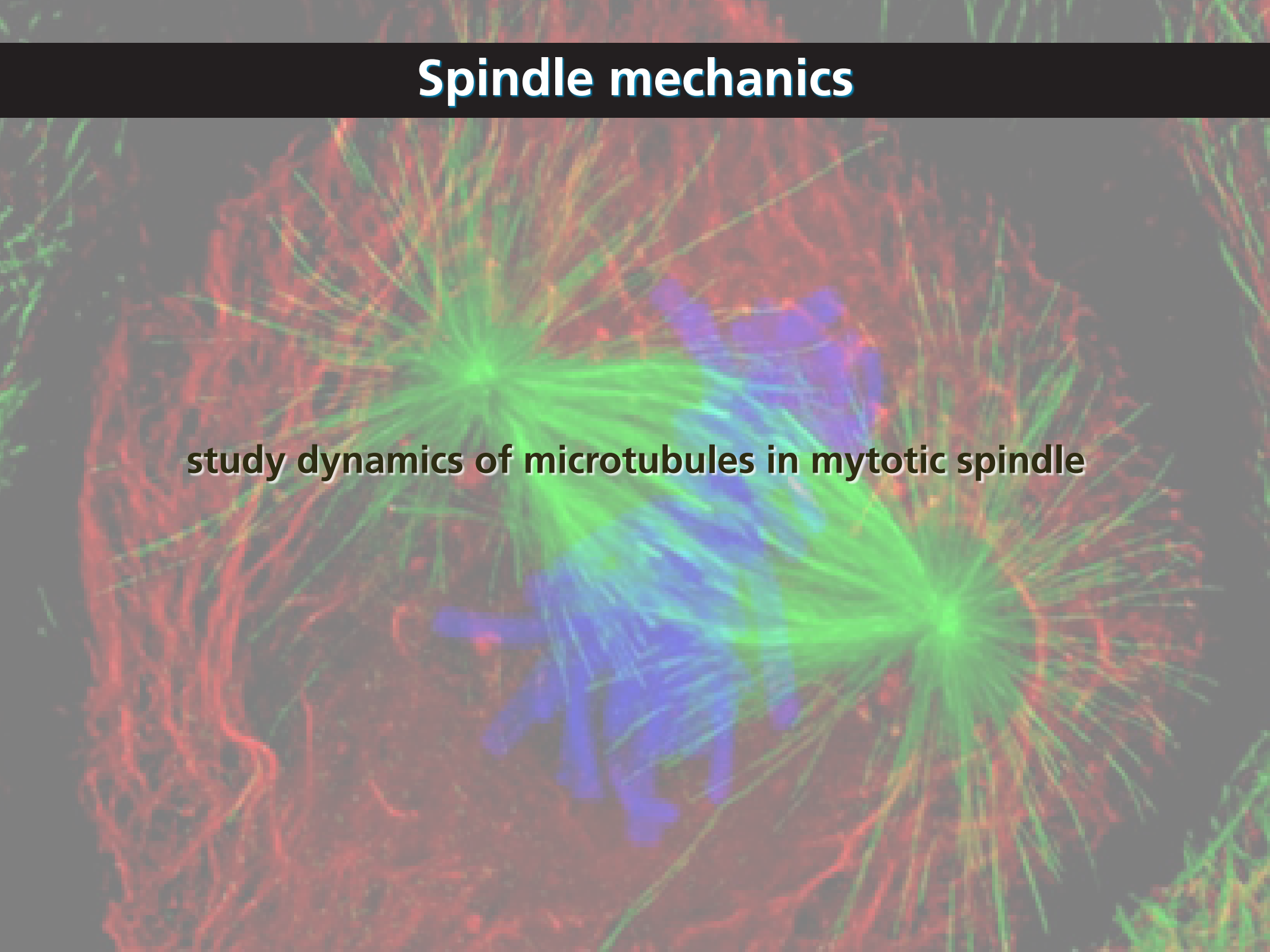


Subcellular surgery

ML7: direct inhibitor of myosin activity



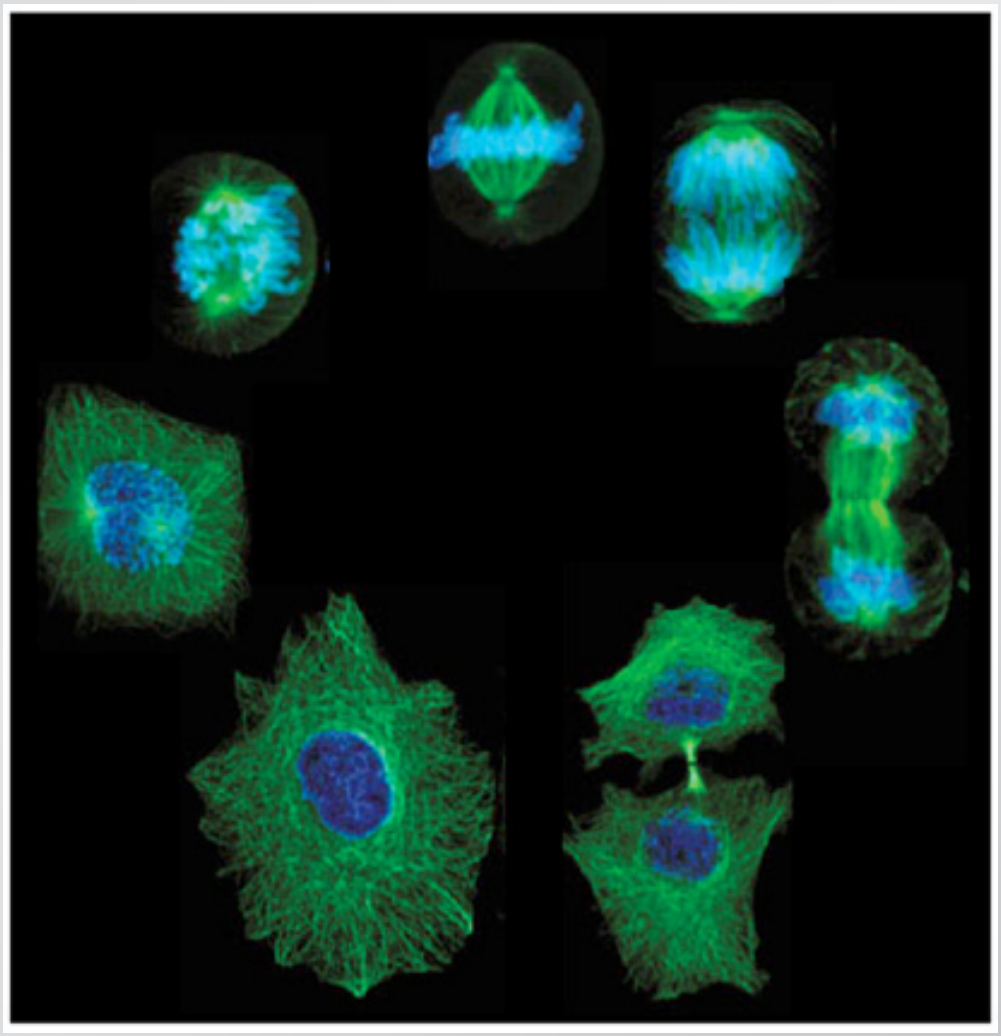
