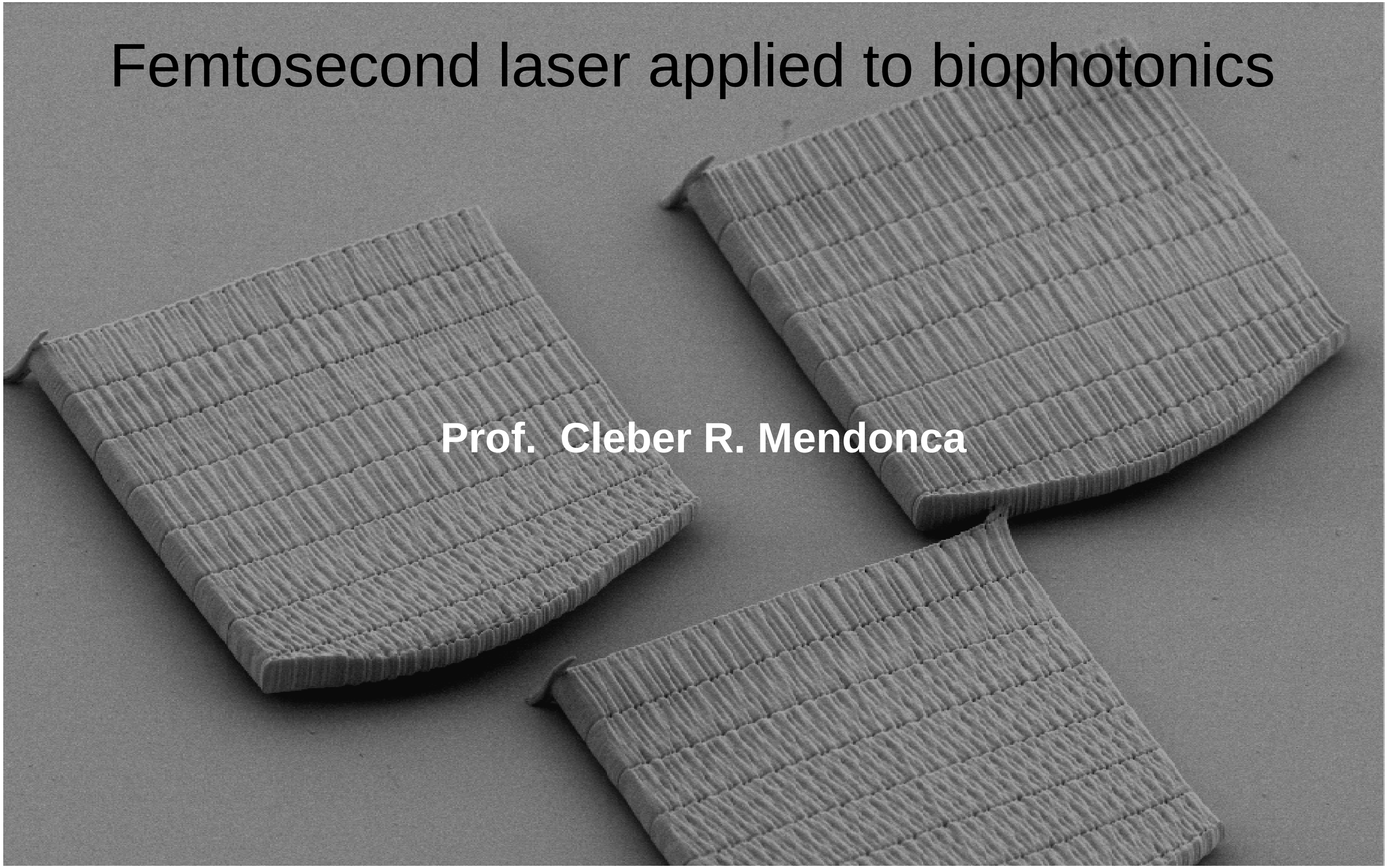
Femtosecond laser applied to biophotonics

Prof. Cleber R. Mendonca



introduction

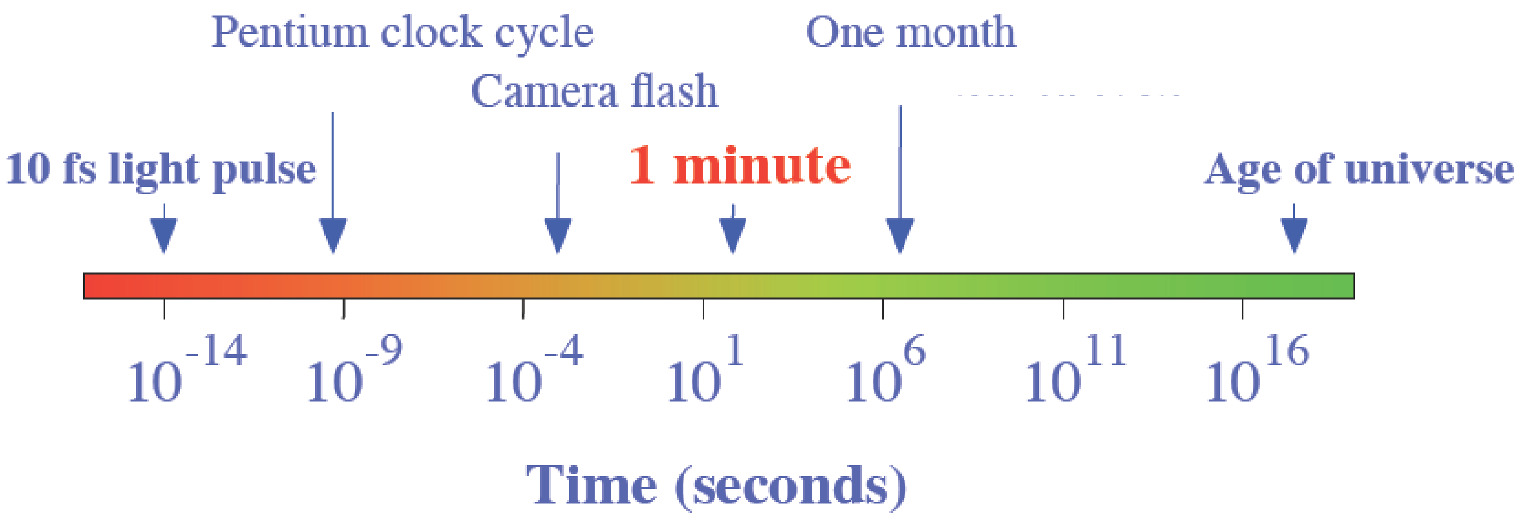
short pulse duration $\longrightarrow$ high intensity

(even at low energy)

introduction

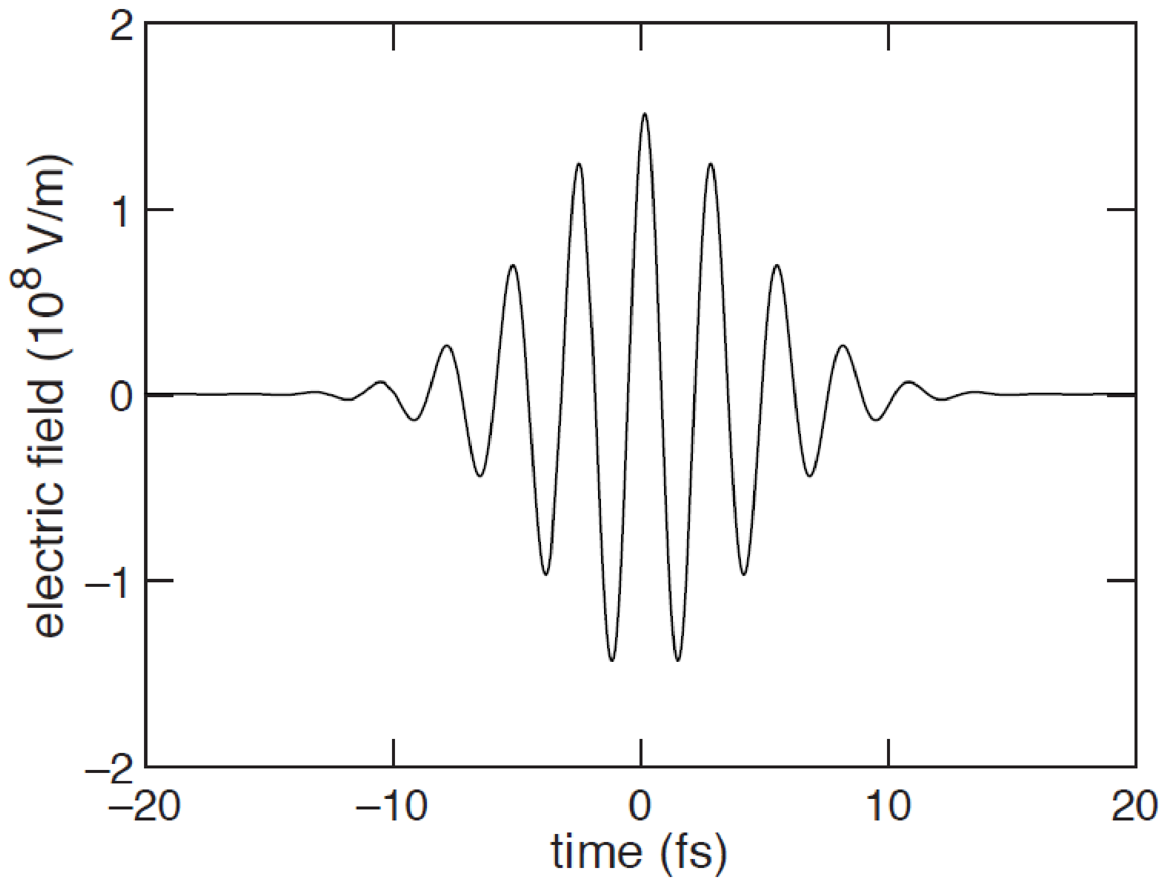
how short is a femtosecond pulse ?

1 fs = 10^{-15} s



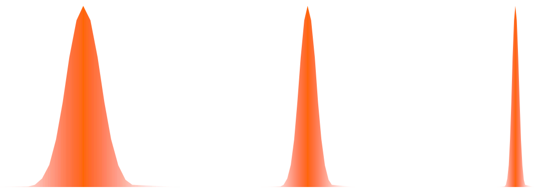
introduction

how short is a femtosecond pulse ?



introduction

Ti:Sapphire lasers



100 fs 50 fs 20 fs

Very intense light

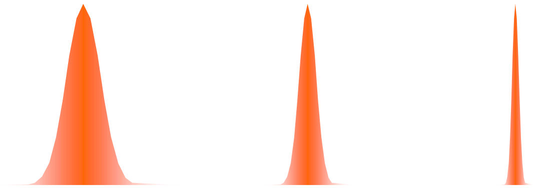
Laser intensities ~ 100 GW/cm^2

1×10^{11} W/cm^2

Laser pointer: 1 mW/cm^2 (1×10^{-3} W/ cm^2)

introduction

Ti:Sapphire lasers

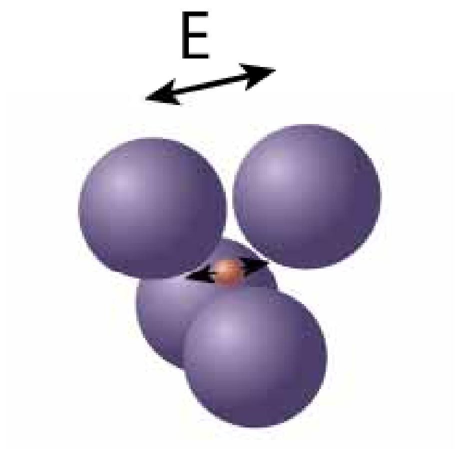


100 fs 50 fs 20 fs

Very intense light

Nonlinear Optical Phenomena

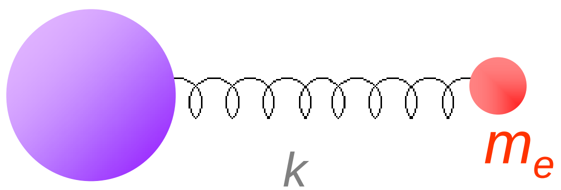
Light matter interaction



harmonic oscillator

Semiclassical treatment

electron on a spring



oscillation frequency

$$\omega_0 = \sqrt{\frac{k}{m_e}}$$

Linear optical processes

$E_{radiation} << E_{interatomic}$

Induced polarization

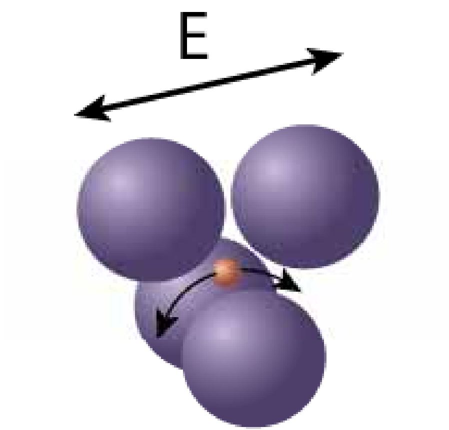
$$P = \chi E$$ linear response

absorption $\alpha = \alpha(\lambda)$

refraction $n = n(\lambda)$

independent of the light intensity

Nonlinear optical processes



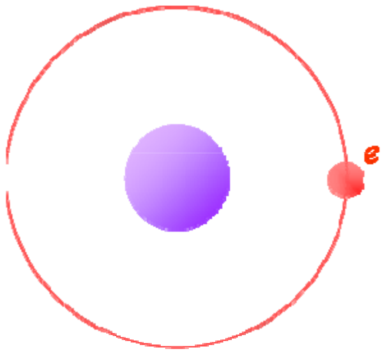
high light intensity

$$E_{rad.} \sim E_{inter.}$$

How high should be the light intensity ?

