Laser processing and manufacturing of micro- and nanoscale biosystems

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An event of the Romanian Presidency of the Council of the European Union

Miniaturized platforms for bio-related applications

Motivation:

1. boosting sensing capabilities by bio-mimicking and reducing sizes in 2D & 3D configurations;

2. replacing conventional methods by innovative technologies to reduce cost and propose new characteristics.

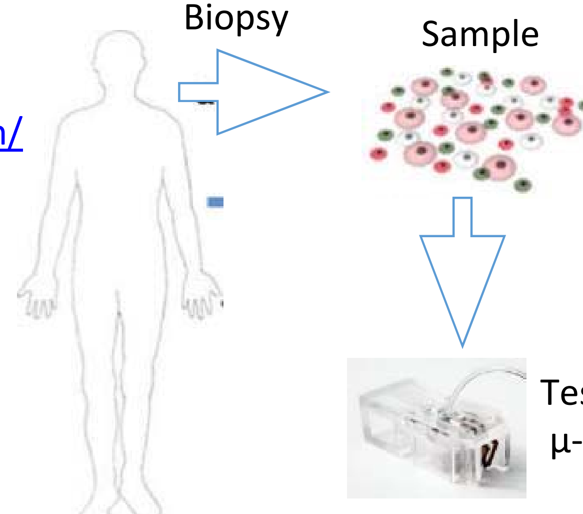
2D

http://web.anl.gov

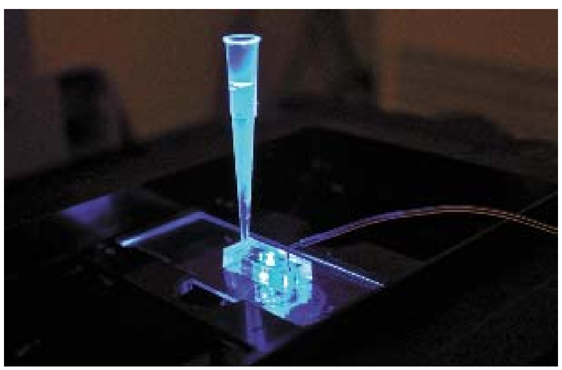
www.beyondpositive.org

Localized implant coatings

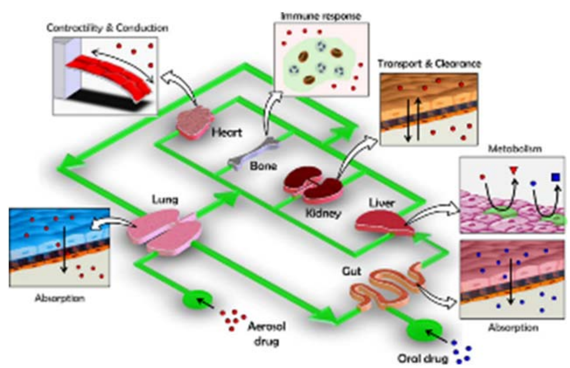
http://kyocera-md.com/
technology/surface/