Spindle mechanics



study dynamics of microtubules in mytotic spindle

Spindle mechanics

spindle forms during cell division



Spindle mechanics

spindle forms during cell division

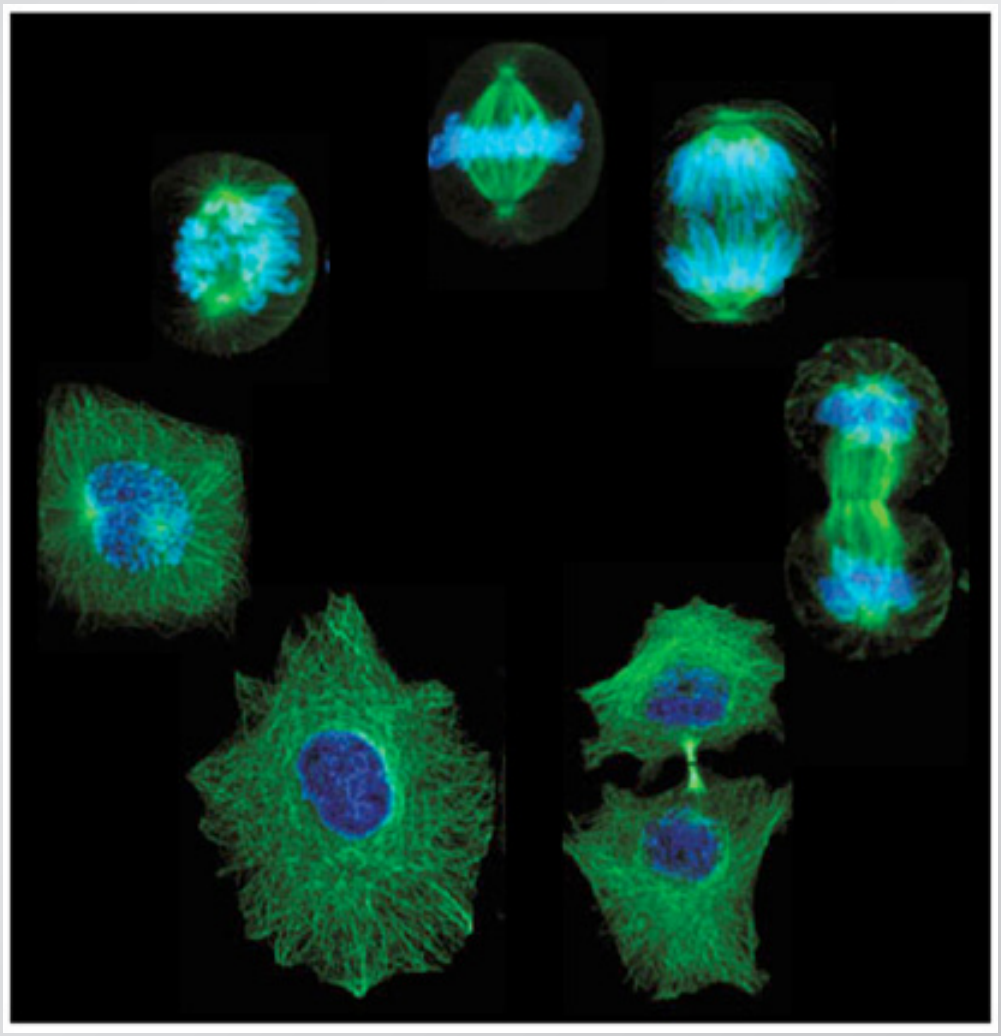
metaphase

prophase

anaphase