Nonlinear Optics

Inter-atomic electric field



e = 1.6×10^{-19} C
r ~ 4 Å

E ~ 1×10^{10} V/m

cw laser

P = 20 W
w_o = 20 μm

$$I = \frac{2P}{\pi w_0^2}$$

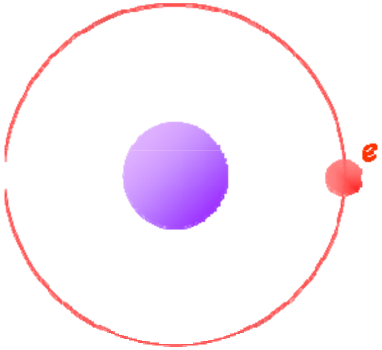
I = 3×10^{10} W/m^2

$$\mathrm{I} = \frac{1}{2} c n \varepsilon_0 E_0^2$$

E_o = 4×10^6 V/m

Nonlinear Optics

Inter-atomic electric field



$e = 1.6 \times 10^{-19}$ C
$r \sim 4$ Å

$E \sim 1 \times 10^{10}$ V/m

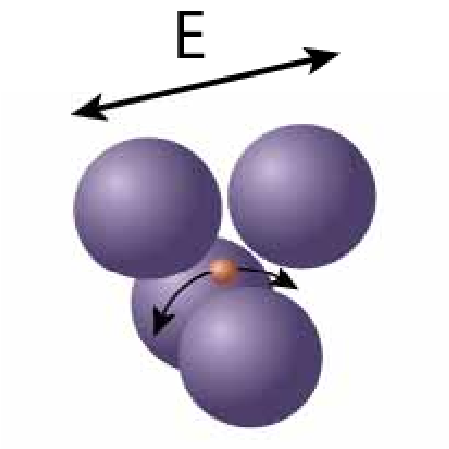
pulsed laser

$I = 10$ GW/cm^2 =
10×10^{13} W/m^2

$$I = \frac{1}{2} c n \varepsilon_0 E_0^2$$

$E_o = 1 \times 10^8$ V/m

Nonlinear Optics



anharmonic oscillator

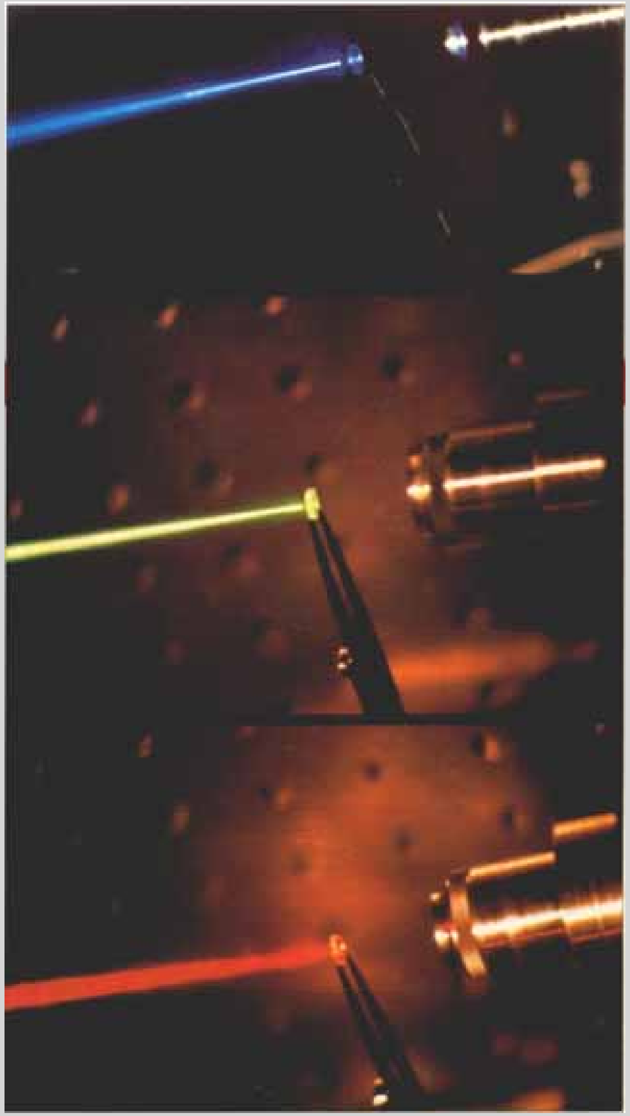
high light intensity

$$E_{rad.} \sim E_{inter.}$$

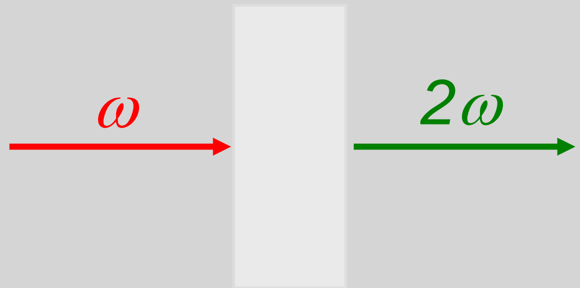
nonlinear polarization response

$$P = \chi^{(1)}E + \chi^{(2)}E^{2} + \chi^{(3)}E^{3} + \ldots$$

Second harmonic generation



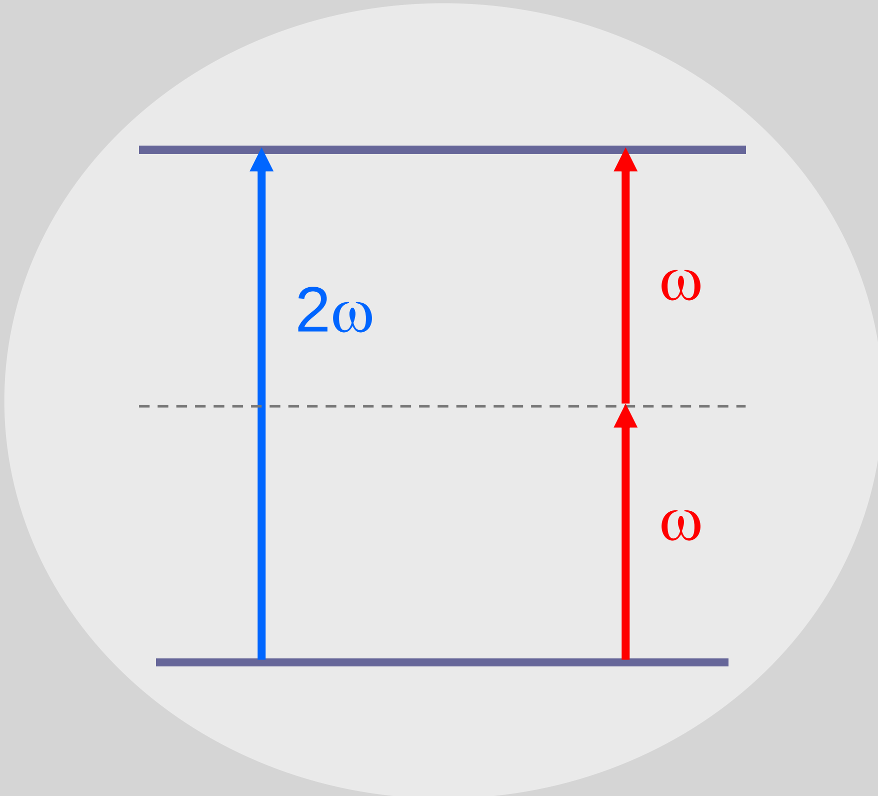
Second order processes $\chi^{(2)}$



Two-photon absorption

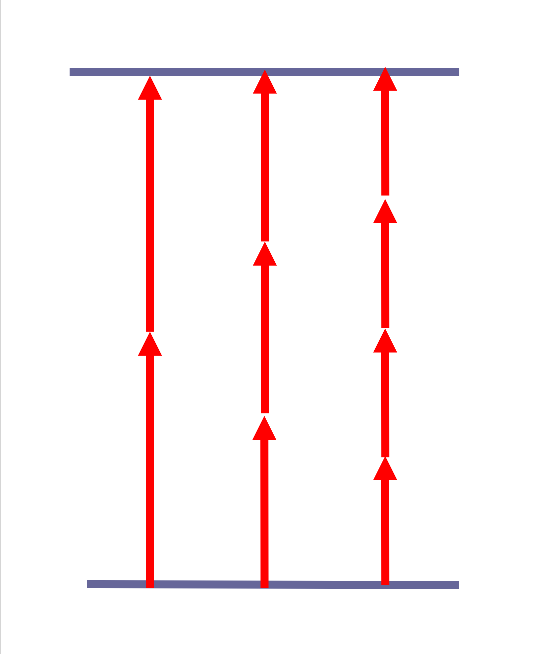
Third order processes $\chi^{(3)}$

$$\alpha = \alpha_0 + \beta I$$



Multi-photon absorption

$$\chi^{(3)}, \chi^{(5)}, \chi^{(7)}, \ldots$$

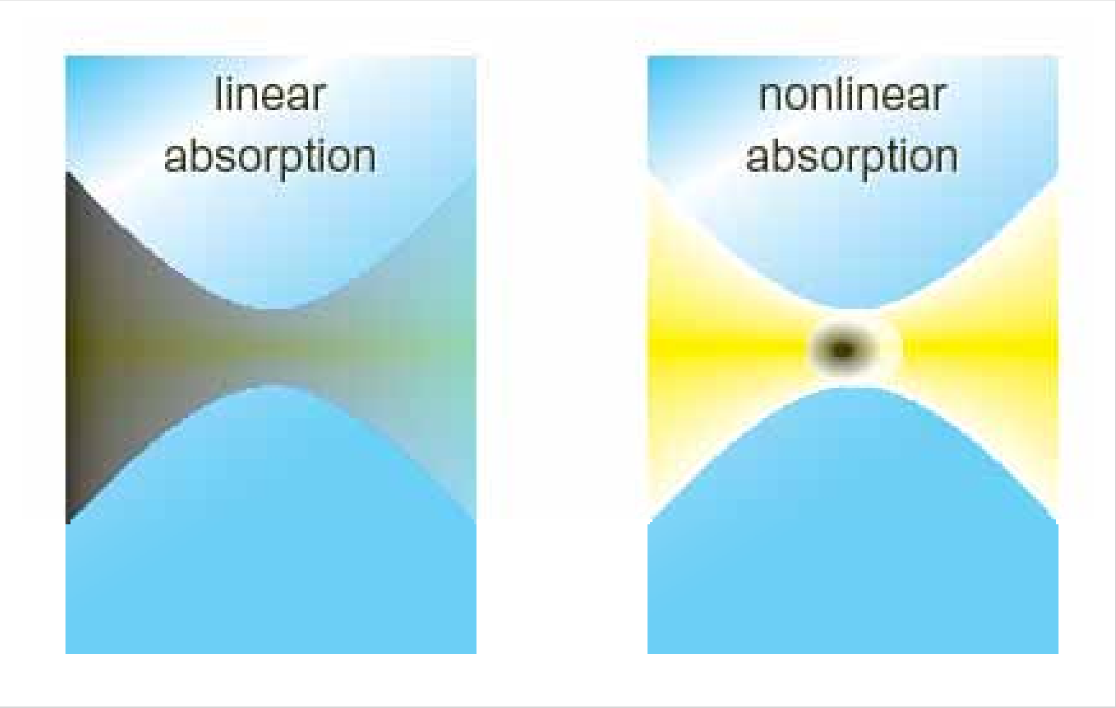


$$\alpha = \alpha_0 + \beta I + \alpha_3 I^2 + \alpha_4 I^3 + \alpha_5 I^4 + \ldots$$