3D



http://www.dailycal.org

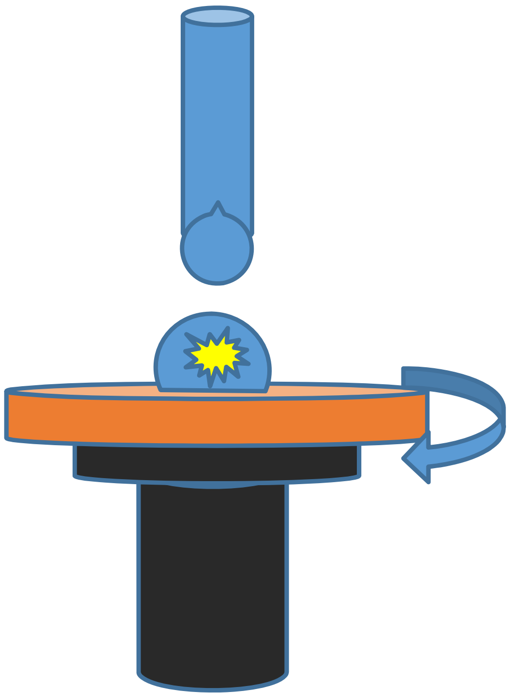


Trends Cell Biol. 2011, 21(12): 745

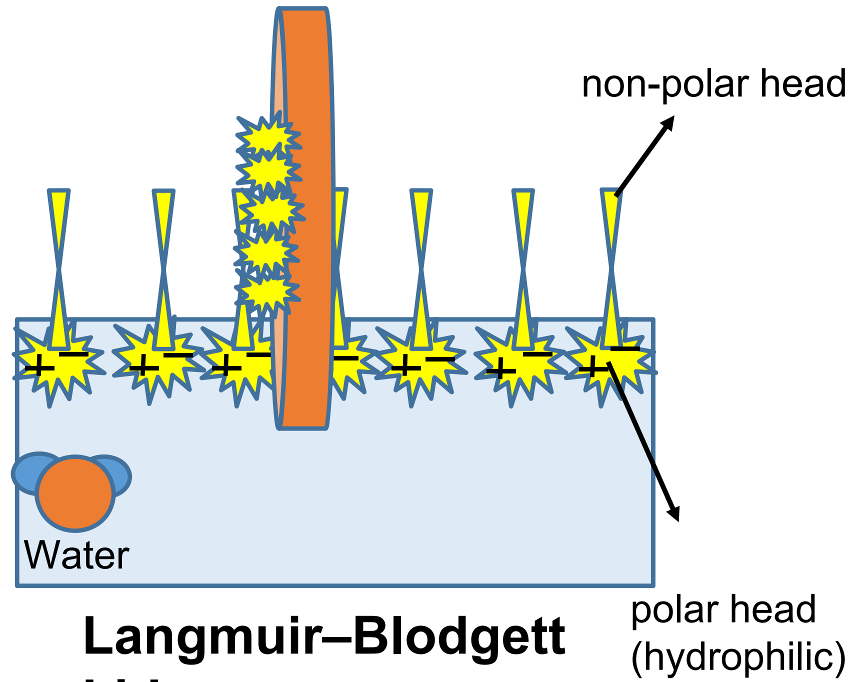
Devices that require robustness, biocompatibility and functionality (if possible, multiple use) – Lab on a Chip (LoC), Point of Care (PoC)

Biomimetic microsystems –
single biochip -
microfluidic circulatory 3D system drug absorption –
evaluate drug efficacy and toxicity.

Conventional techniques to deposit thin organic coatings



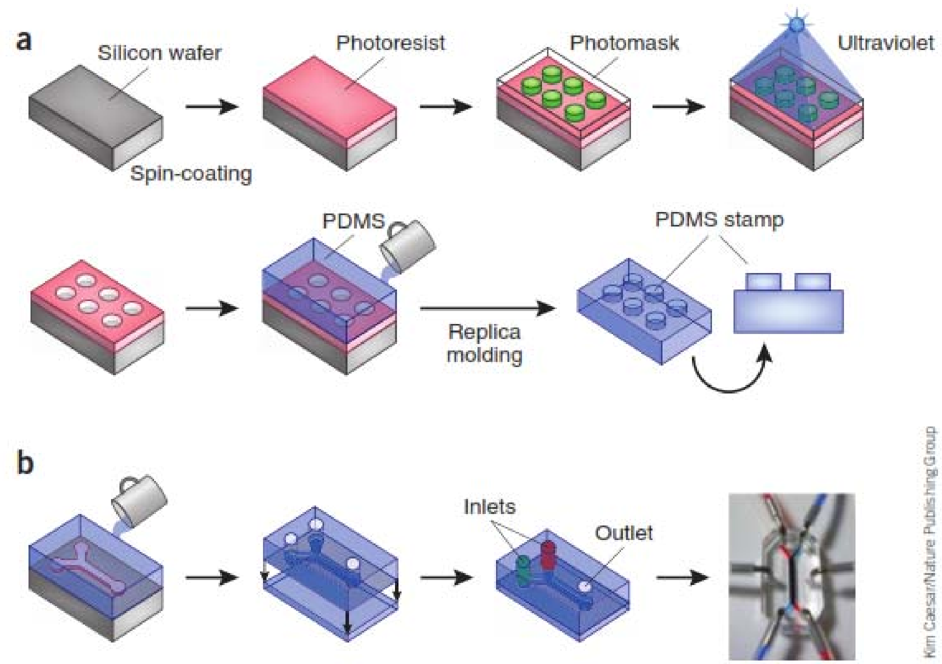
Spin coating



Langmuir–Blodgett
LbL

Material consuming techniques;
Substrate limited;
Difficult to obtain multistructure-layers

Conventional biochip 3D platforms: current approach and limitations of 3D cell culture to organs-on-chips



PDMS - poly-dimethylsiloxane

Nature Biotechnology, 32, 760 (2014)