telophase

interphase

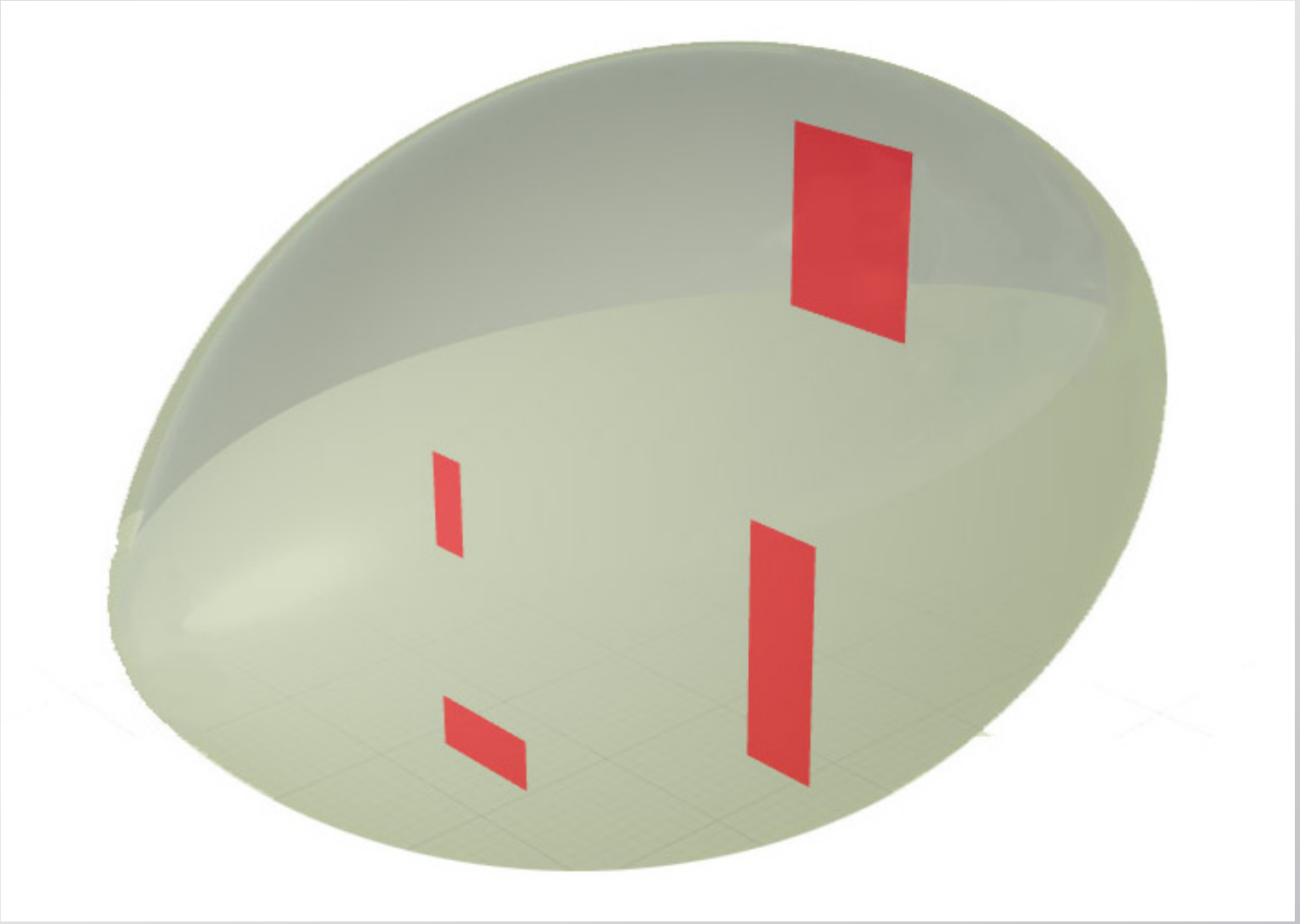


Spindle mechanics

can we determine polarity and length of microtubules?