Spatial confinement of excitation

Nonlinear interaction provides spatial confinement of the excitation

fs-microfabrication



$$\alpha = \alpha_0 \qquad\qquad \alpha = \alpha_0 + \beta I$$

fs-laser micromachining

- microstructuring
- microfabrication

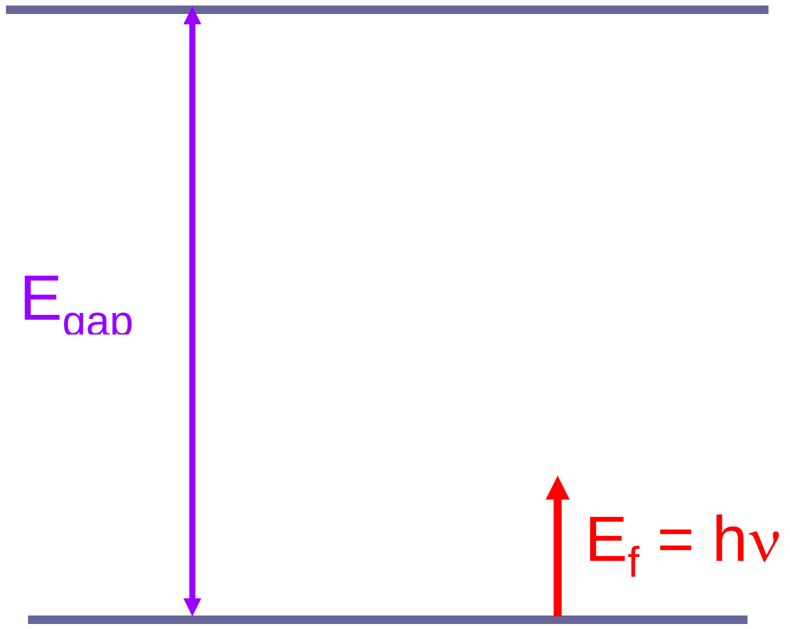
fs-laser microstructuring

photon energy < bandgap

nonlinear interaction

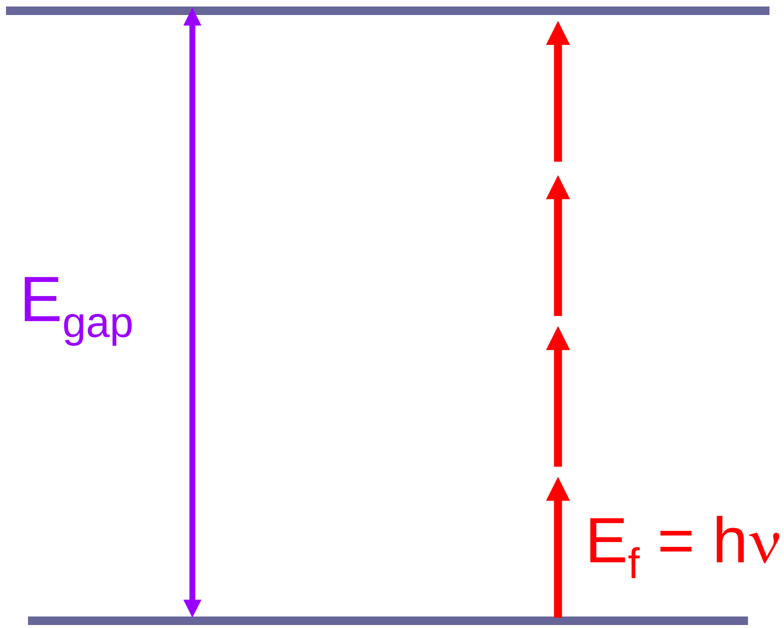
fs-laser microstructuring

nonlinear interaction



fs-laser microstructuring

nonlinear interaction

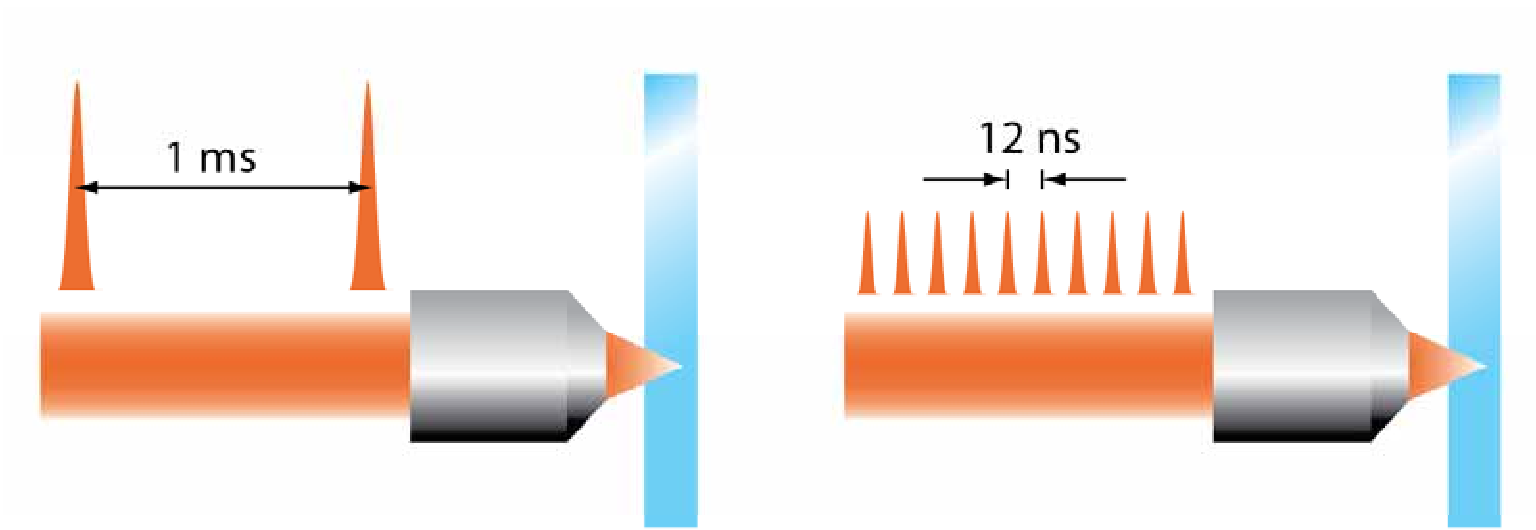


multiphoton absorption

fs-laser microstructuring

amplified laser

oscillator

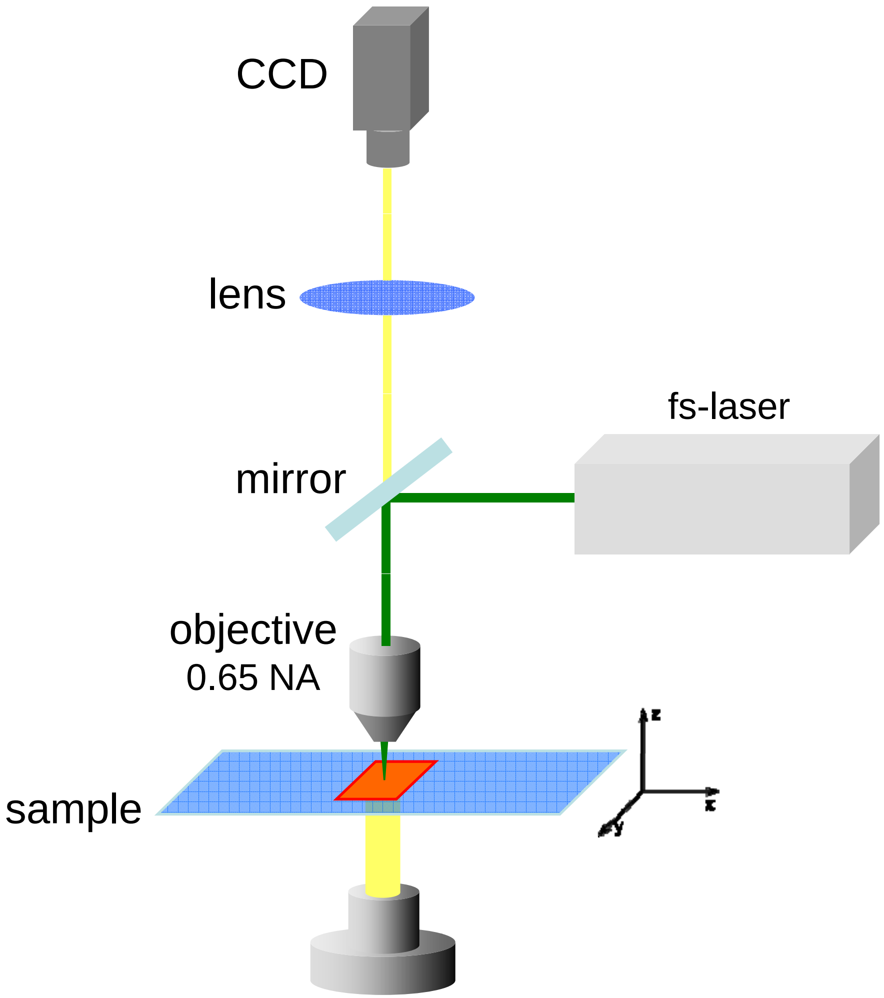


repetitive

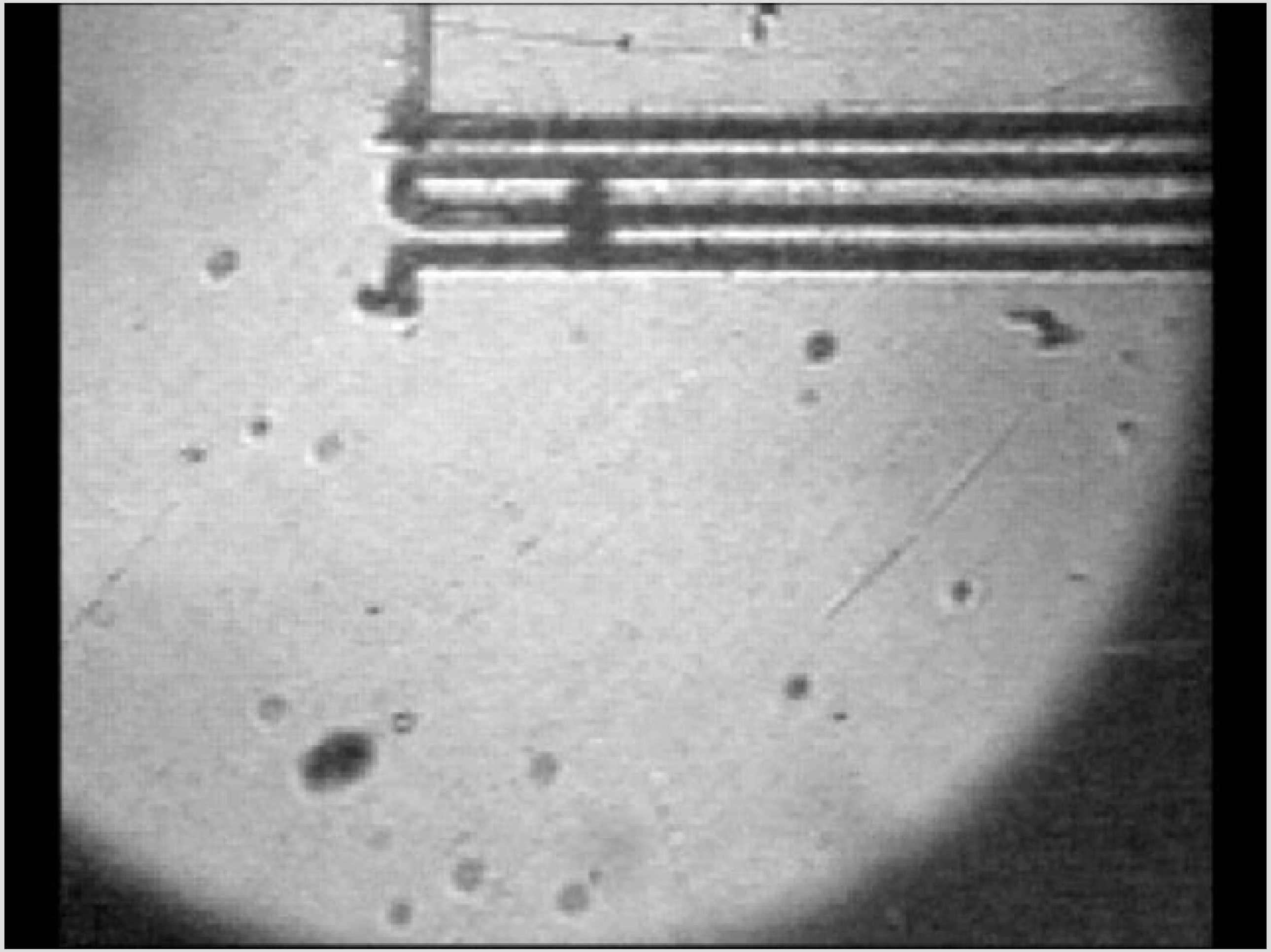
cumulative

Micromachining the sample´s **Volume** or **Surface**

microstructuring surfaces



microstructuring surfaces



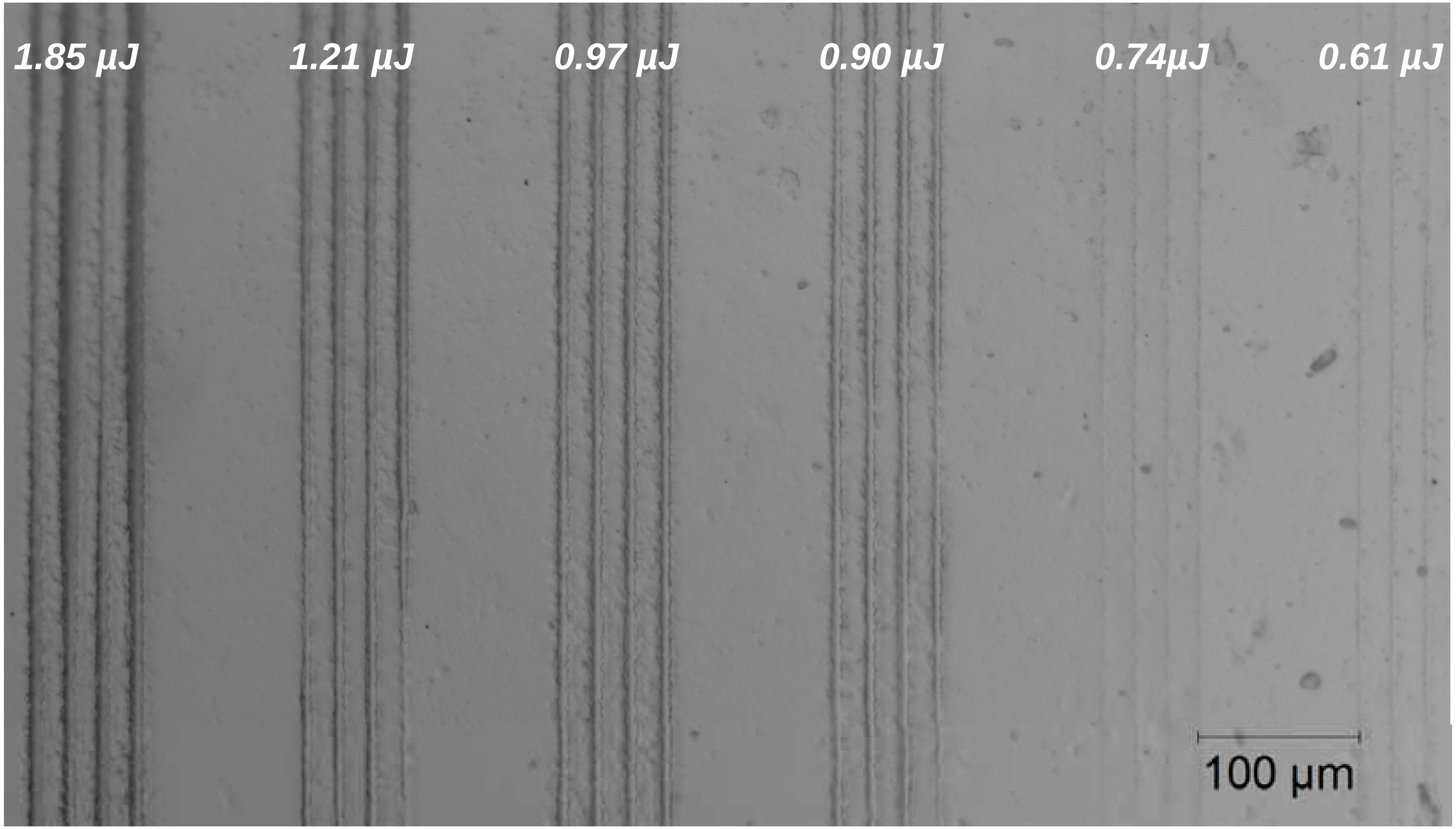
fs-laser micromachining

Latex - natural rubber of the clones: GT 1

Production of latex-based scaffolds for cellular growth

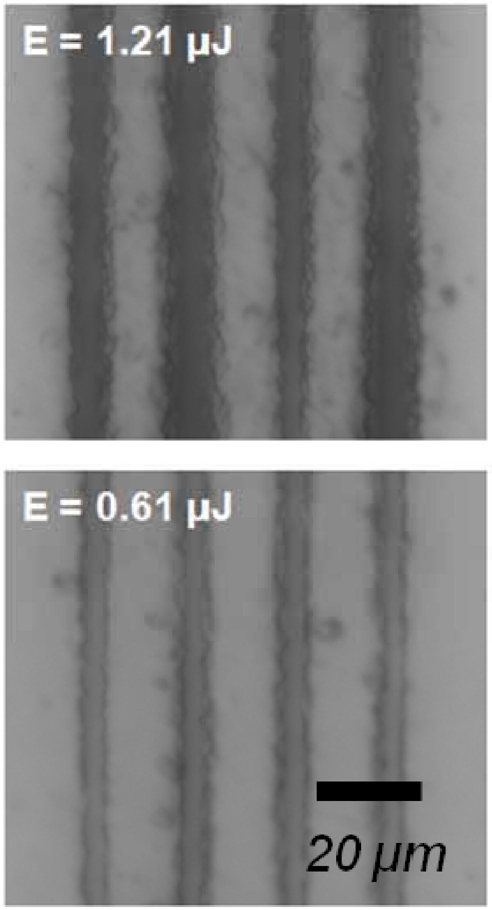
Microstructuring Latex

influence of pulse energy in the micromachining of Latex



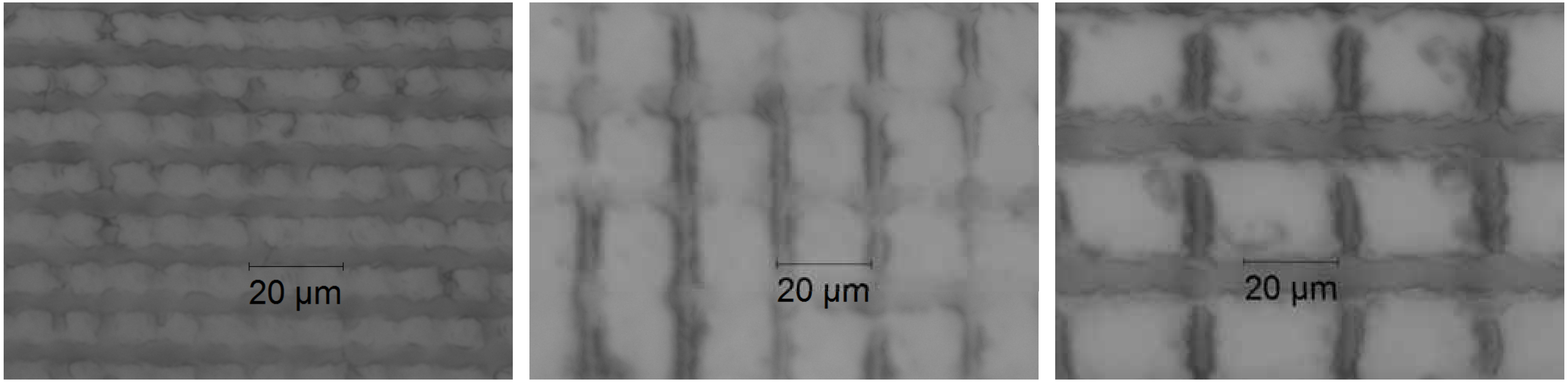
Microstructuring Latex

High resolution and small collateral damage



Microstructuring Latex

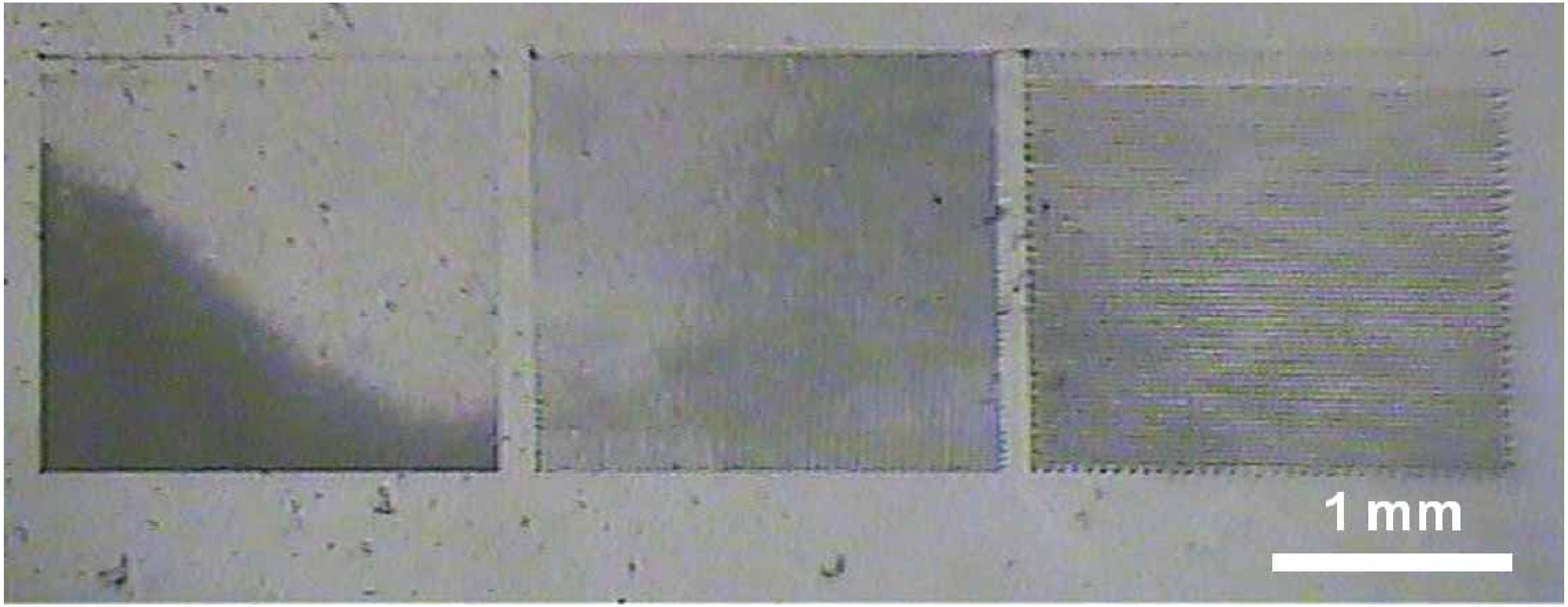
Some of the surface patterning produced on latex



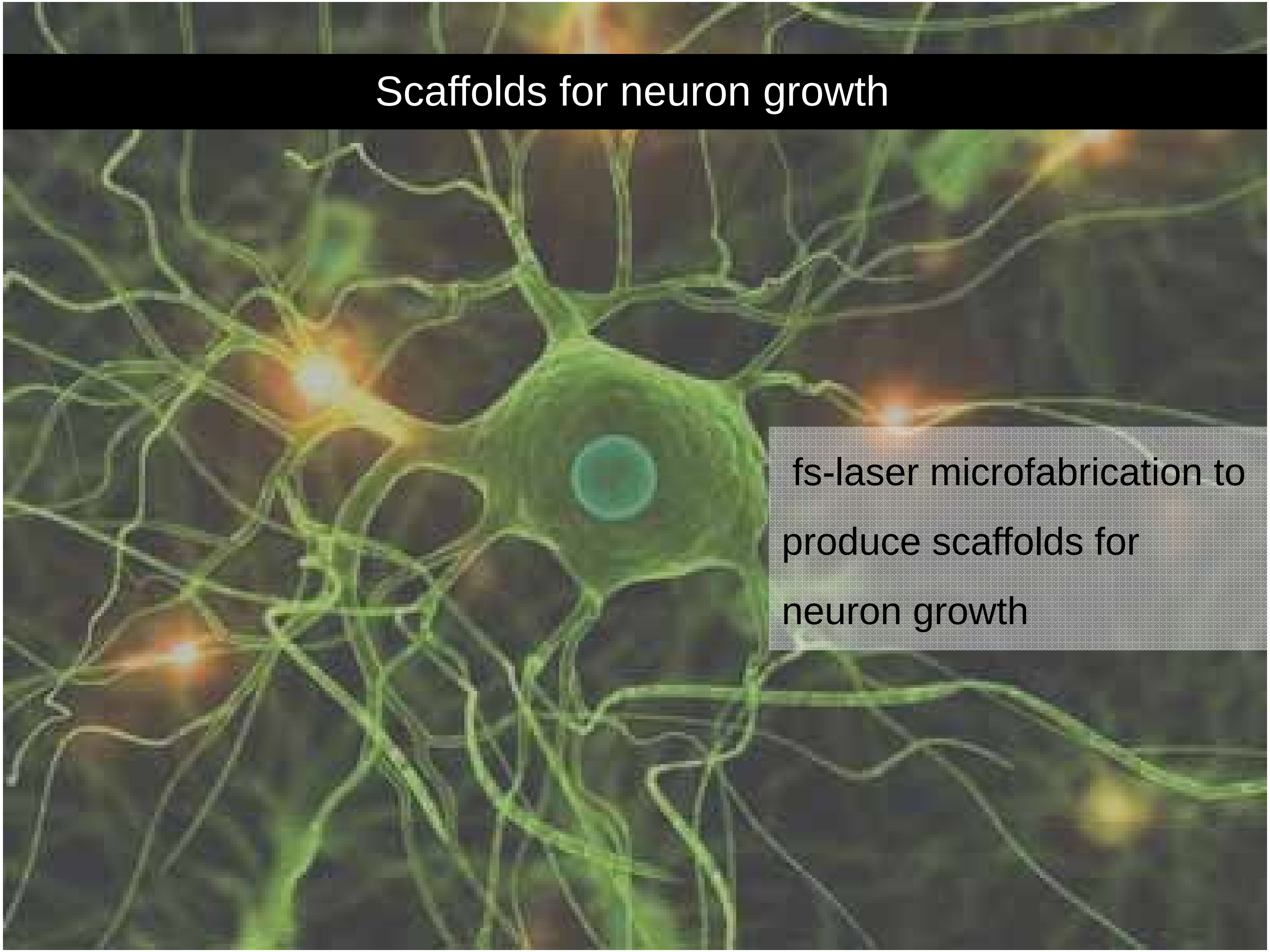
no carbonization of the latex has been observed

Microstructuring Latex

Relatively large areas can be produced with this method

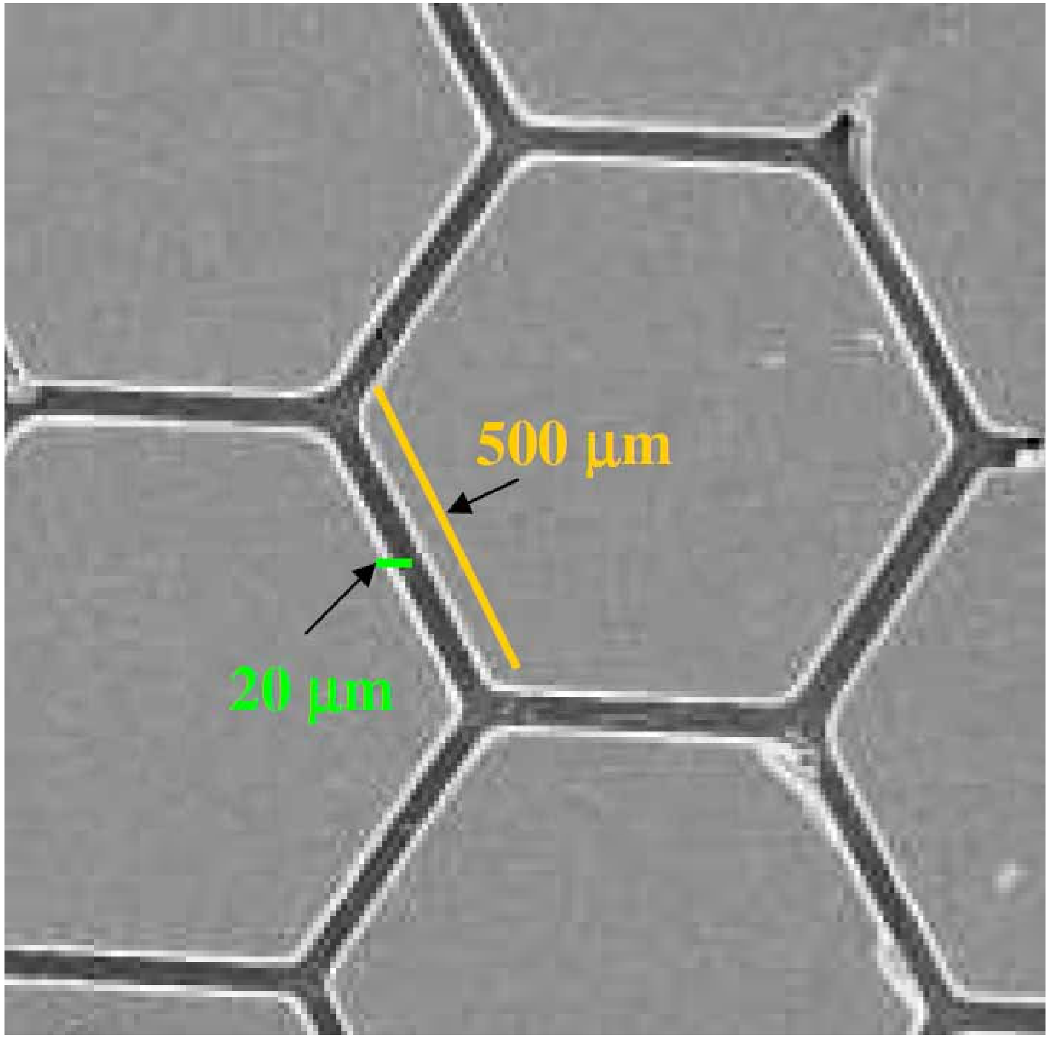


Scaffolds for neuron growth



fs-laser microfabrication to produce scaffolds for neuron growth

Scaffolds for neuron growth



Scaffolds for neuron growth

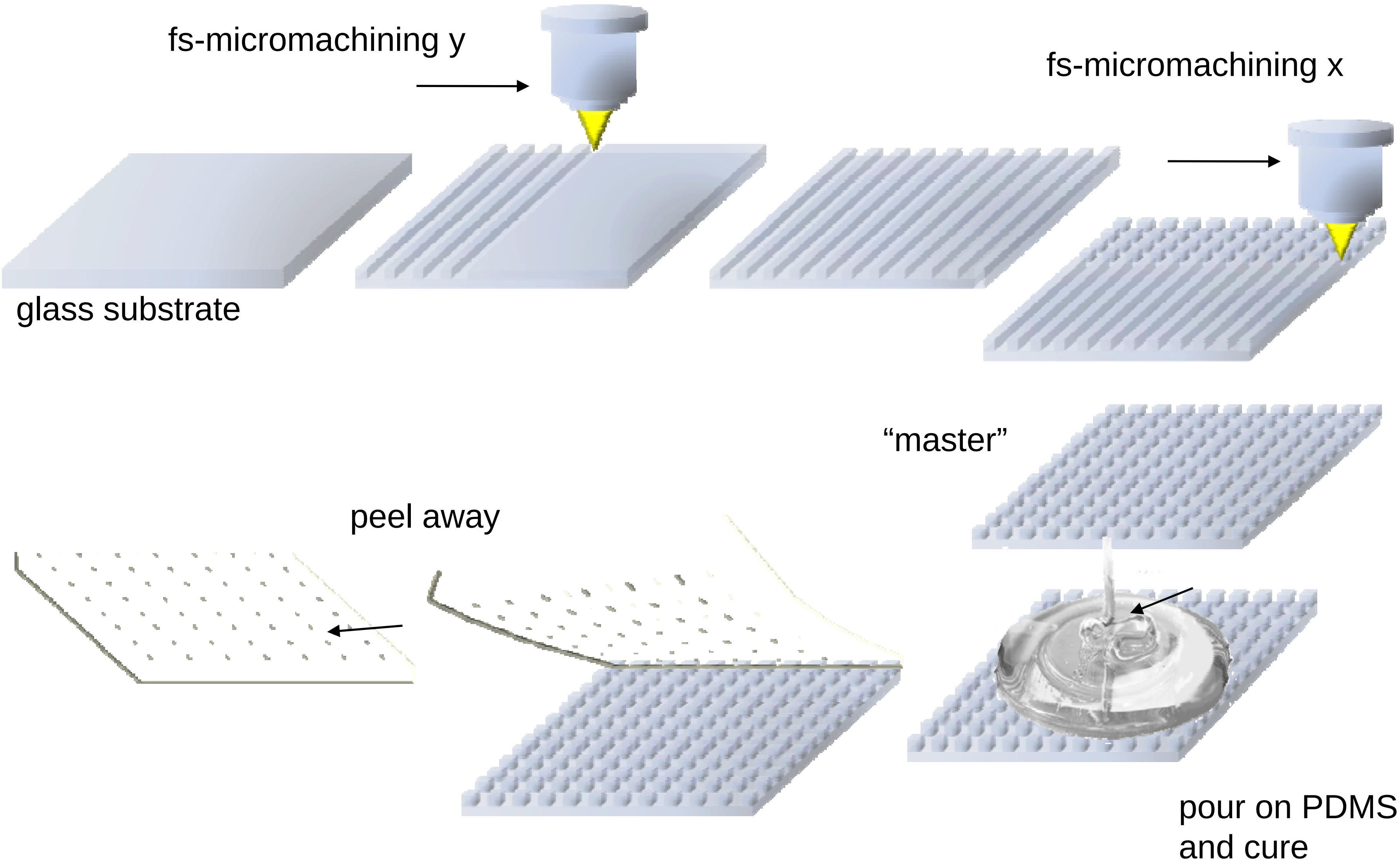
Neuron growth platforms need very specific biopolymers