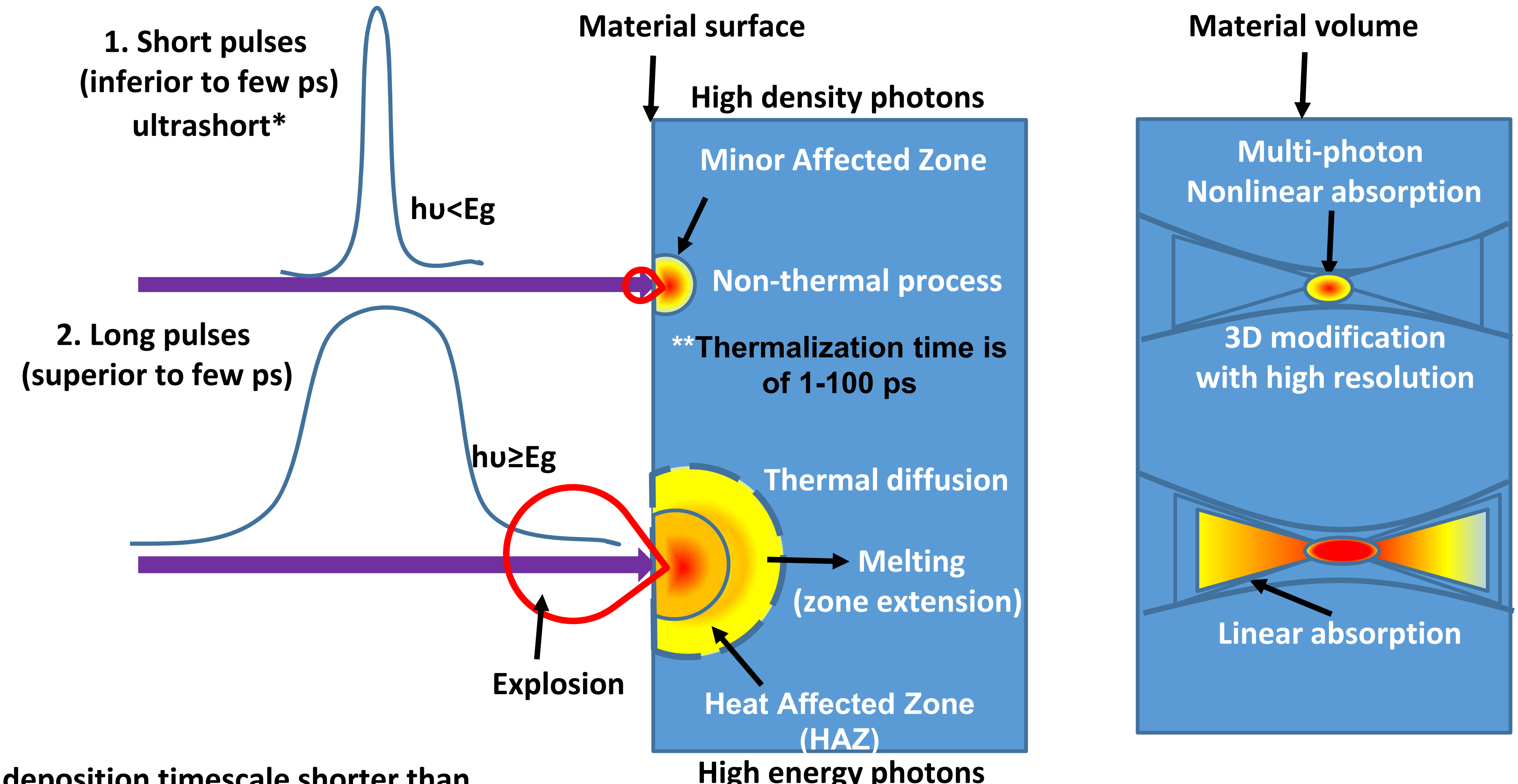
Fabrication methods for microfluidic chips.

Pros: Most chips are made out of PDMS because it is easy to use and has high optical clarity, gas permeability and biocompatibility.

Cons: PDMS can adsorb small organic compounds, including many drugs, and its high gas permeability can hinder some applications.

Unconventional: laser pulses

non-continuous wave with power concentrated in a pulse of some duration

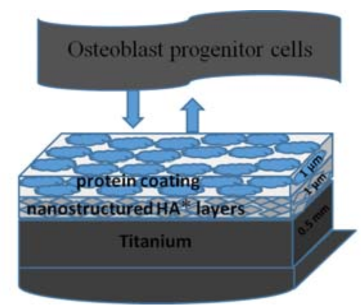


**energy deposition timescale shorter than electron-phonon coupling processes

*Ultrashort = femtosecond (fs) and few picosecond (ps)