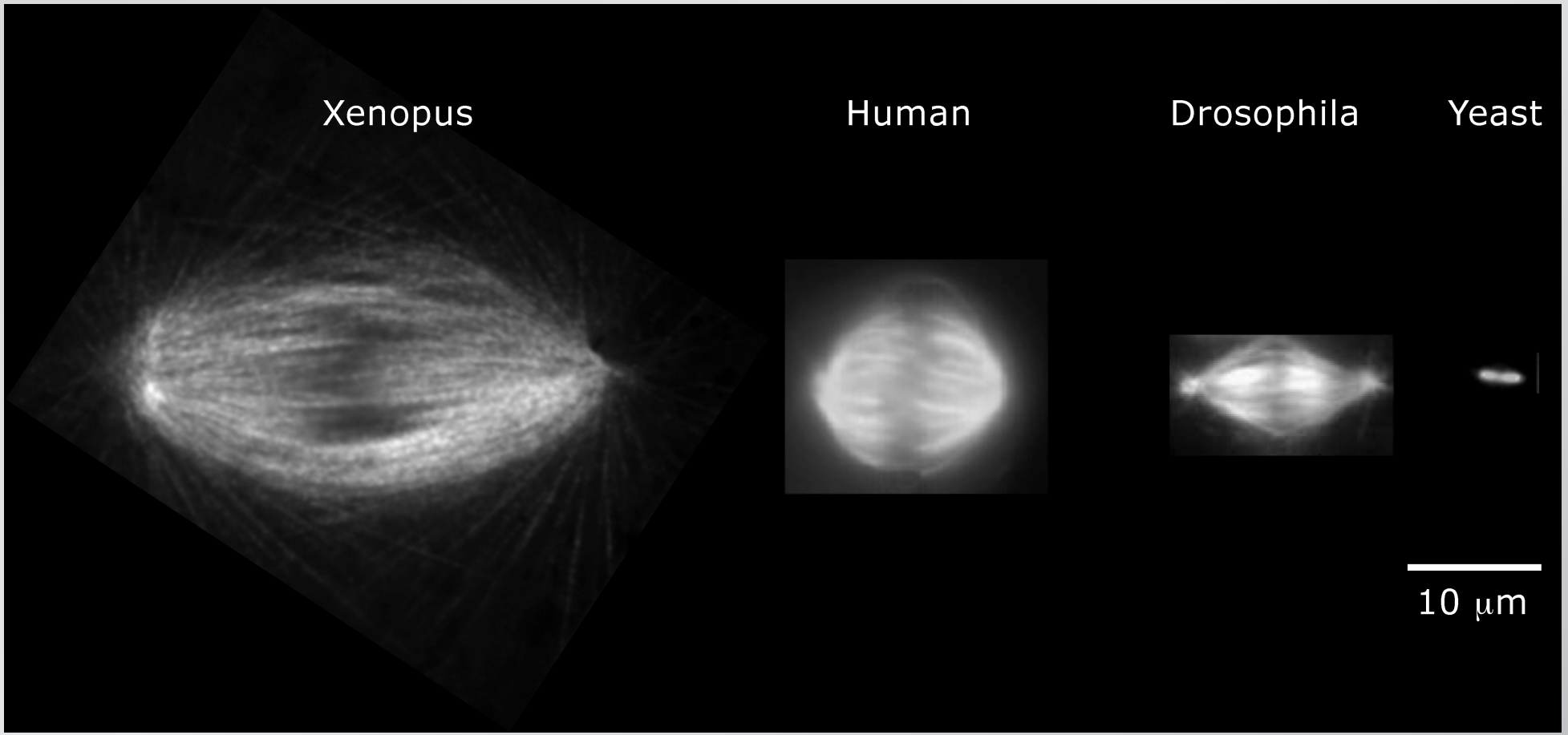
Spindle mechanics

observe depolymerization dynamics after planar cut(s)



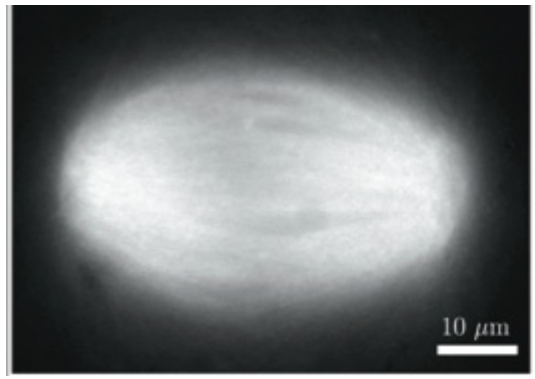
Spindle mechanics

spindles from frog egg extract



Spindle mechanics

direct observation of depolymerization wave



Spindle mechanics

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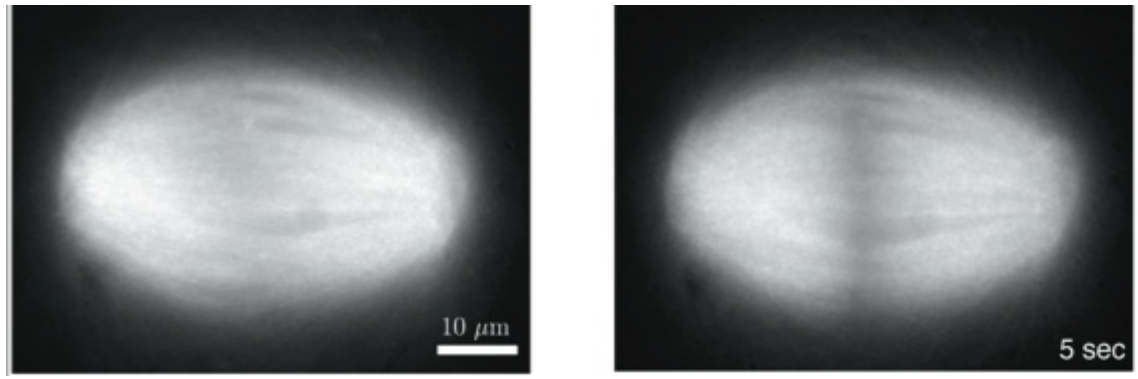