approach

1 - Microstructure glass surface

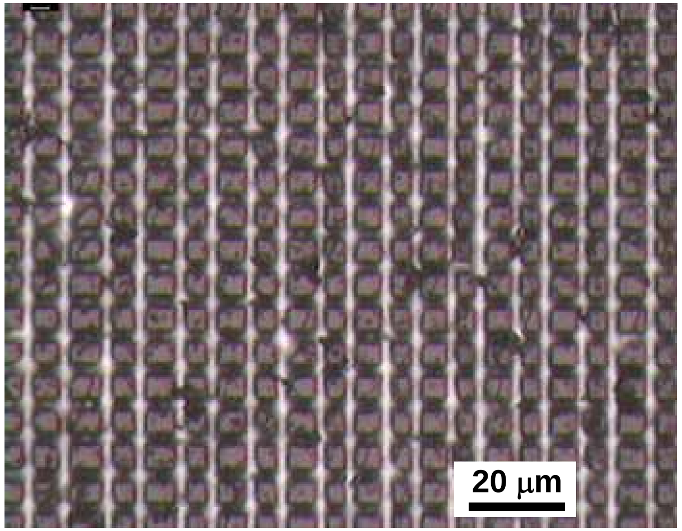
2 – Stamping with PDMS

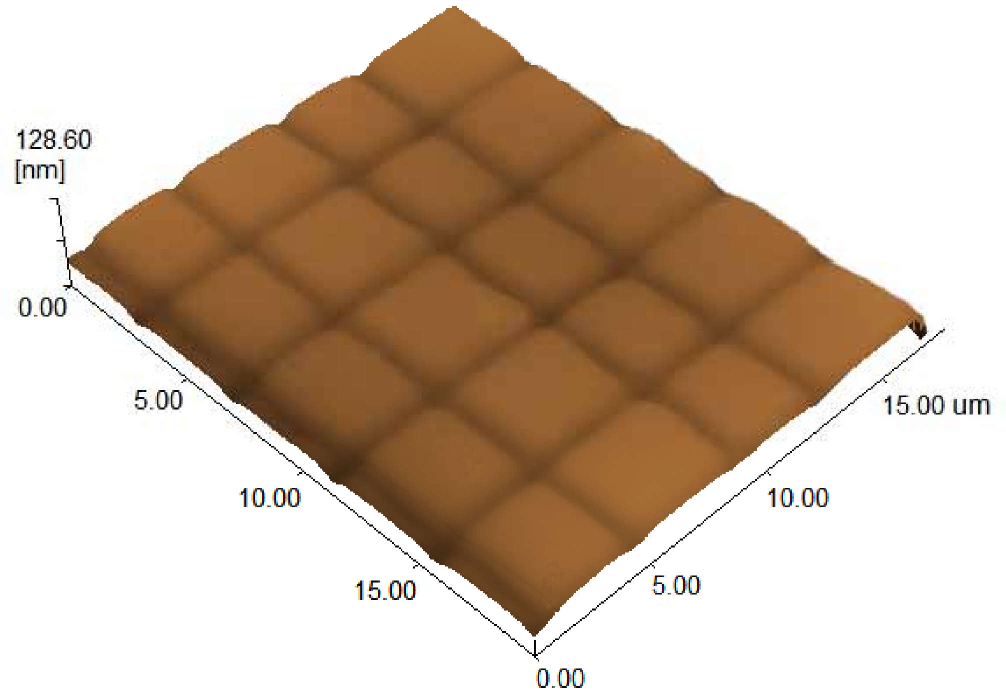
Fabrication of the molds



Fabrication of the molds

examples of micromachined surfaces in glass

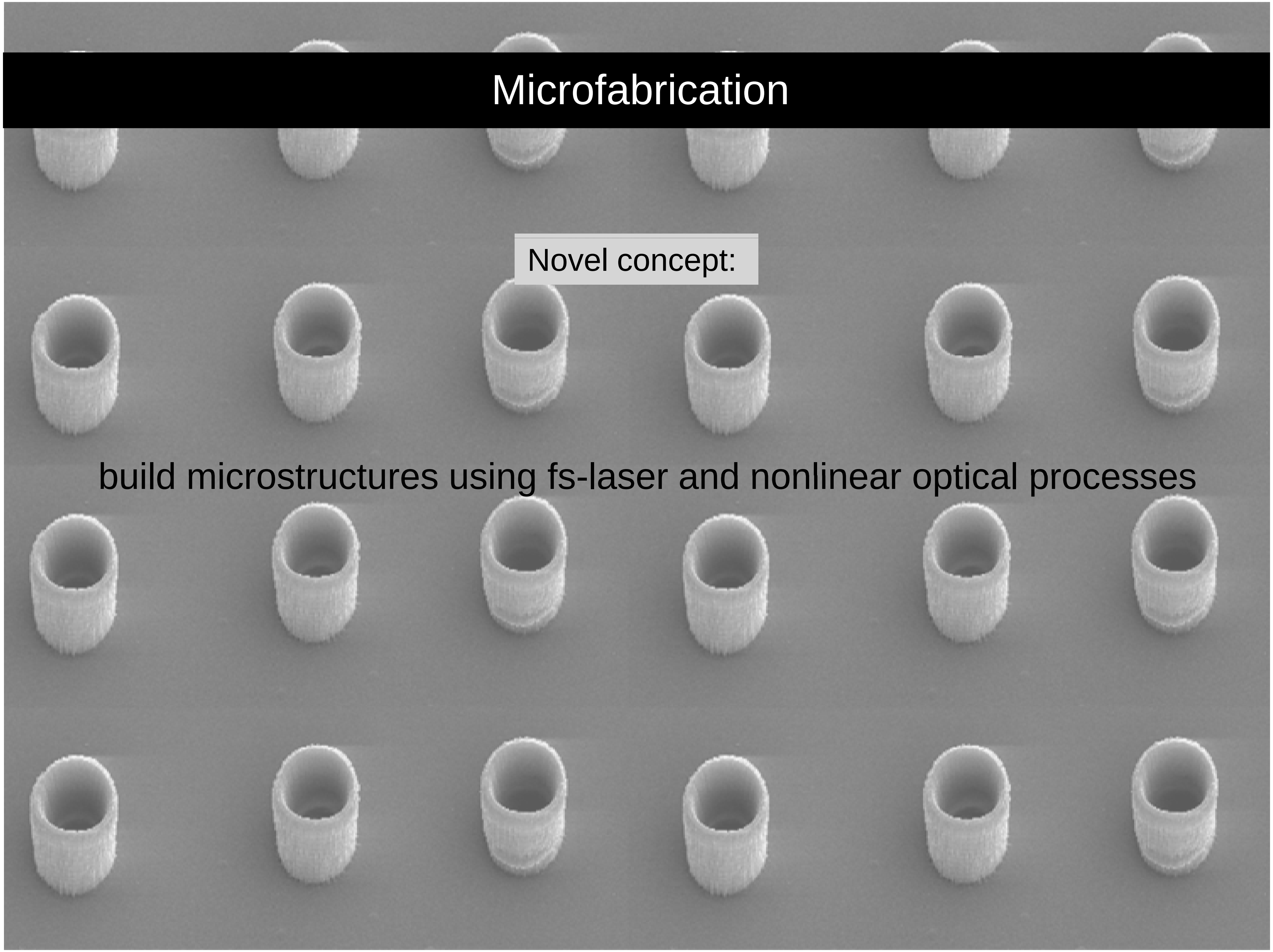




Microfabrication

Novel concept:

build microstructures using fs-laser and nonlinear optical processes

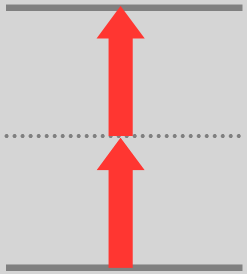


Two-photon polymerization

Monomer + Photoinitiator $\rightarrow$ *Polymer*

light

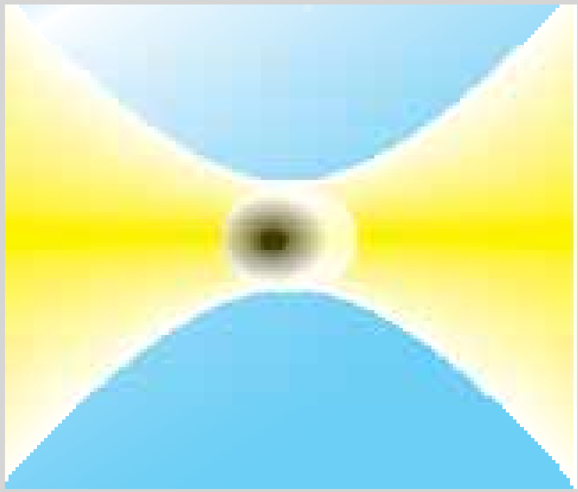
Photoinitiator is excited by ***two-photon absorption***



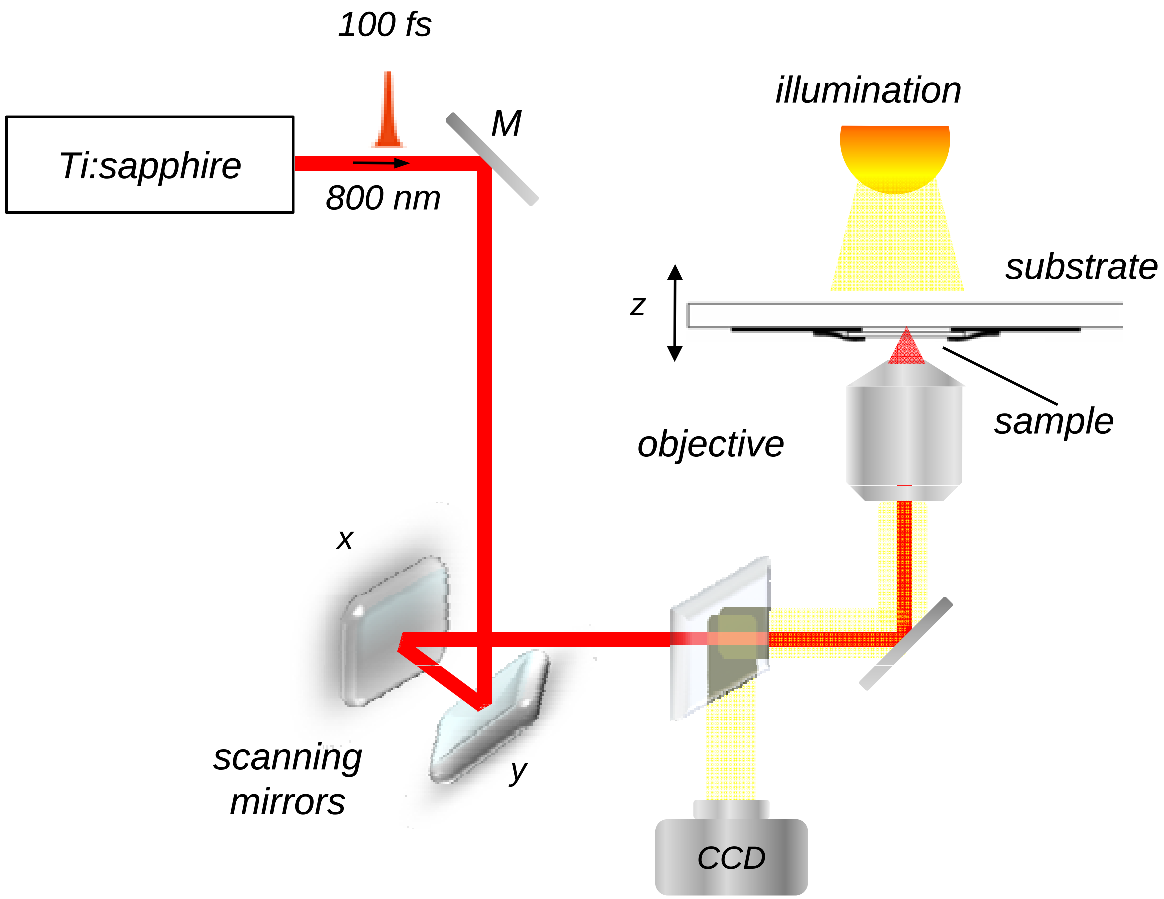
$$R_{2PA} \propto I^{2}$$

The polymerization is confined to the focal volume.

High spatial resolution



Two-photon polymerization setup



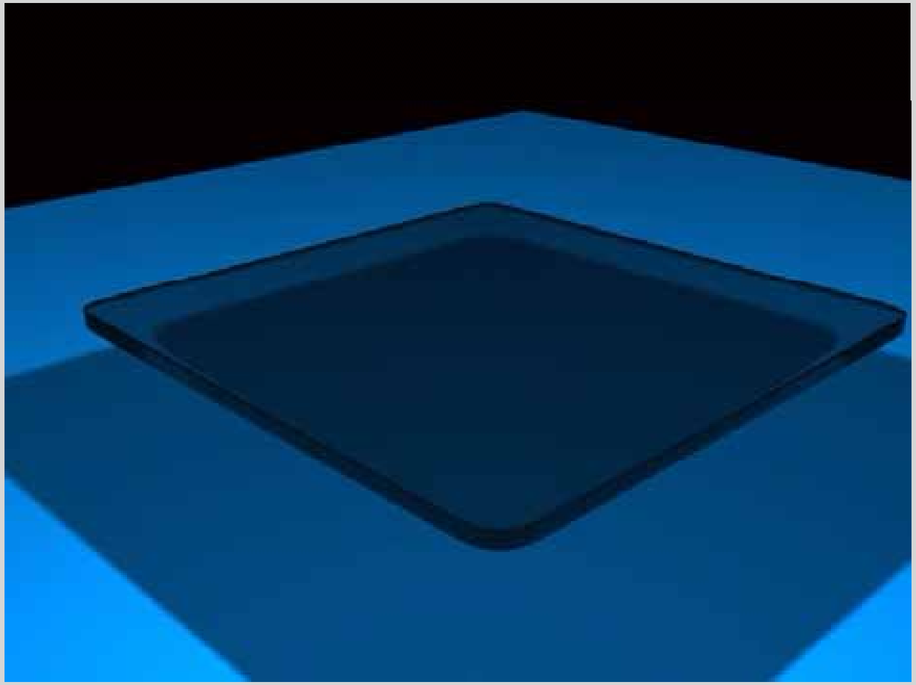
Ti:sapphire laser oscillator

- 130 fs
- 800 nm
- 76 MHz
- 20 mW

Objective

40 x
0.65 NA

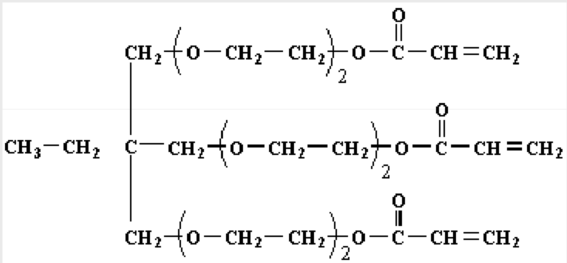
Two-photon polymerization



Resin preparation

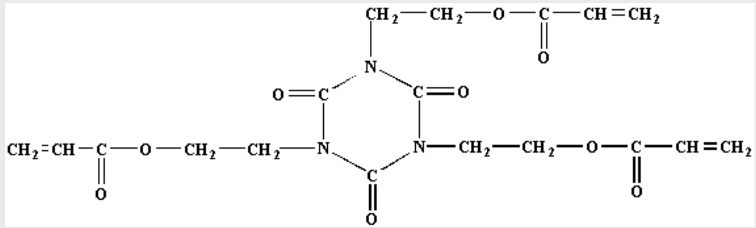
Monomers

Monomer A



reduces the shrinkage upon polymerization

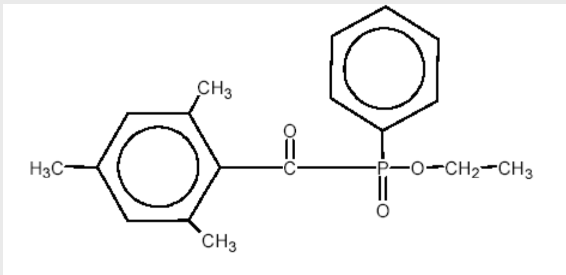
Monomer B



gives hardness to the polymeric structure

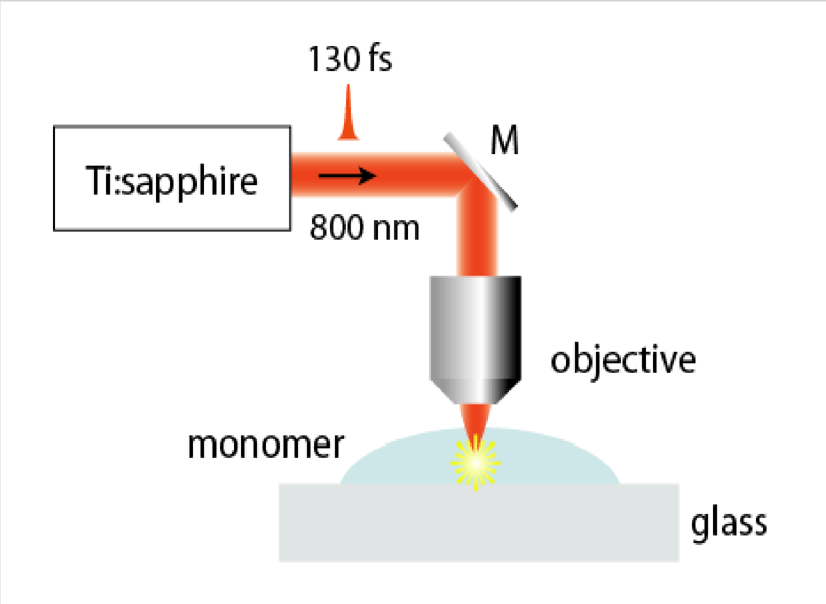
Photoinitiator

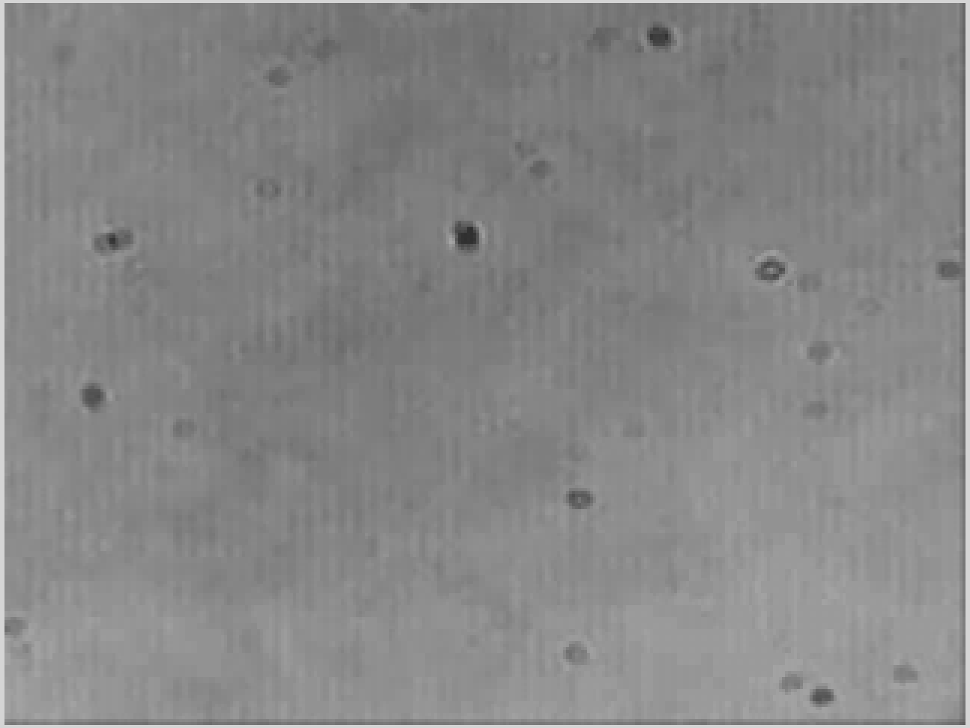
Lucirin TPO-L



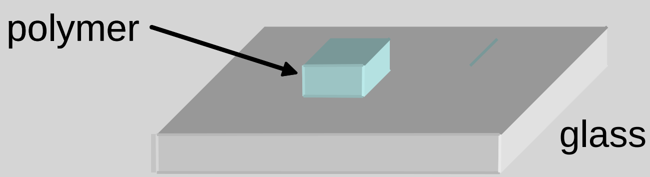
Appl. Phys. A, 90, 633–636 (2008)

Two-photon polymerization

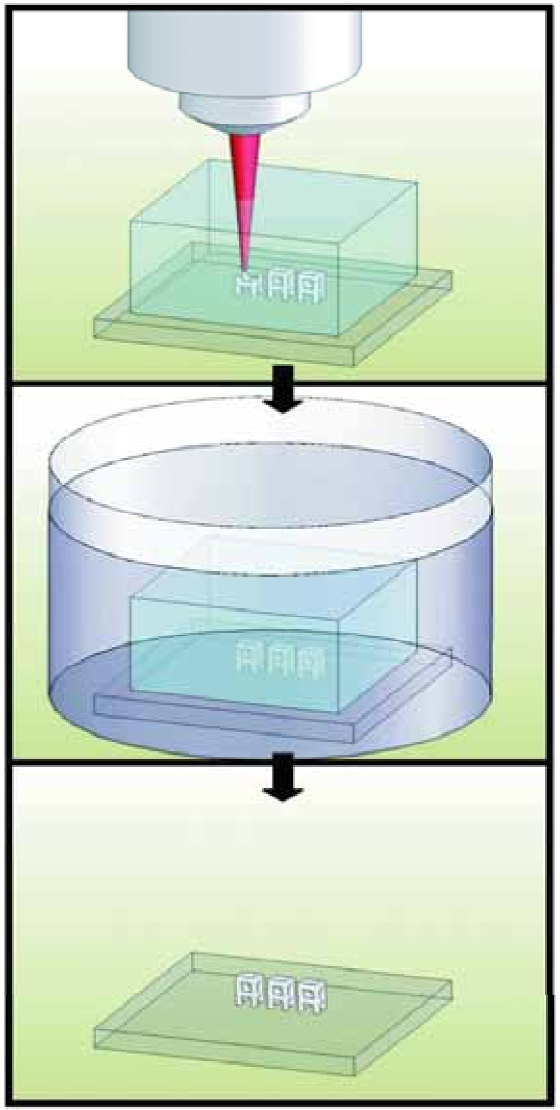




30 µm x 30 µm x 12 µm cube



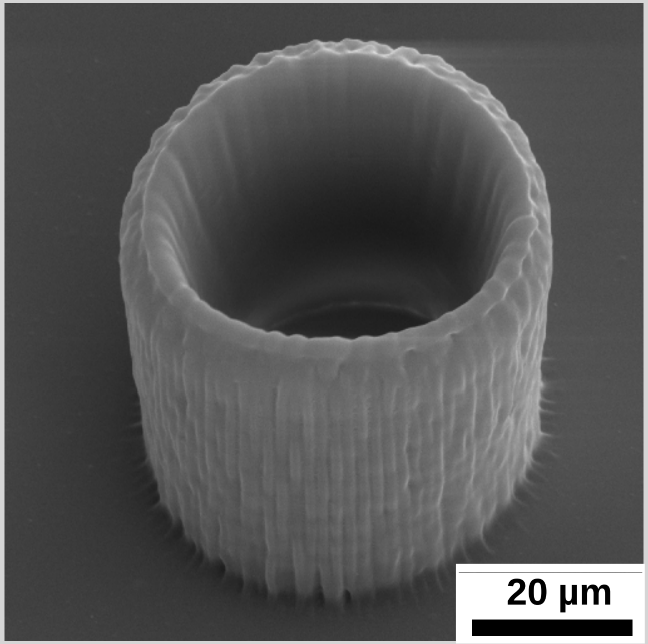
Two-photon polymerization

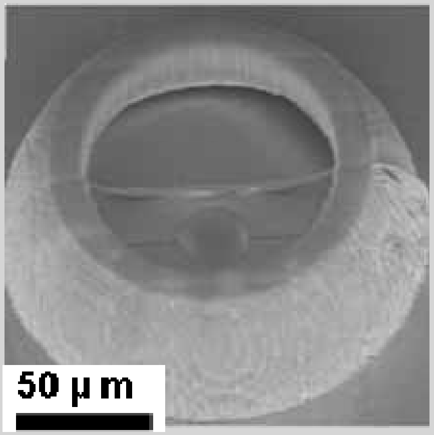


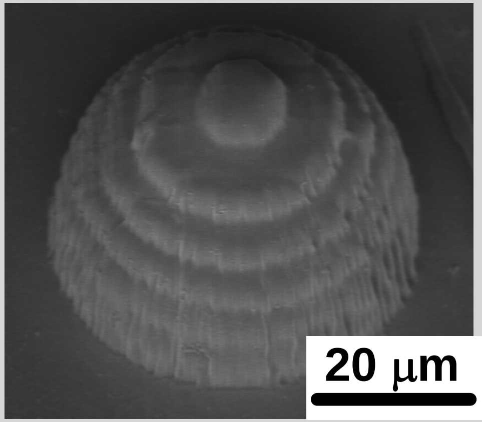
After the fabrication, the sample is immersed in ethanol to wash away any unsolidified resin and then dried

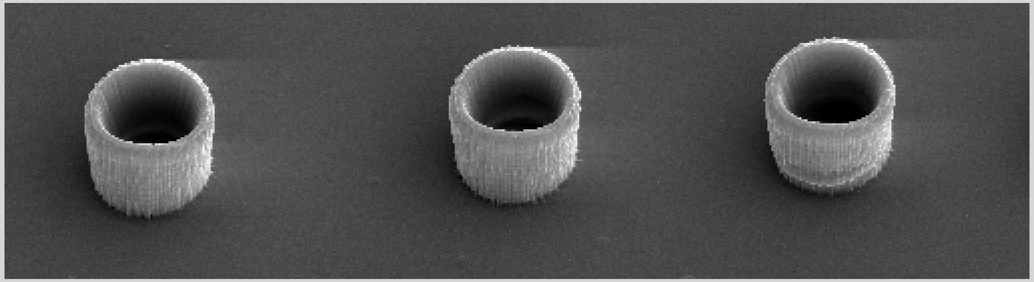
Two-photon polymerization

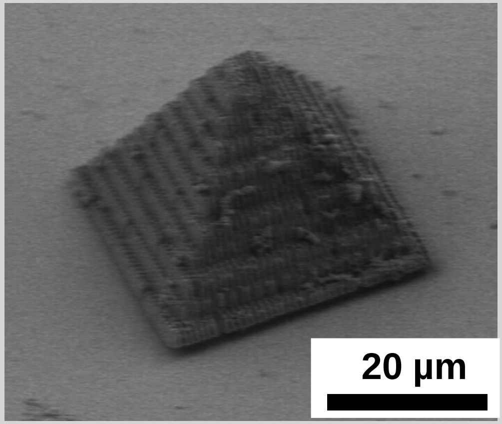
Microstructures fabricated by two-photon polymerization