Spindle mechanics

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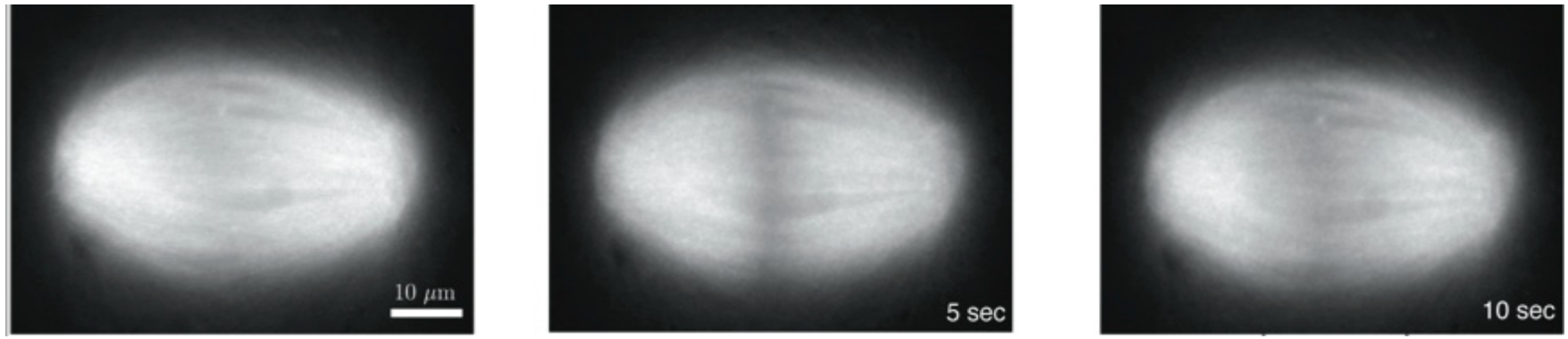
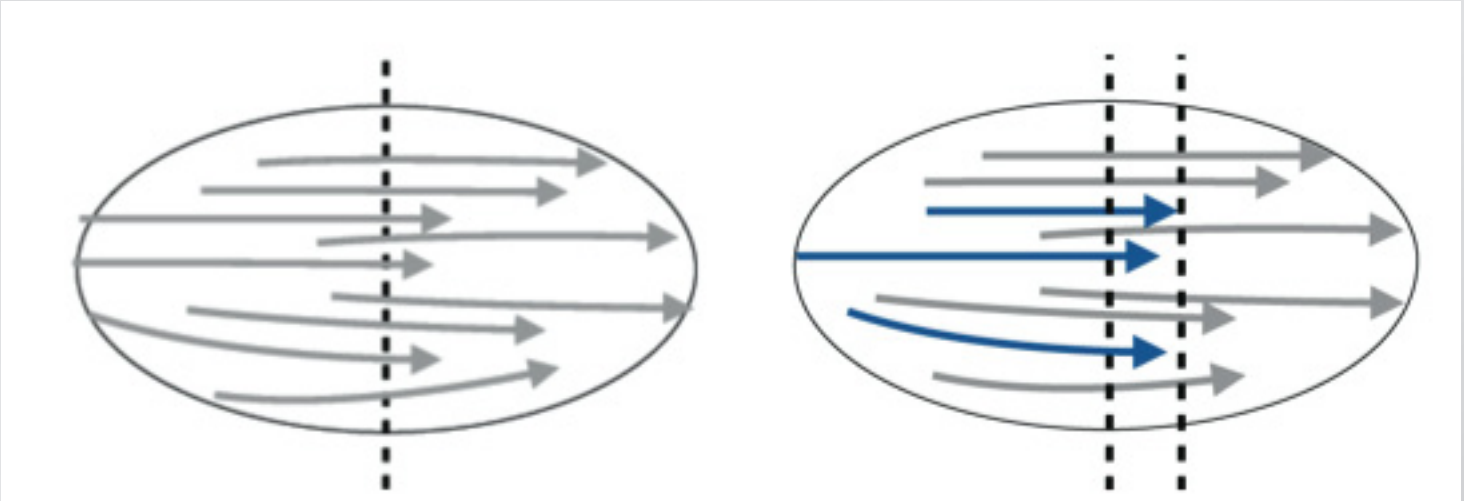


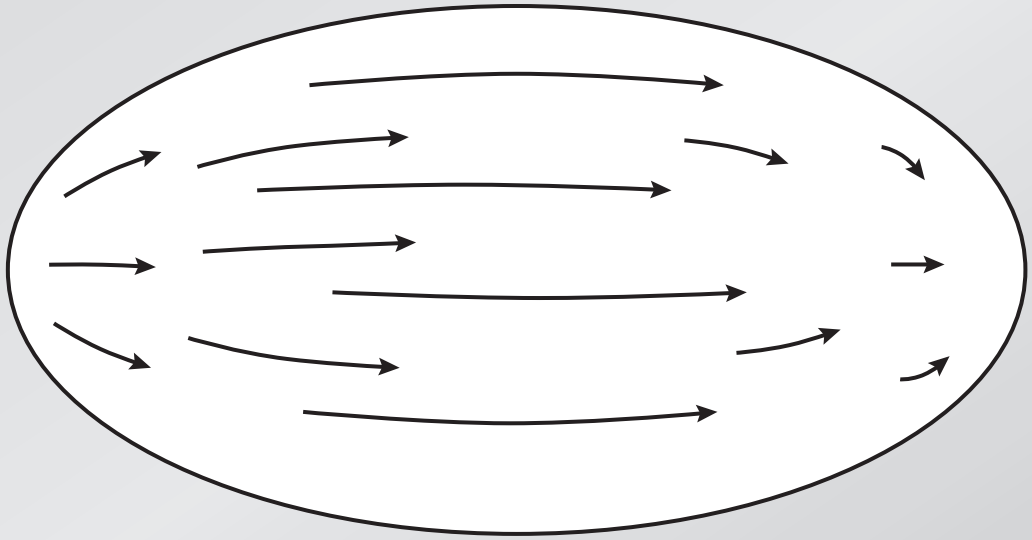
Spindle mechanics

double cuts provide information on mean length



Spindle mechanics

spindle organization



70% 50% 30%

polarity & length distributions varies across cell

Spindle mechanics

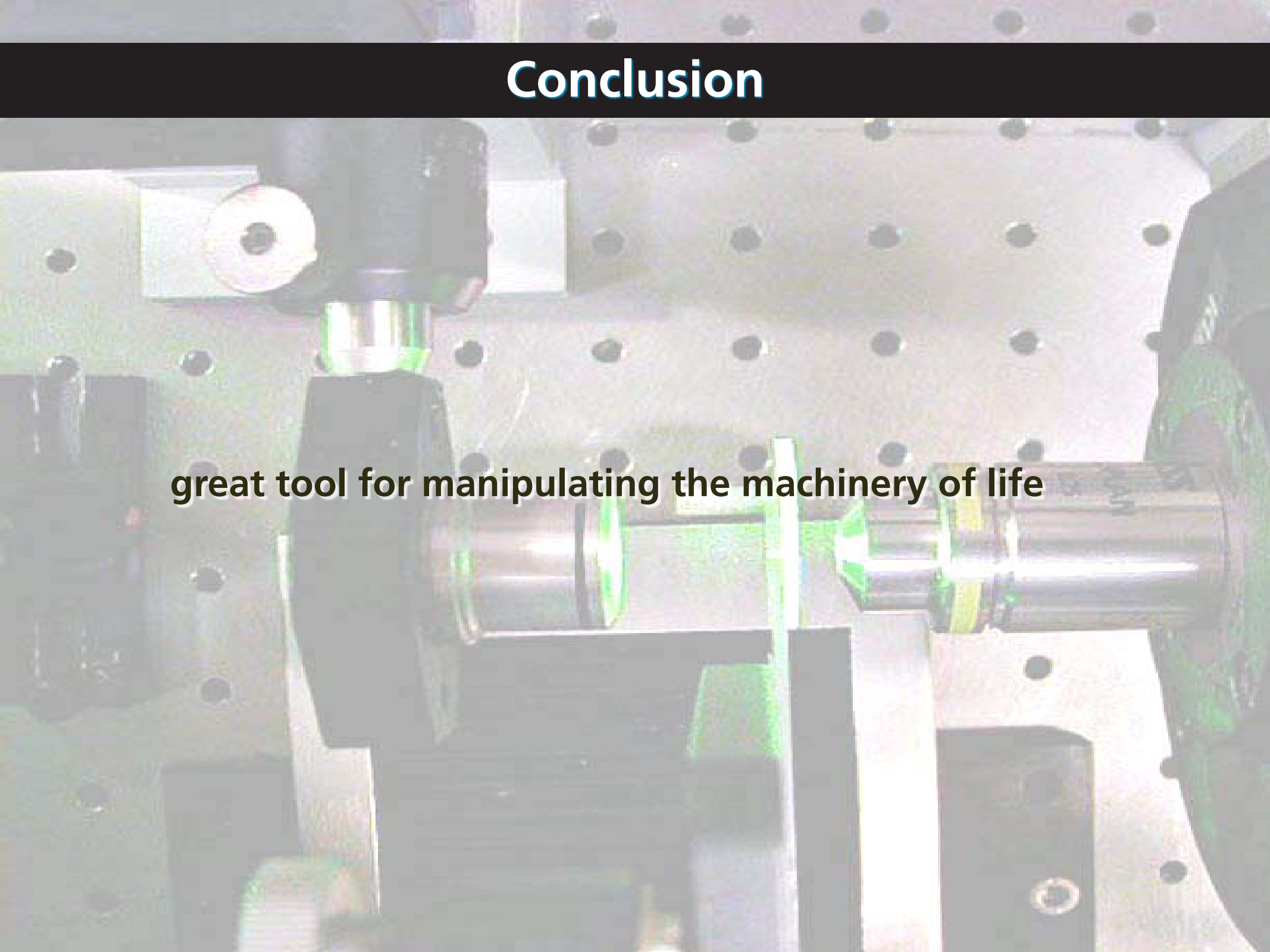
full details:

Paper 7897-4 Monday @ 9:10 am

Summary

- manipulate on subcellular, submicrometer scale