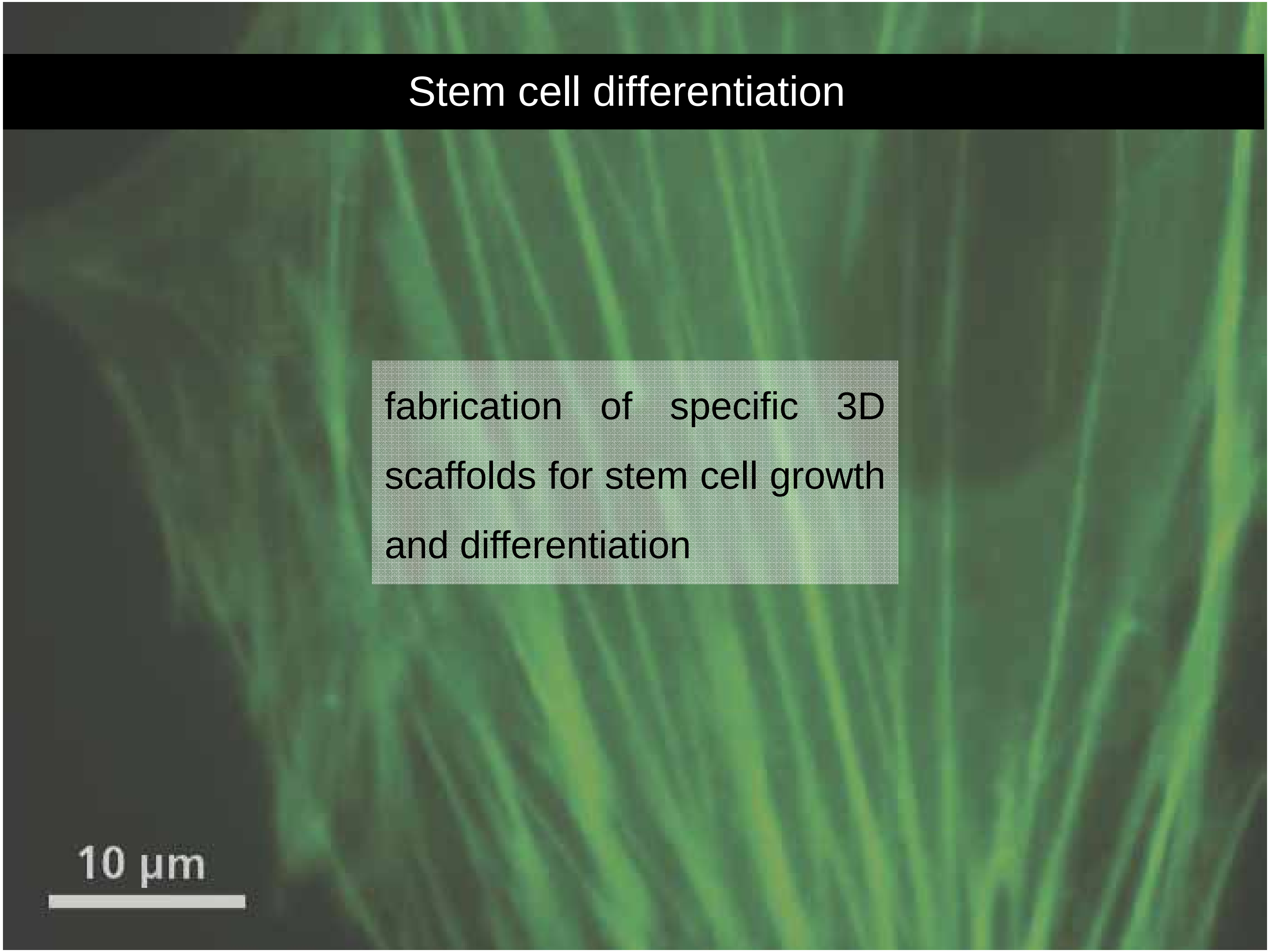




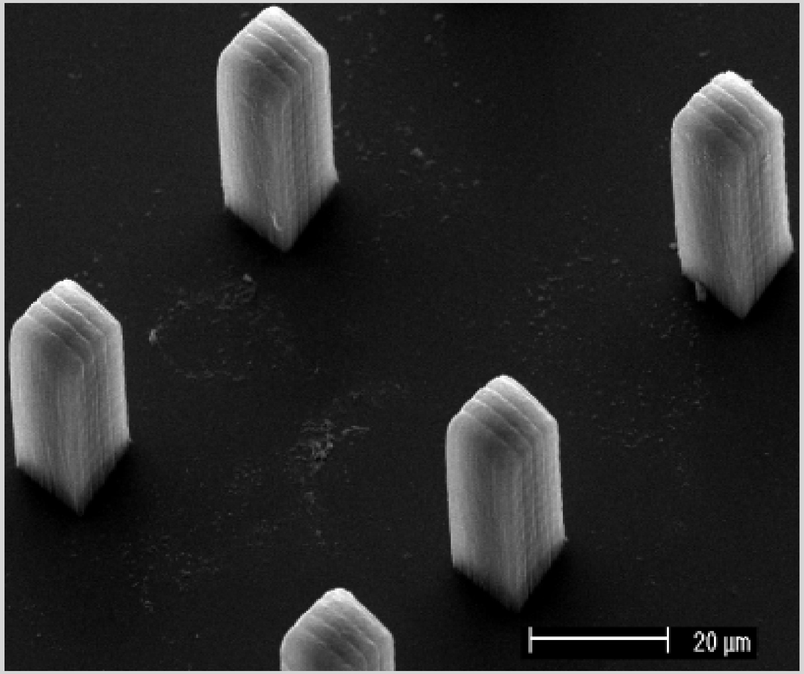


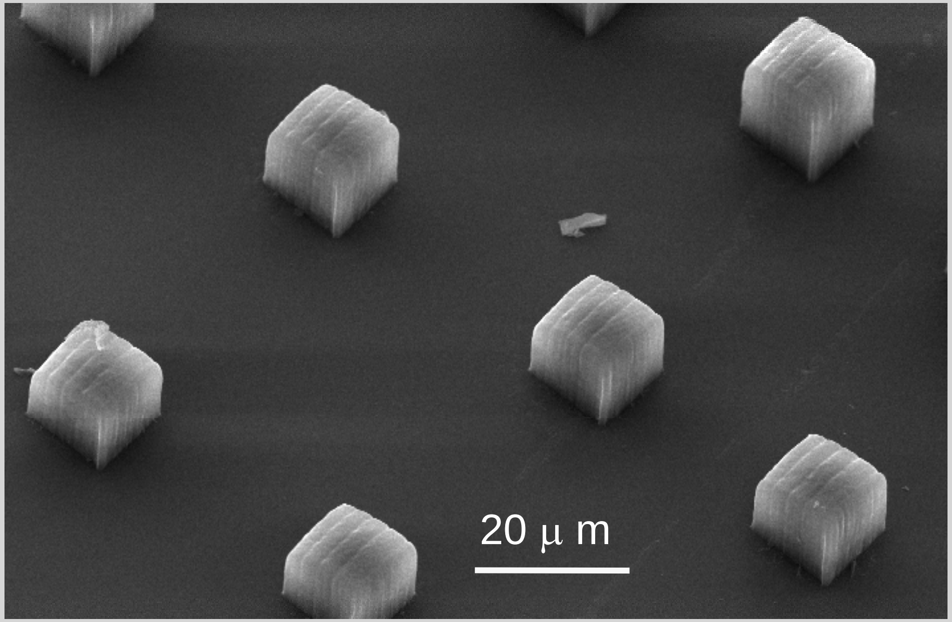
Stem cell differentiation

fabrication of specific 3D scaffolds for stem cell growth and differentiation

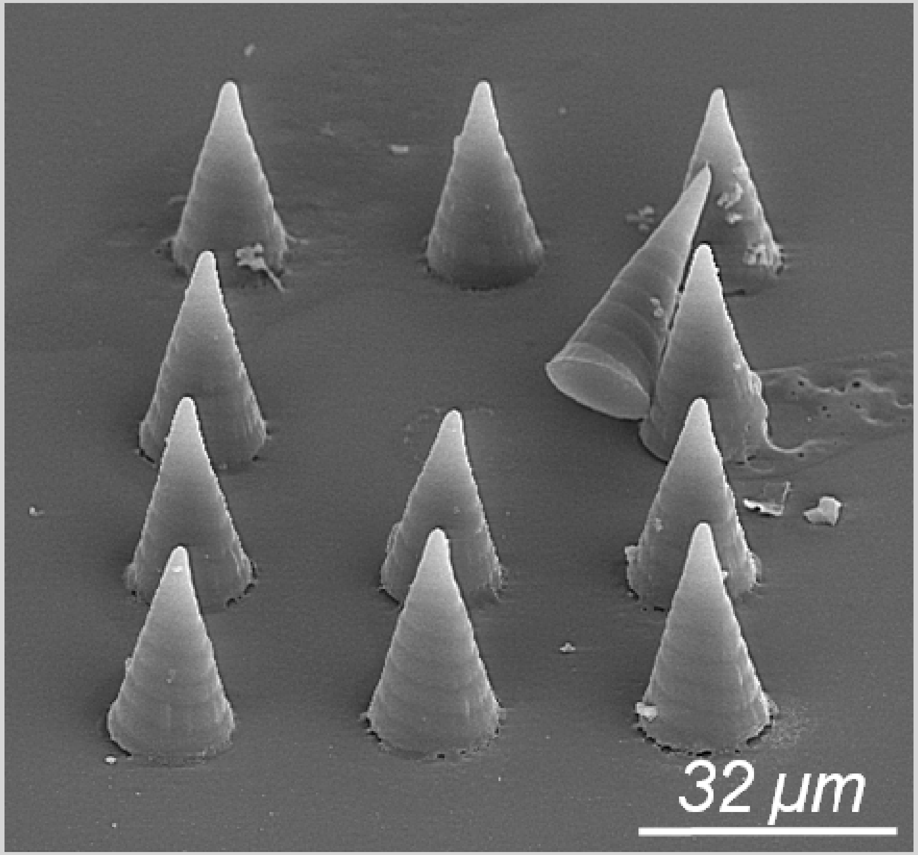


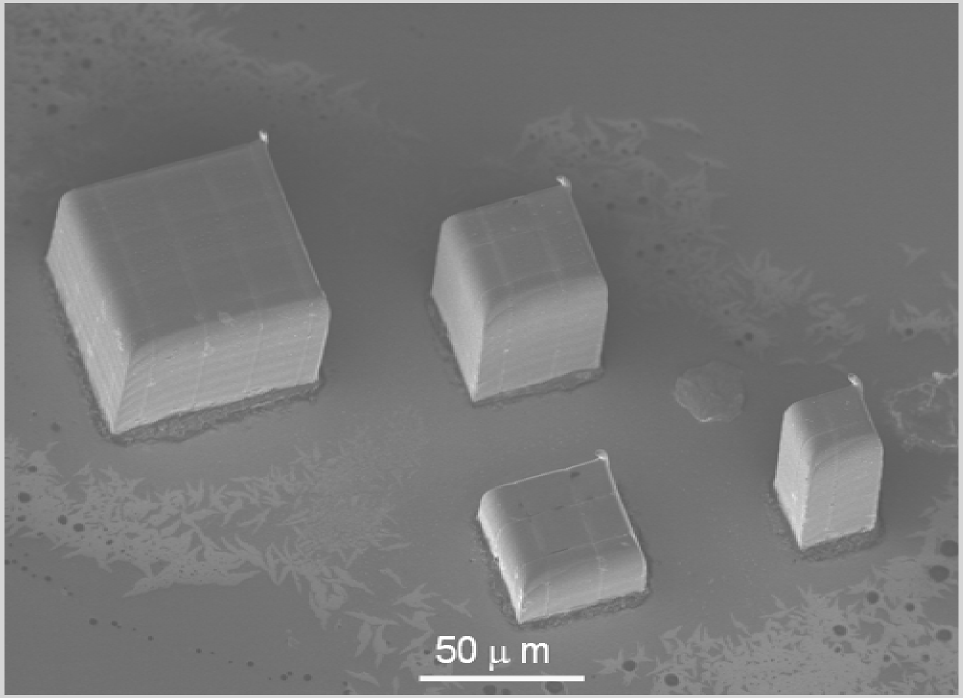
Stem cell scaffolds



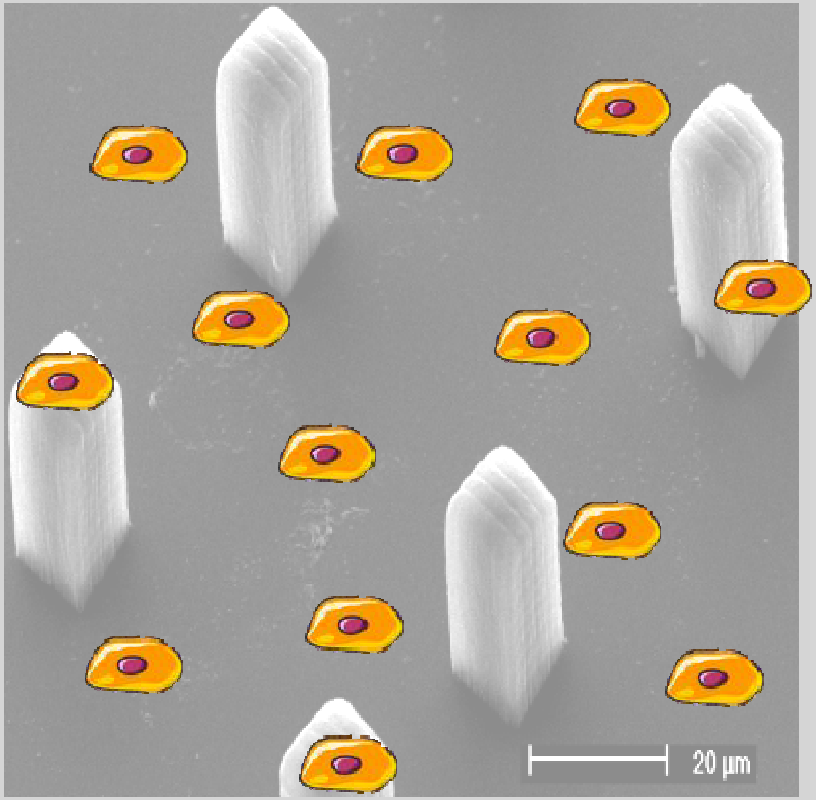


Stem cell scaffolds



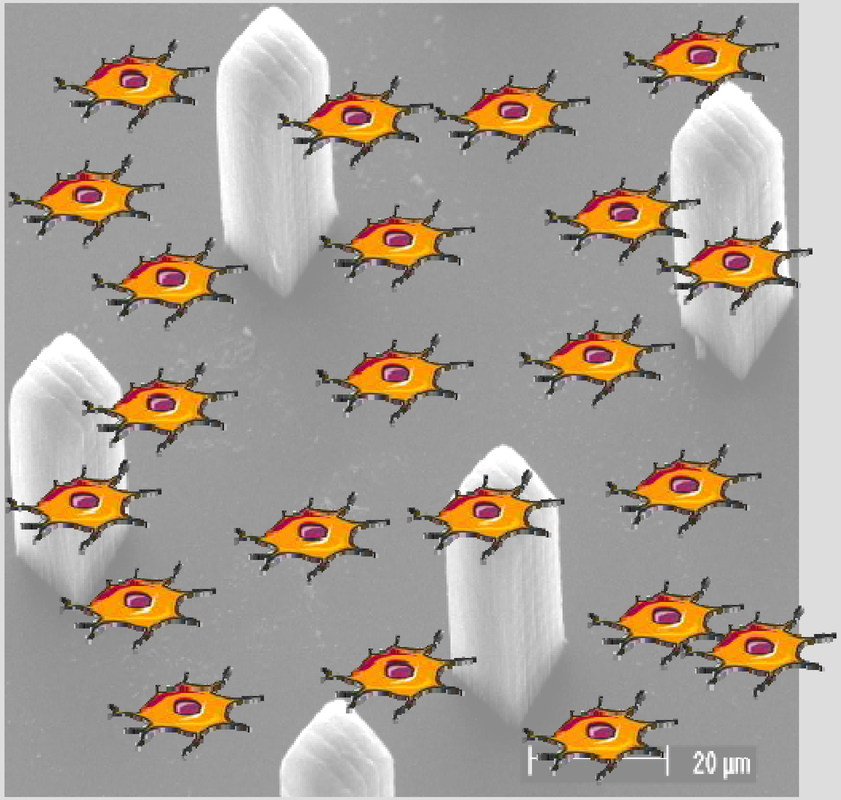


Stem cell differentiation



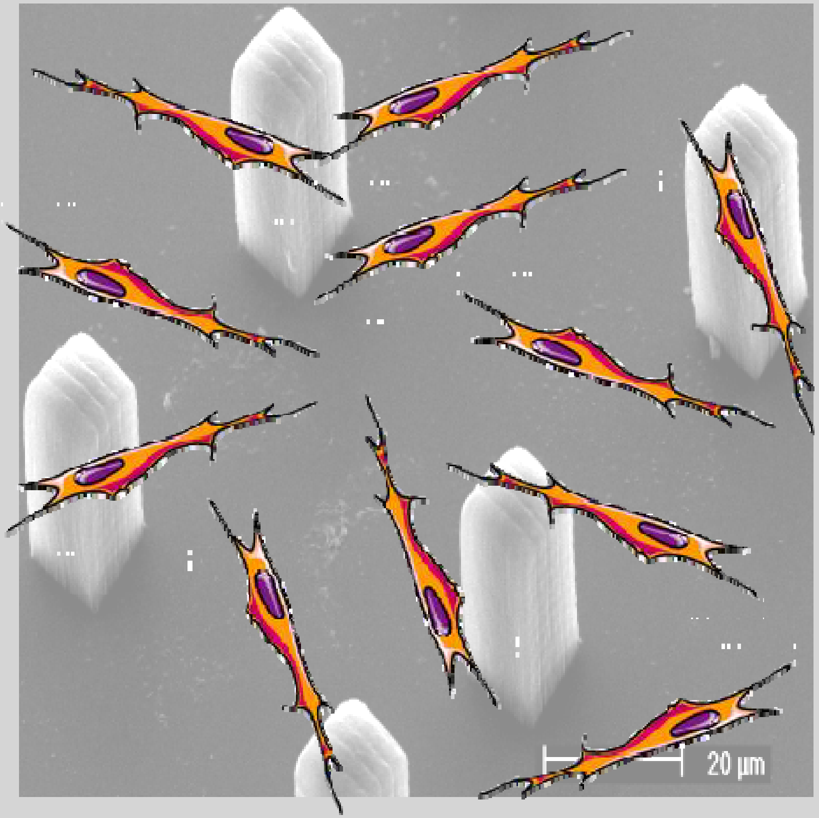
Adhesion

Stem cell differentiation



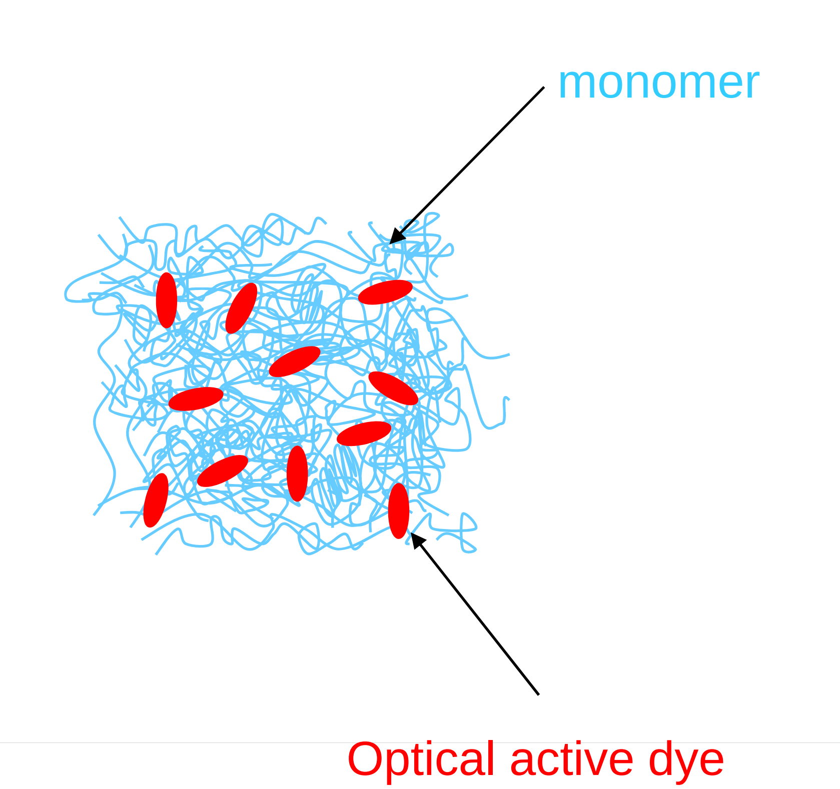
Proliferation

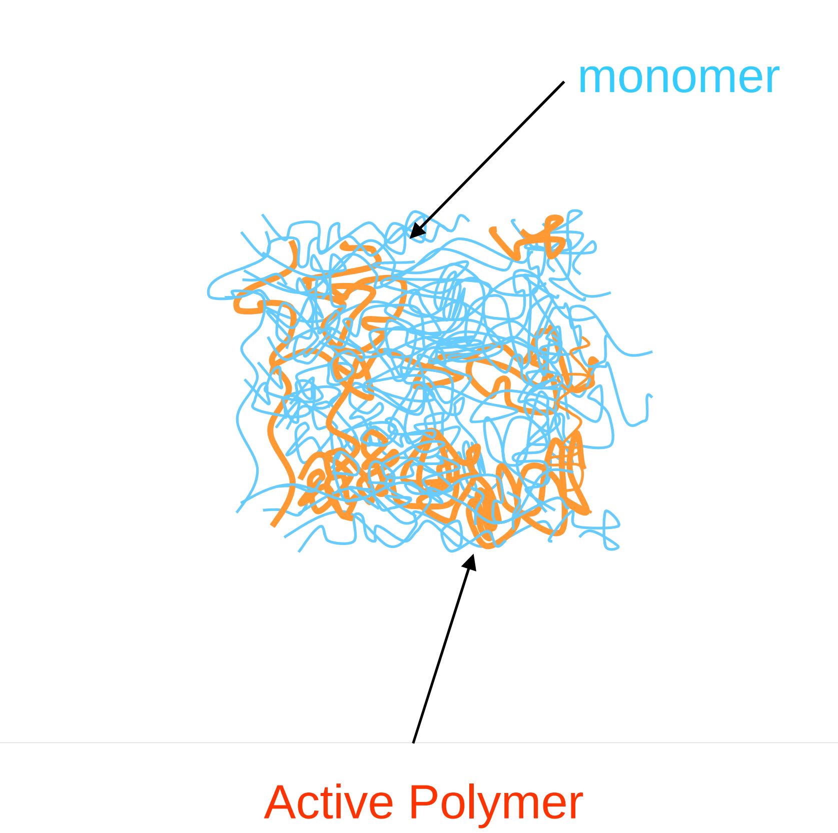
Stem cell differentiation



Differentiation

Microstructures with active compounds



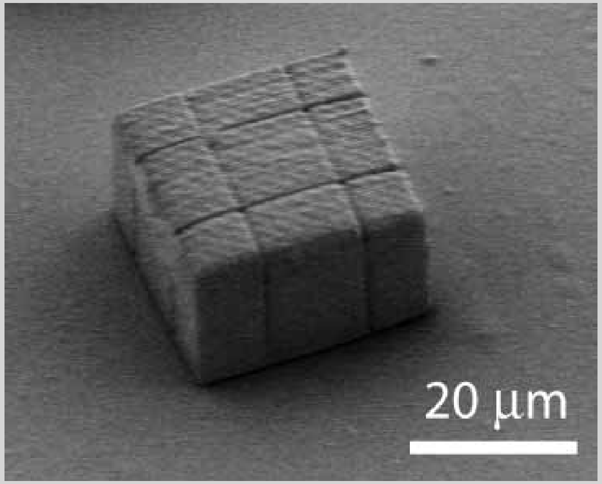


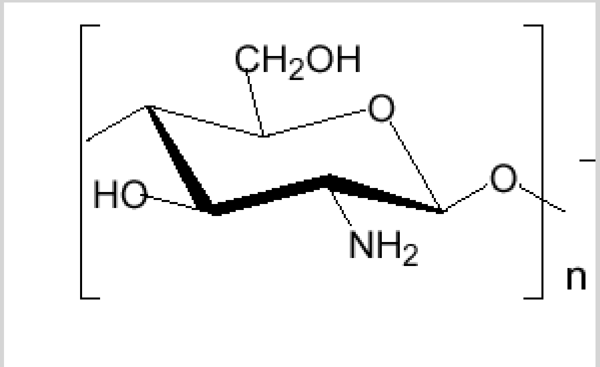
Doping microstructures

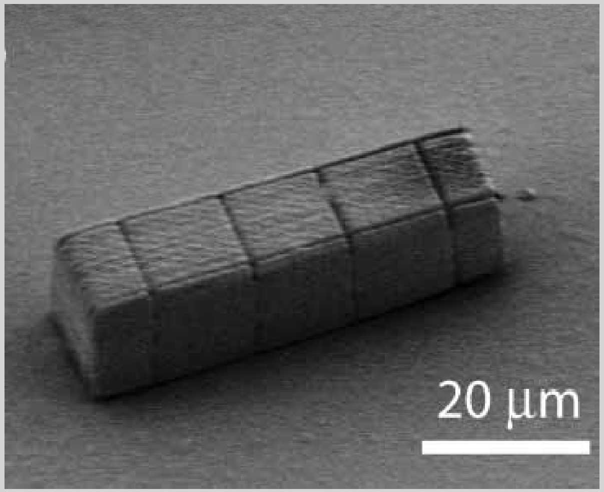
Fabrication of microstructures with special topological and chemical design for bio-relates applications