- penetrate in bulk without compromising viability
- perform high-efficiency, high-throughput transfection
- study spindle mechanics during cell division

Conclusion



great tool for manipulating the machinery of life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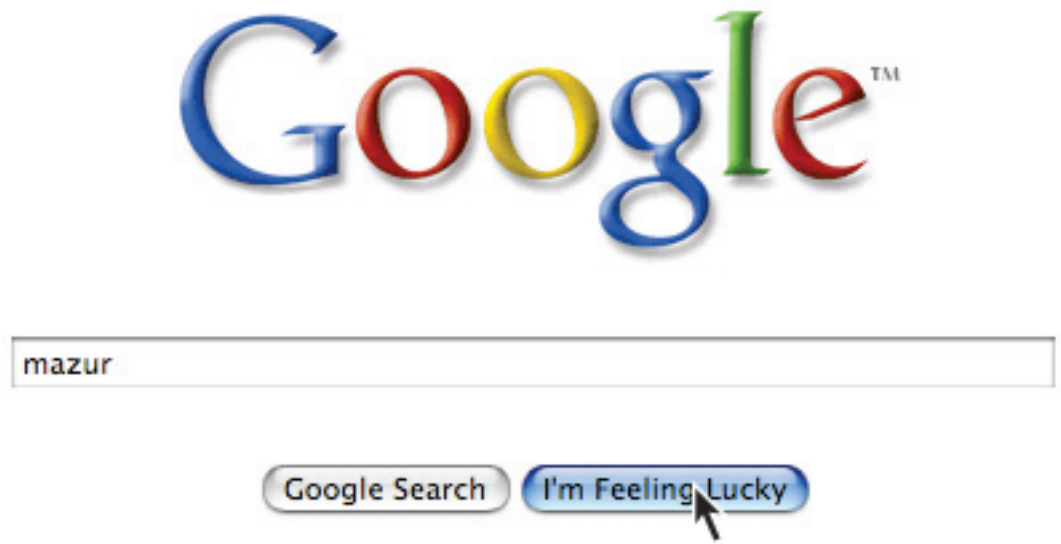
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