Doping microstructures

- microstructures containing biopolymer - chitosan

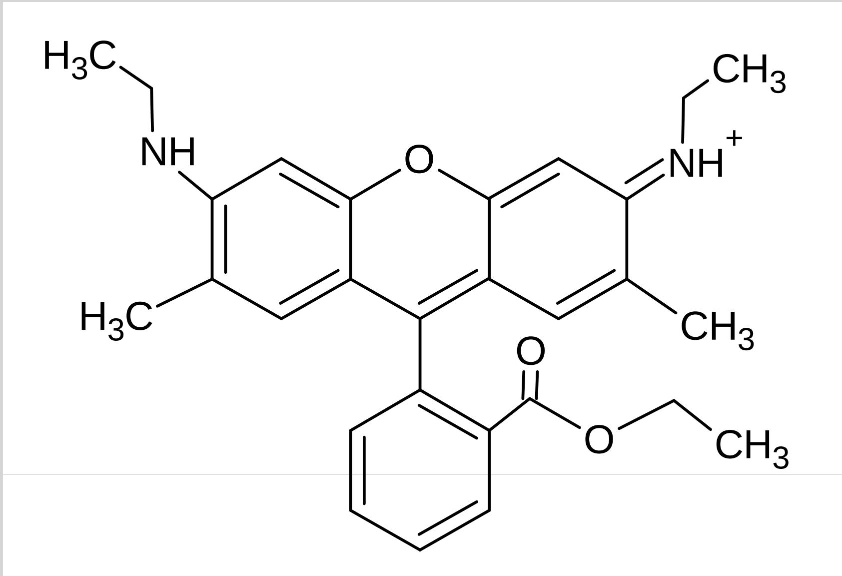






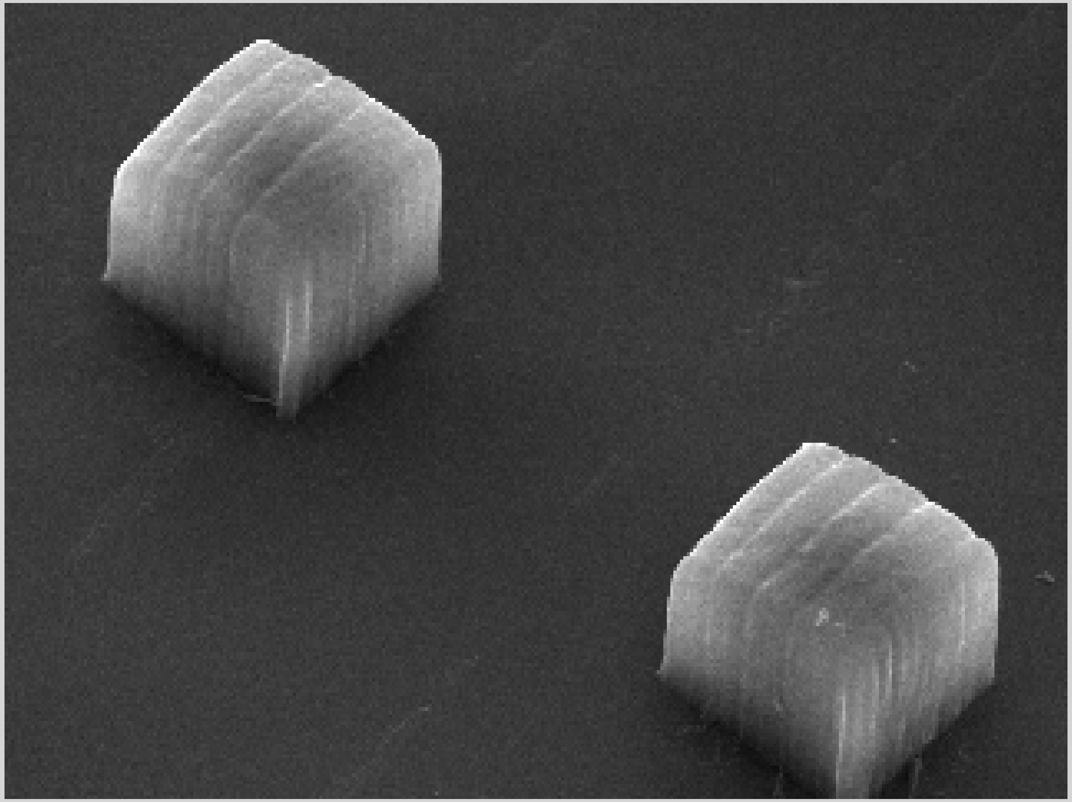
Microstructures containing Rhodamine

Rhodamine 6G

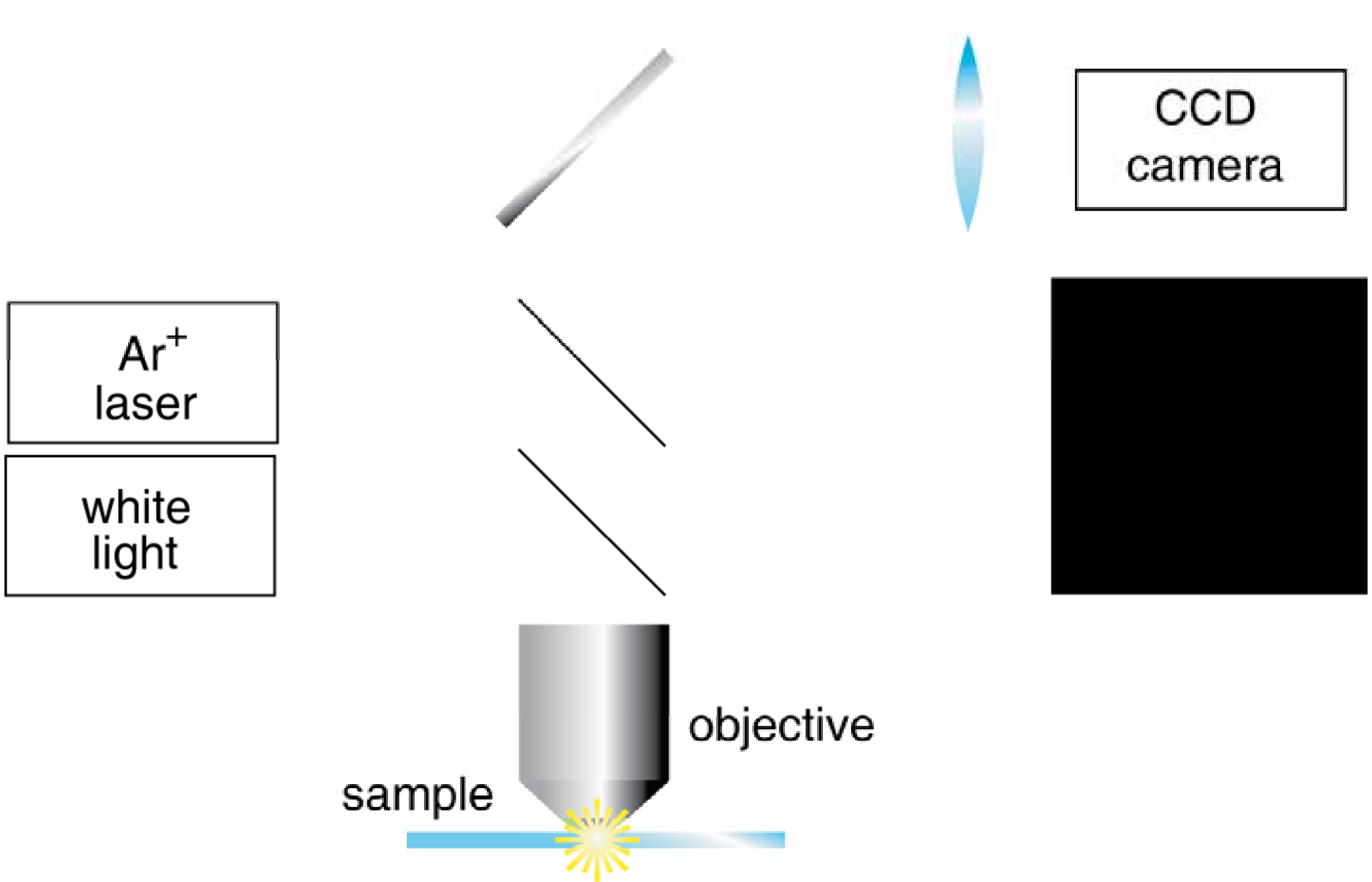


- *High luminescence*
- *Used as dye laser gain medium*

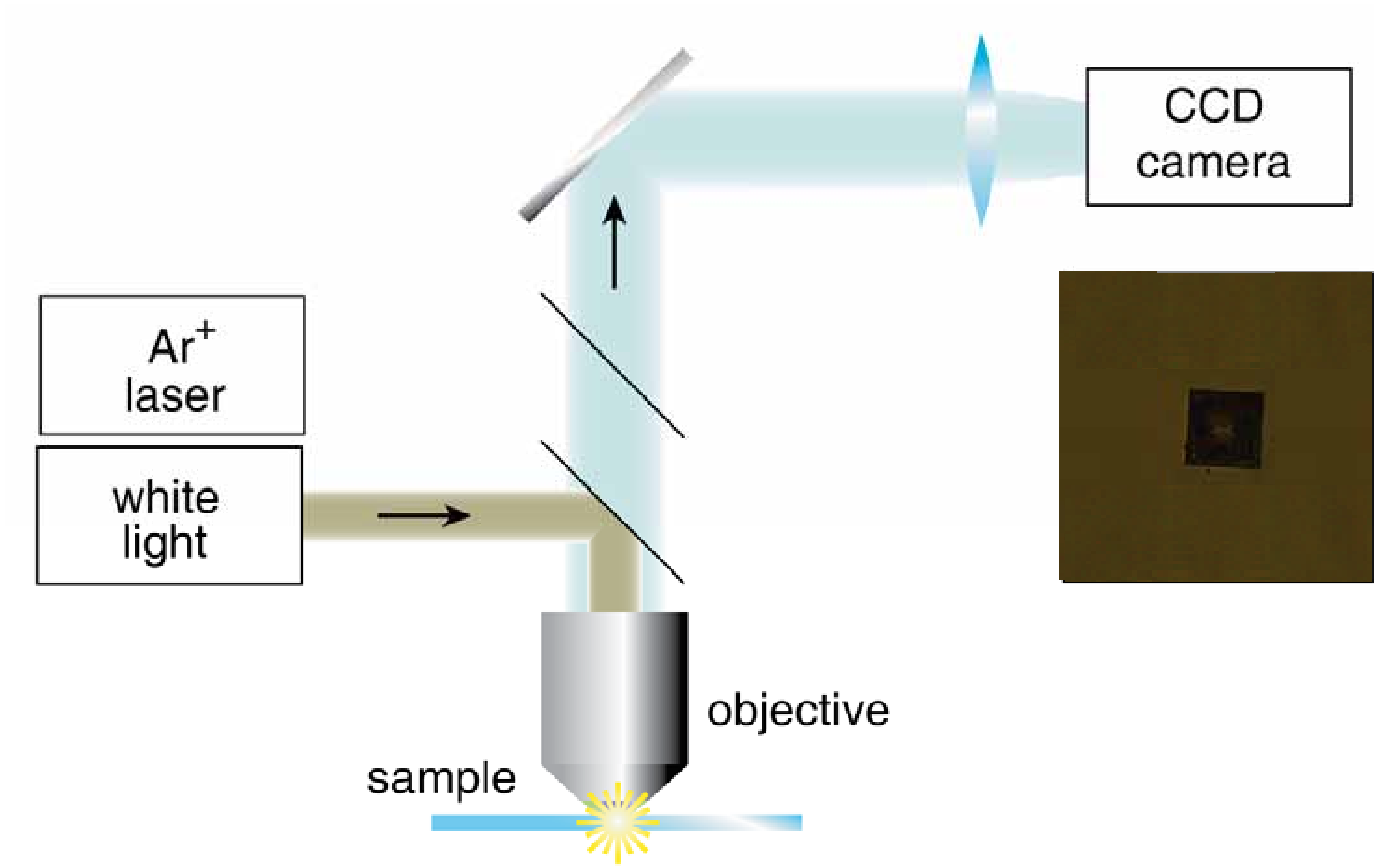
Microstructure containing Rhodamine



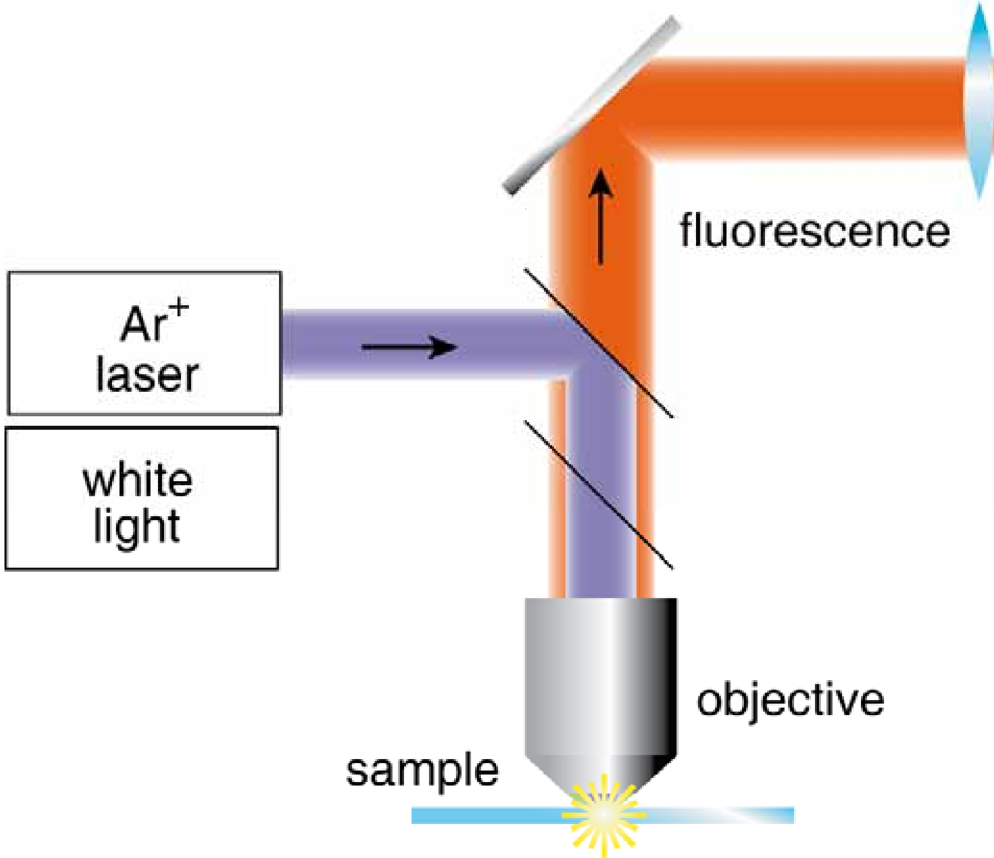
Microstructure containing Rhodamine

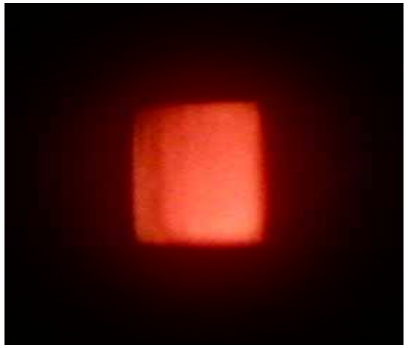


Microstructure containing Rhodamine

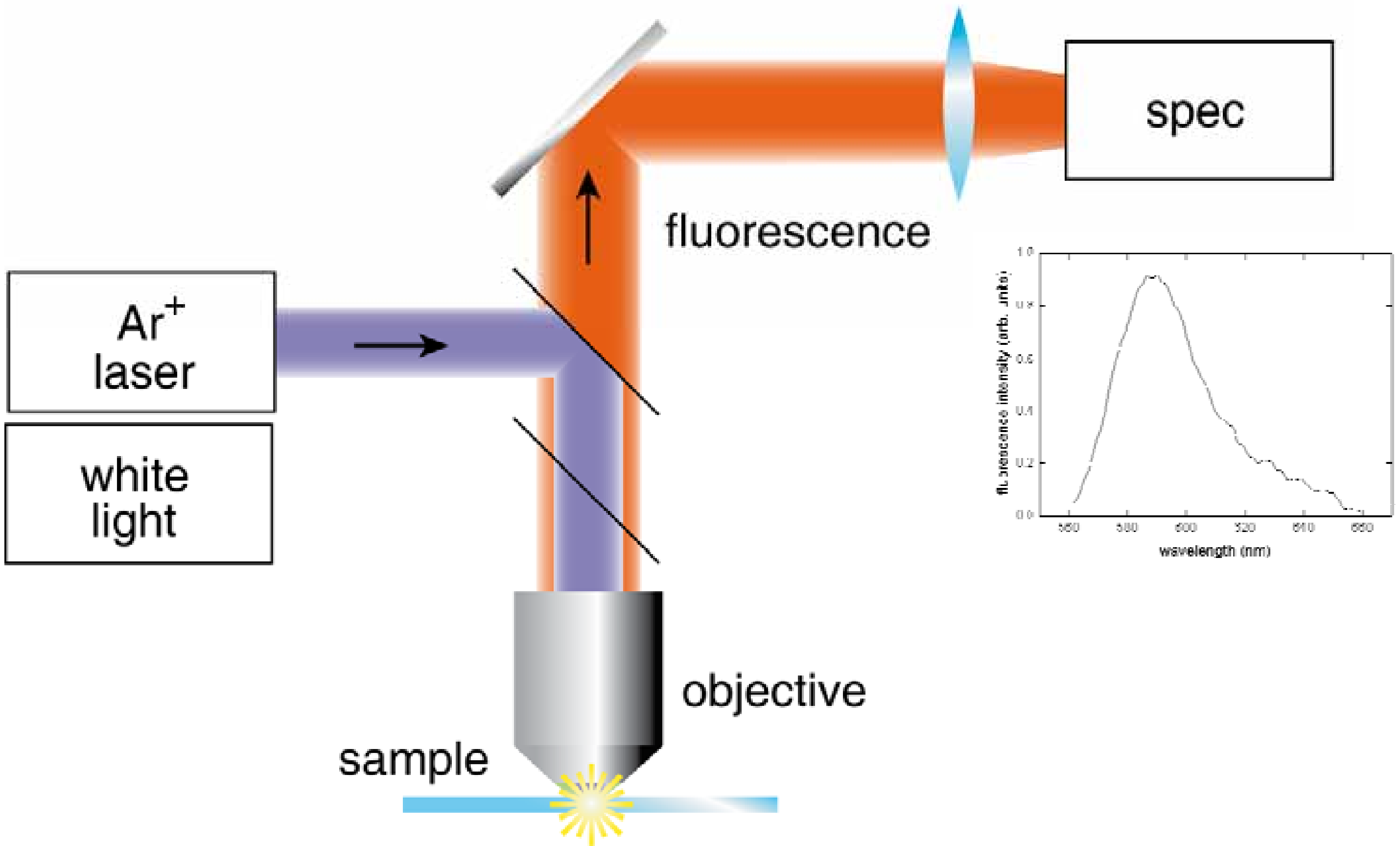


Microstructure containing Rhodamine

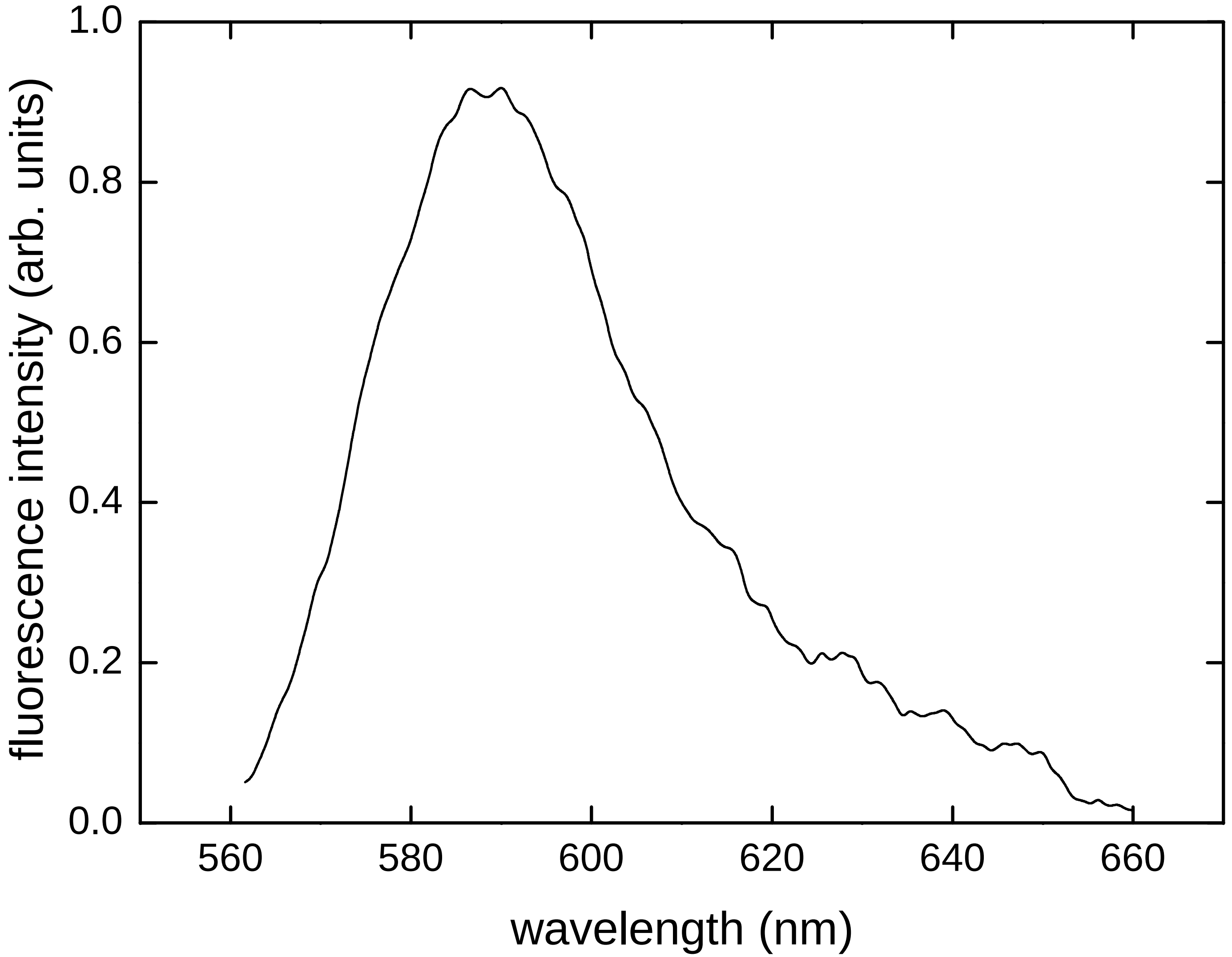




Microstructure containing Rhodamine

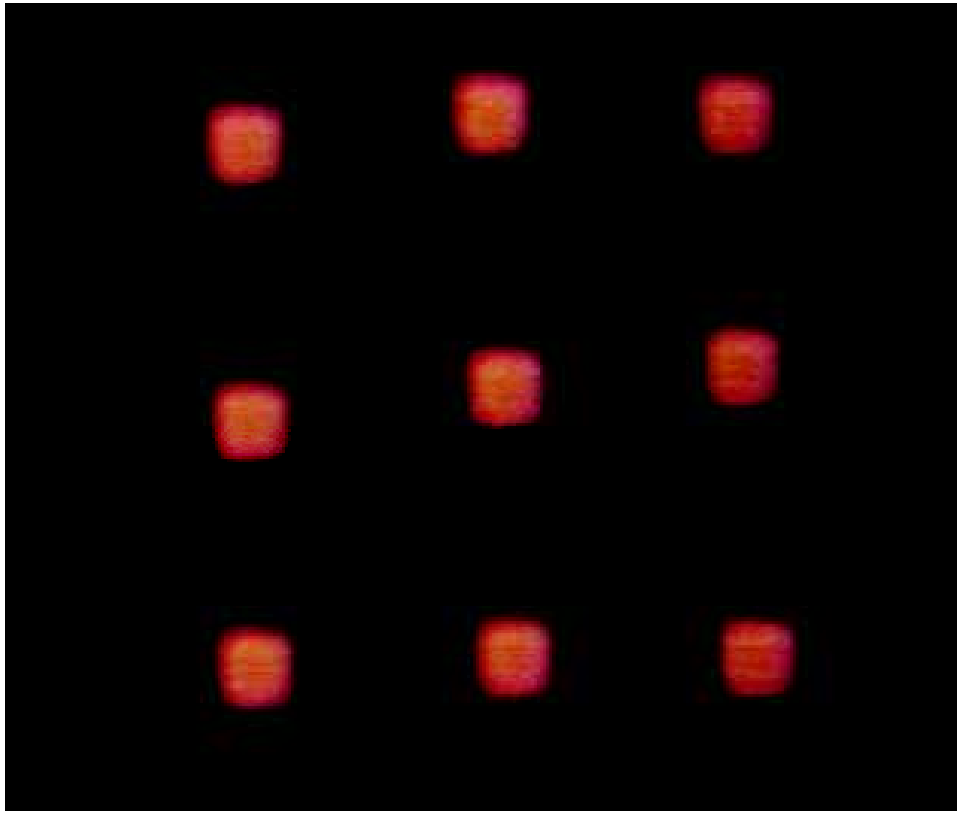


Microstructure containing Rhodamine



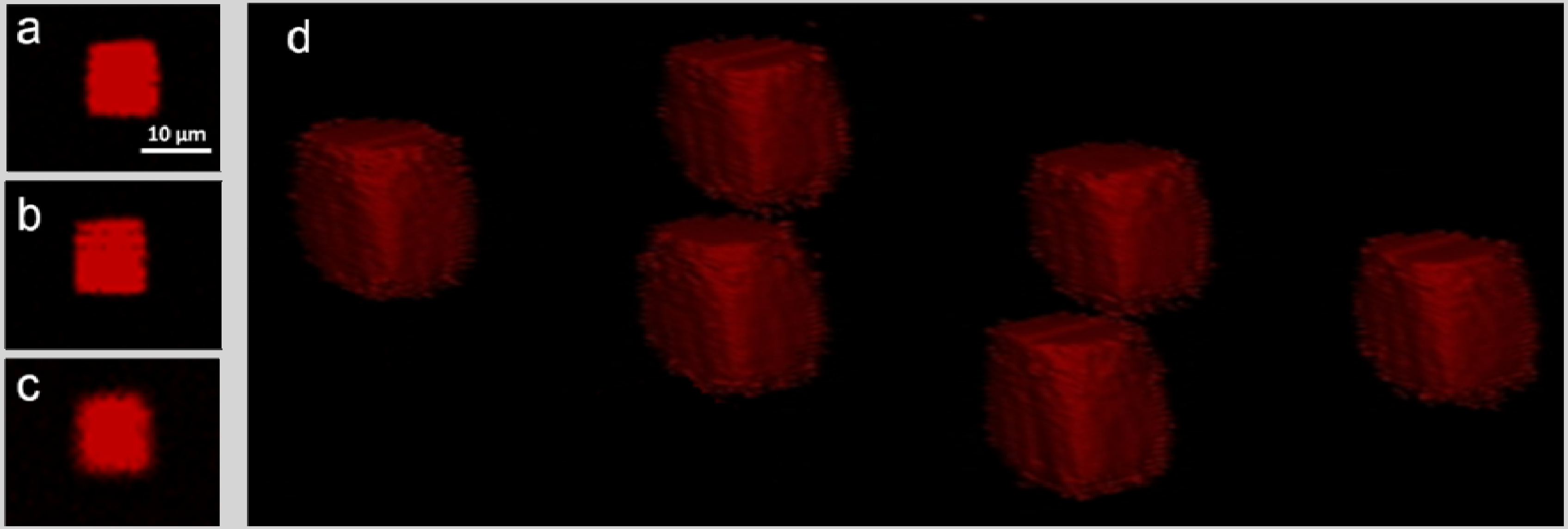
Microstructure containing Rhodamine

fabrication of array of doped microstructures



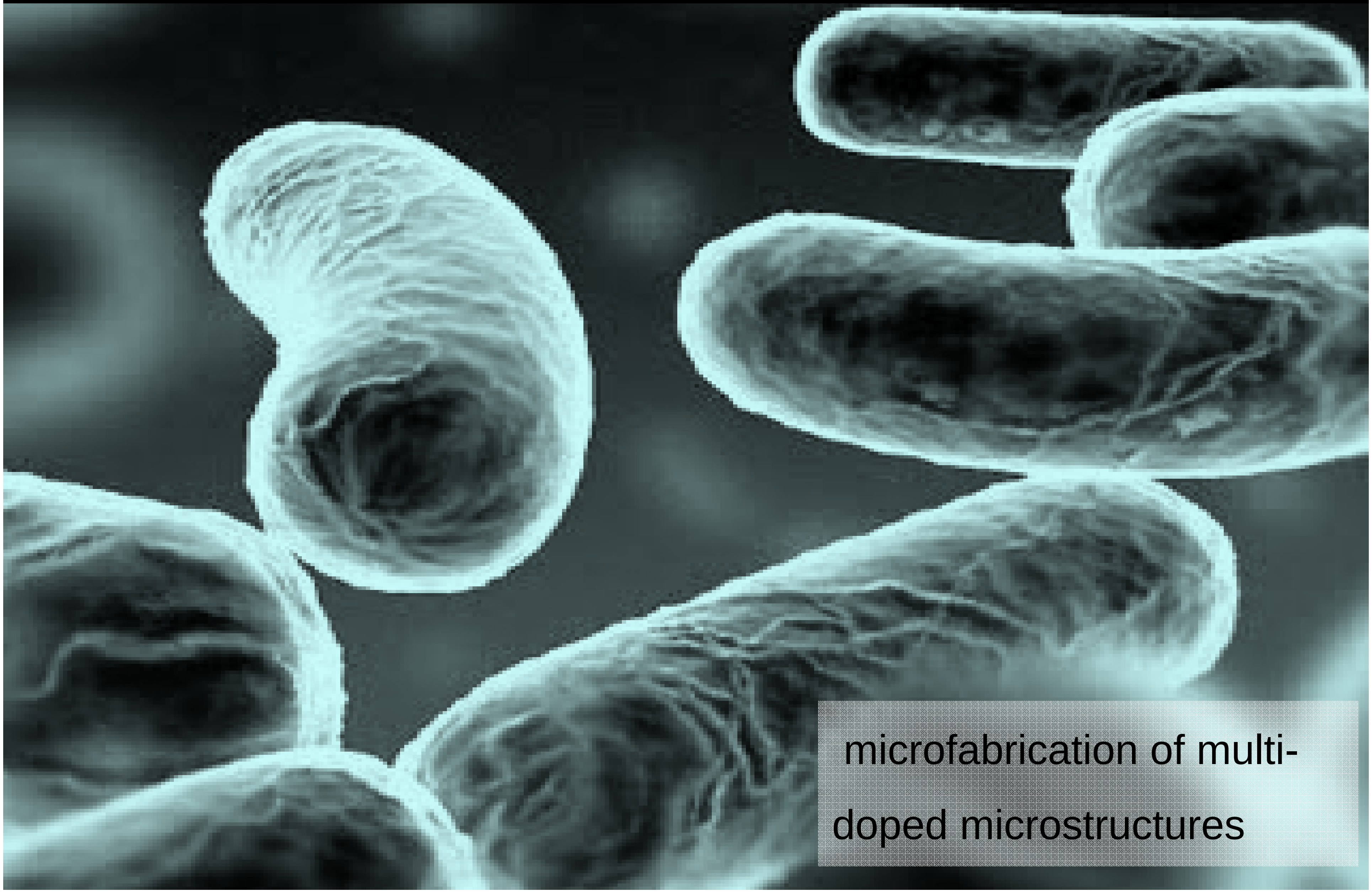
Microstructure containing Rhodamine

Fluorescent confocal microscopy



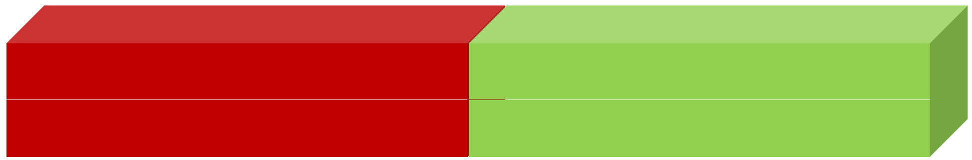
planes separated by 6 μm

Guiding bacterial growth in a micro-environment

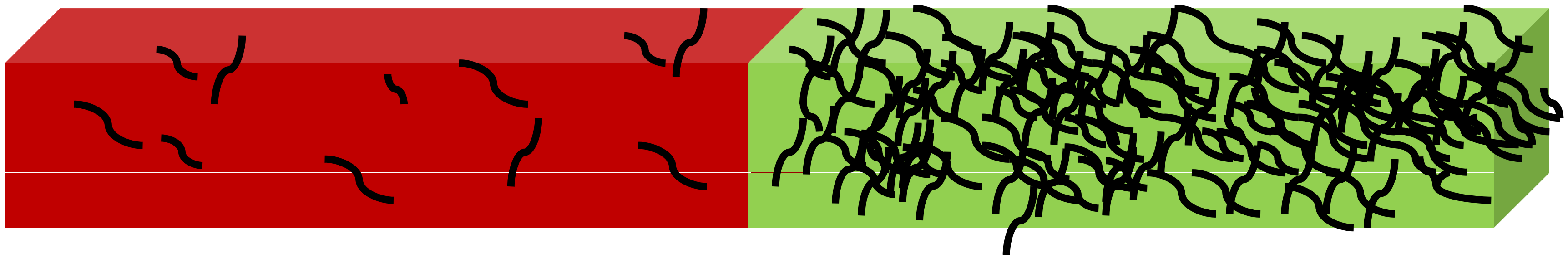


microfabrication of multi-doped microstructures

Guiding bacterial growth in a micro-environment

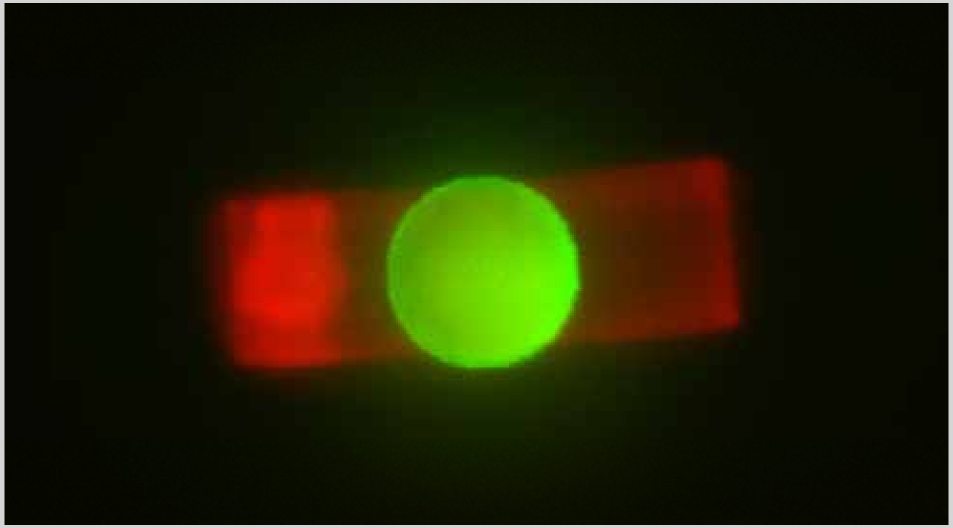


double doped microstructure



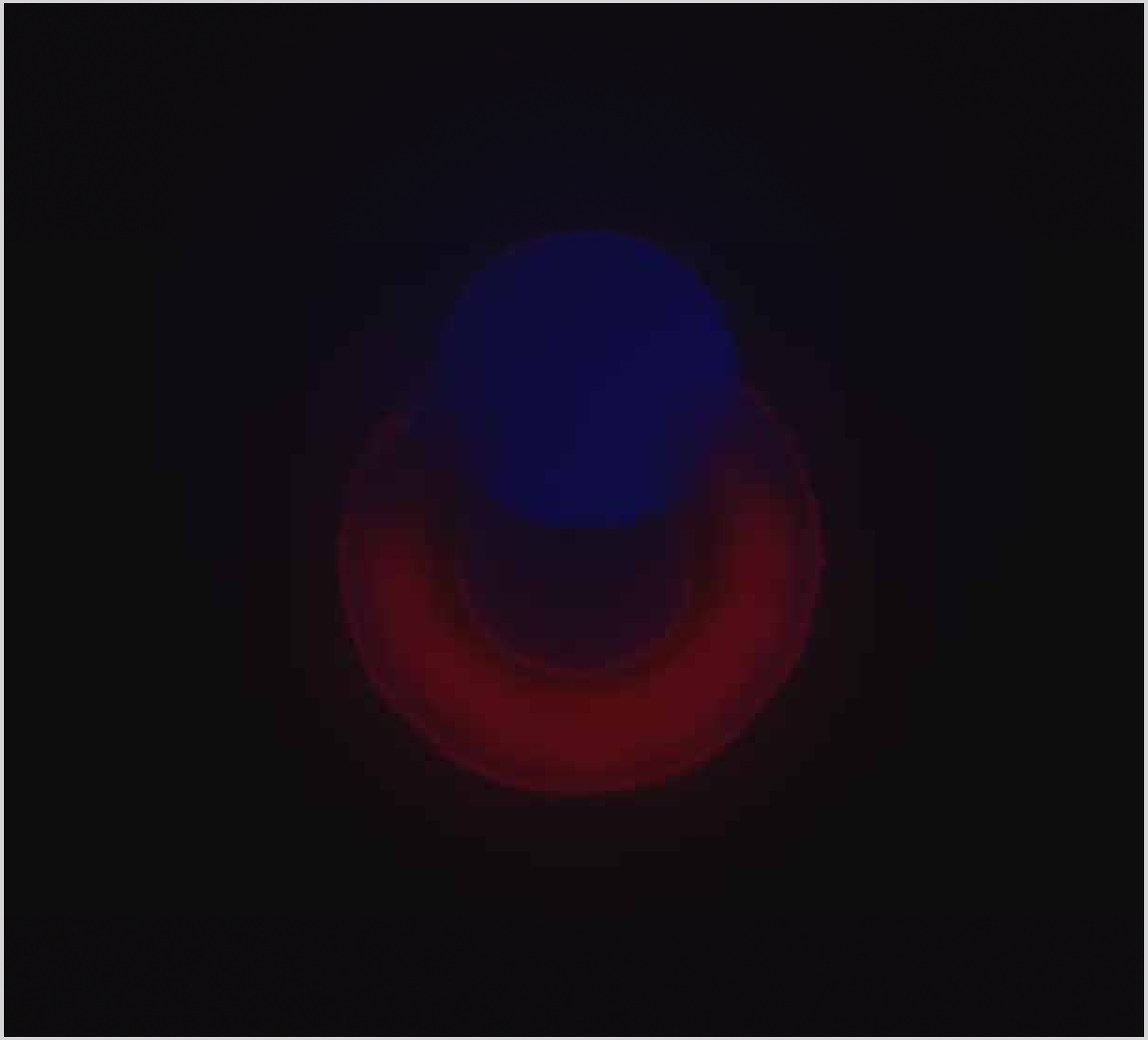
Induce cell growth in distinct regions

Double doped microstructures fabrications

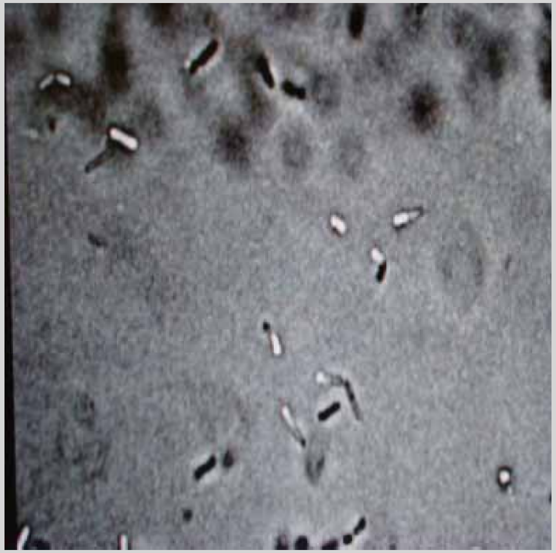


microstructure containing Fluorescein and Rhodamine

Double doped microstructures fabrications



Viability of the Lactobacilus in the resin

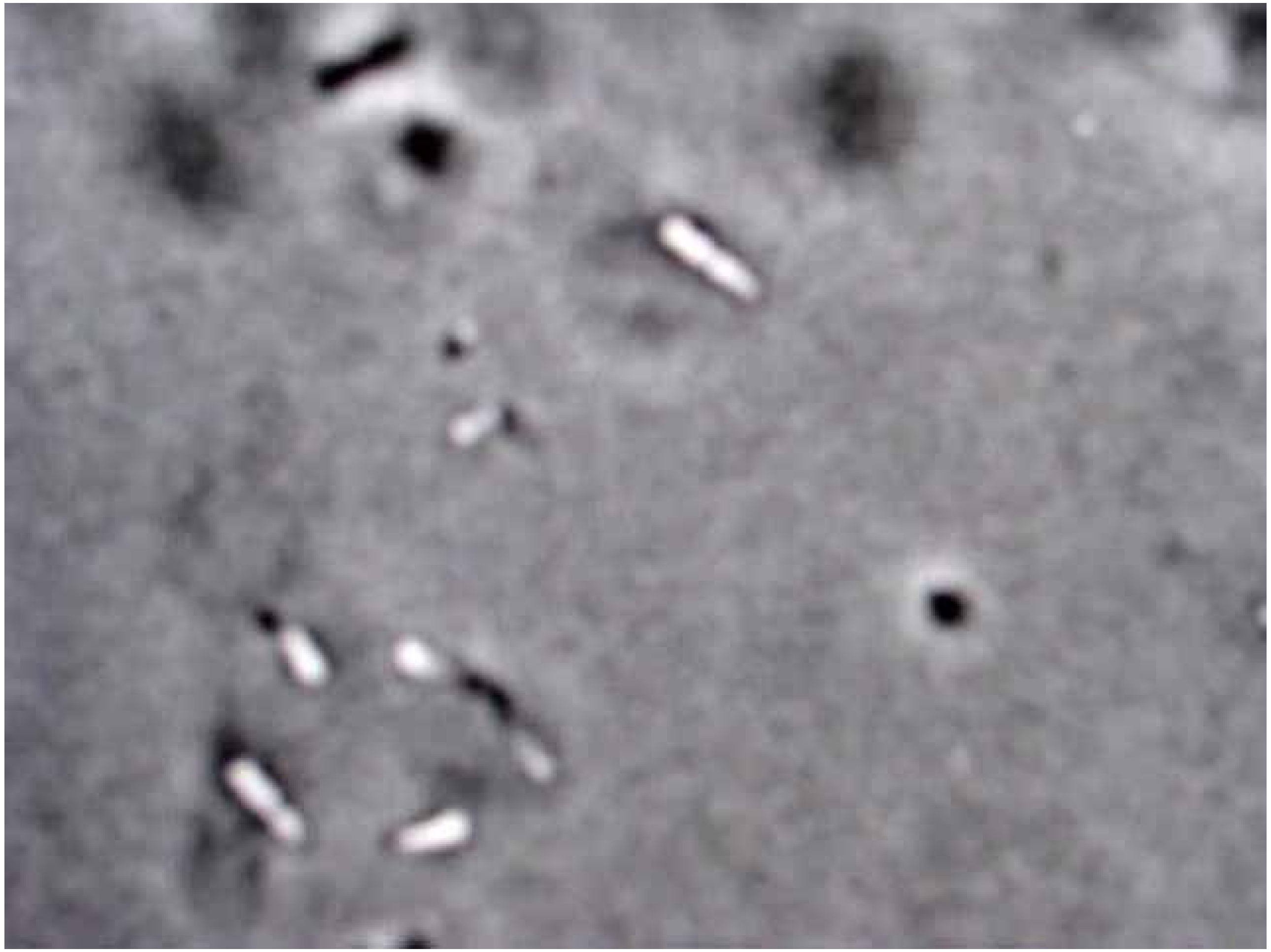


day 0

day 3

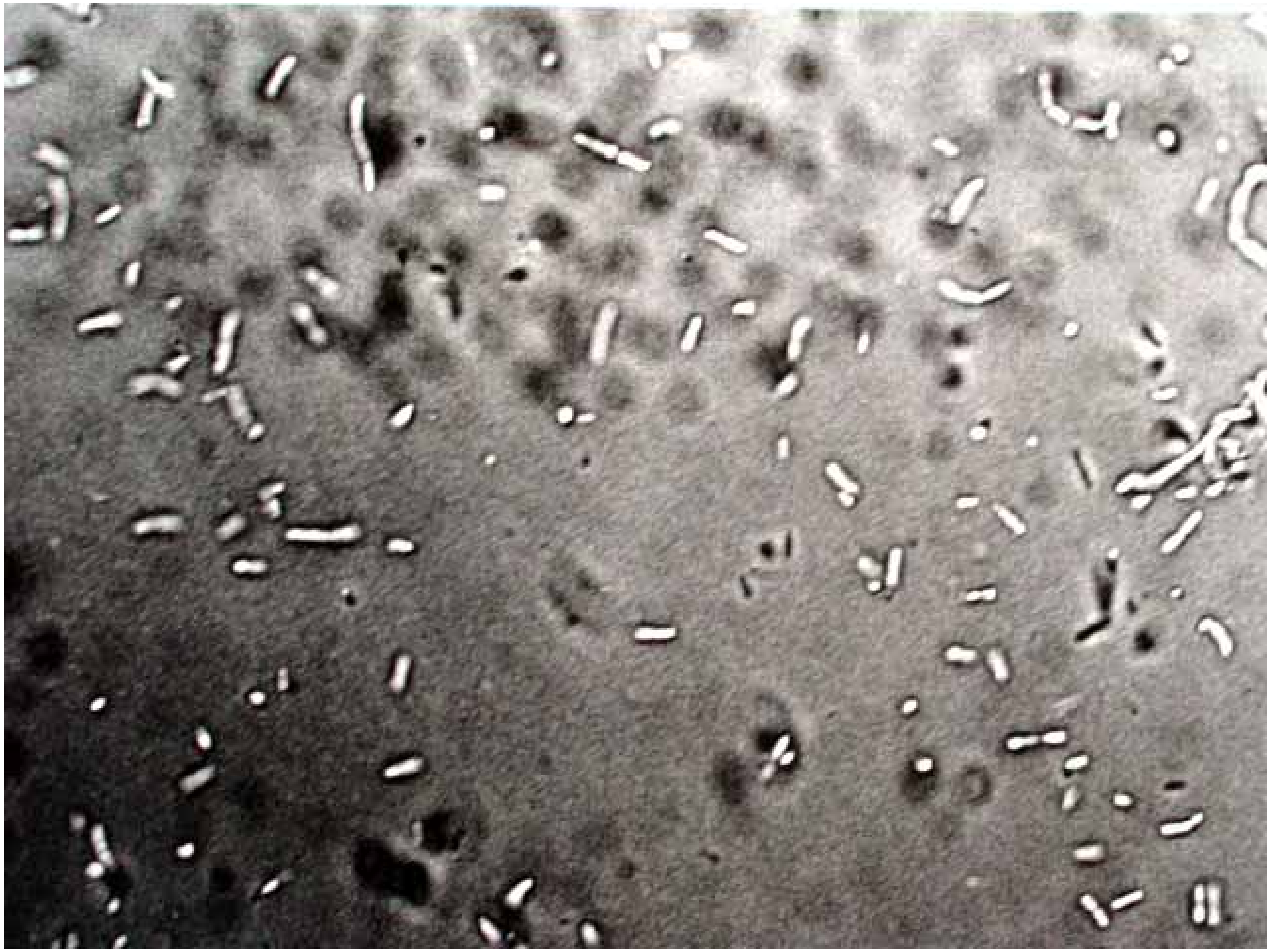
day 7

Viability of the Lactobacilus in the resin



day 0

Viability of the Lactobacilus in the resin



day 3

fs-laser spectroscopy of bio-materials

multi-photon absorption

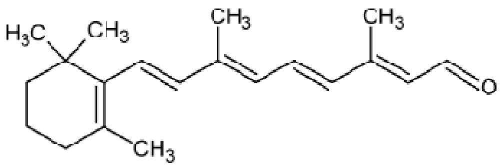
nonlinear refraction

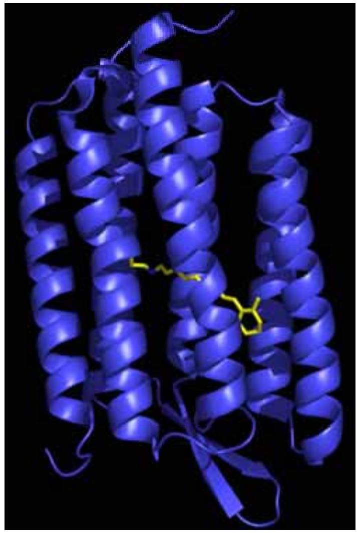
excited state absorption processes

dynamics of ultrashort optical processes

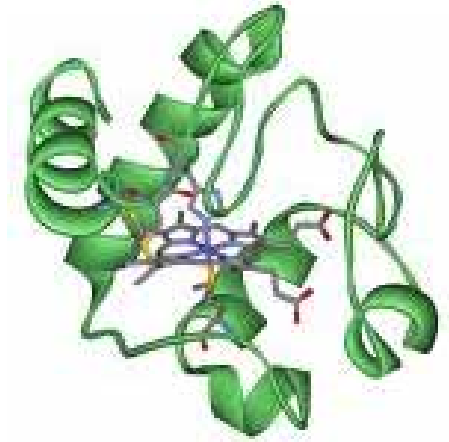
fs-laser spectroscopy of bio-materials

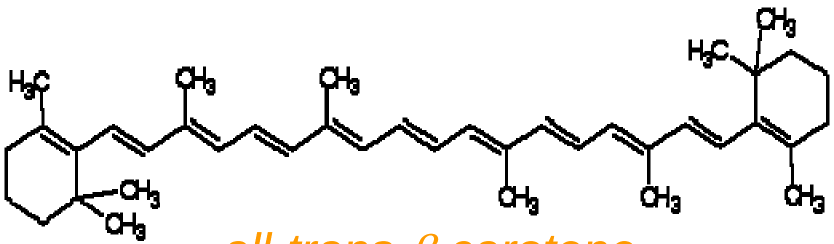
all-trans retinal



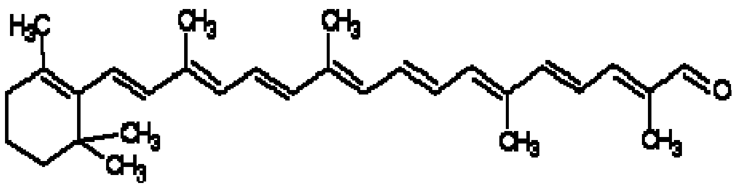


cytochrome c





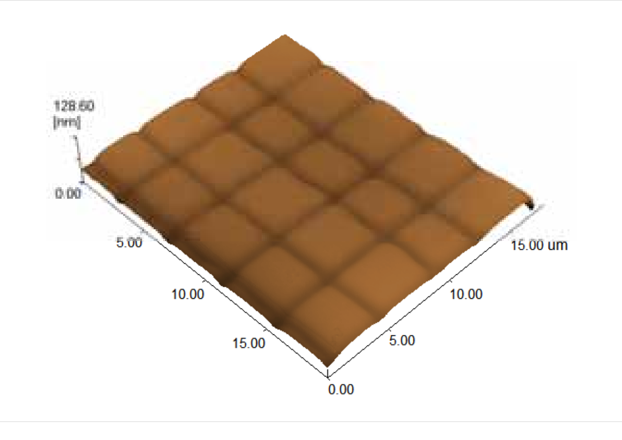
all-trans β-carotene

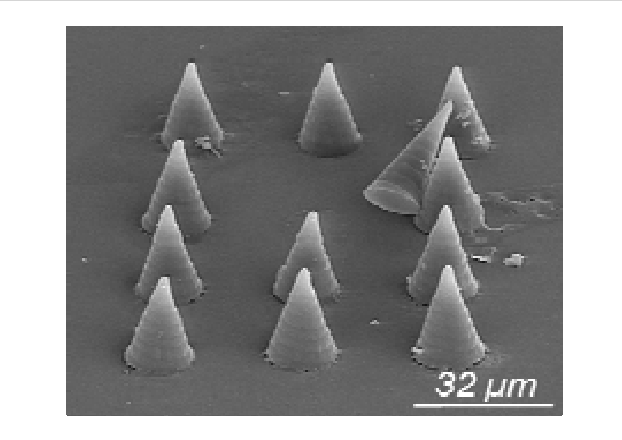


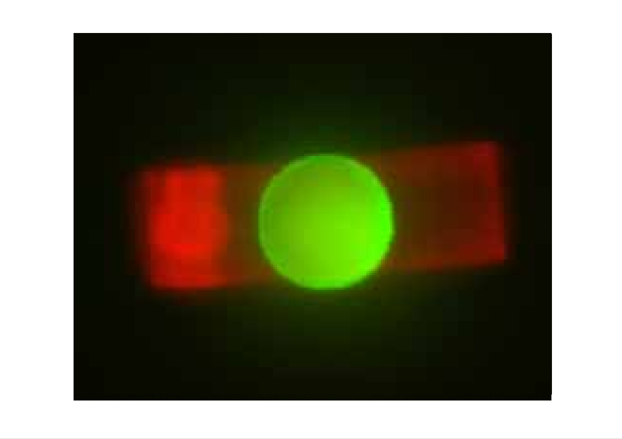
trans β-apo-8´carotenal

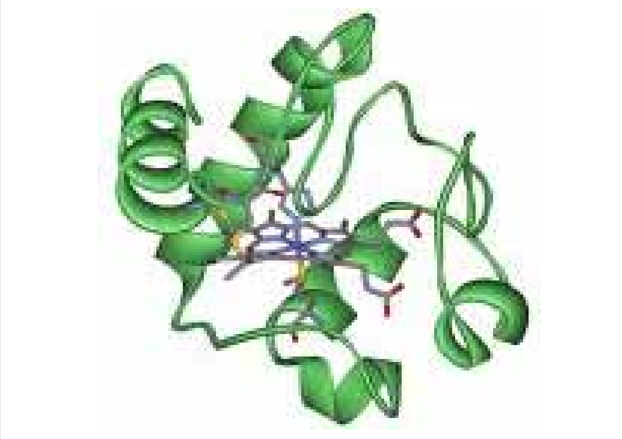
Poster :
Marcelo G. Vivas

Summary









Acknowledgments

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