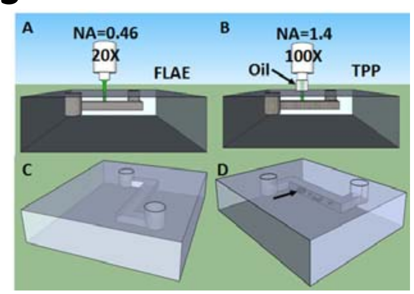
Outline

1. Biomimetic 2D coatings grown by pulsed lasers – long energetic pulses



Biomimetism related to composition – structure - properties

2. Biomimetic 3D environments fabricated by pulsed lasers – ultrashort pulses

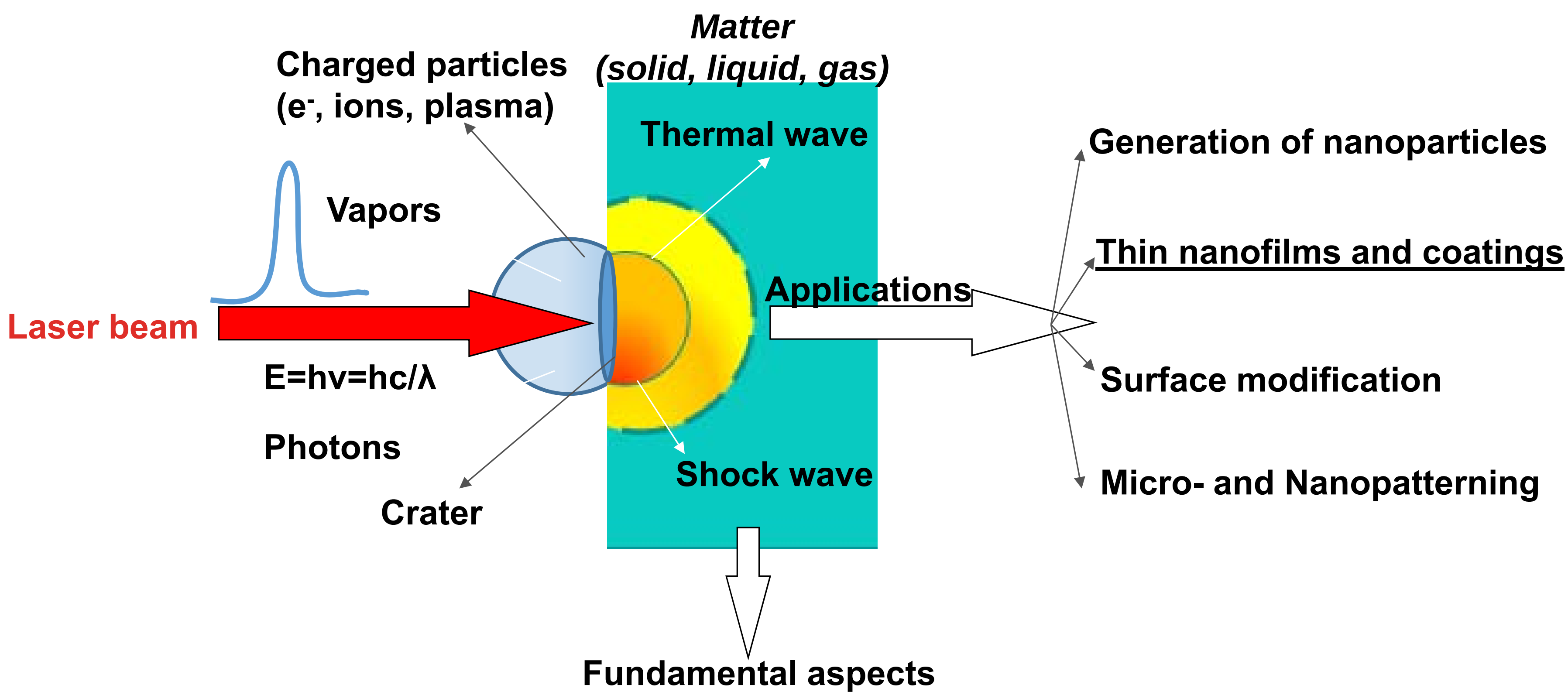


Biomimetism related to architectural and fluid flow aspects

Part I

Biomimetic 2D coatings grown by pulsed laser depositions techniques

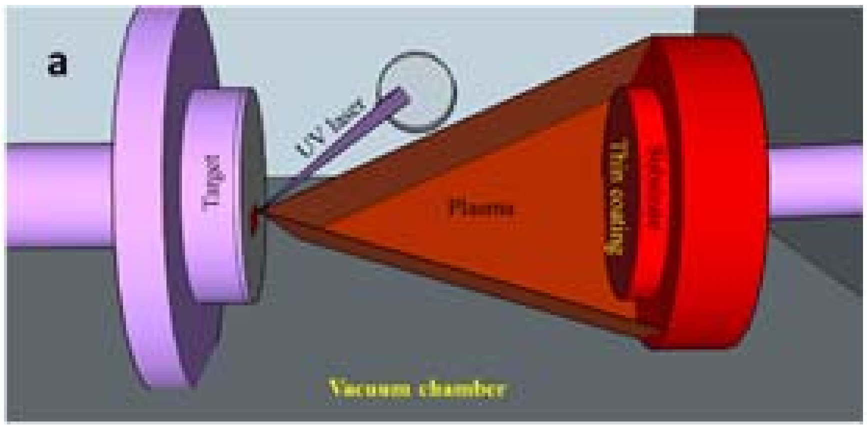
Laser-matter interaction – long pulses

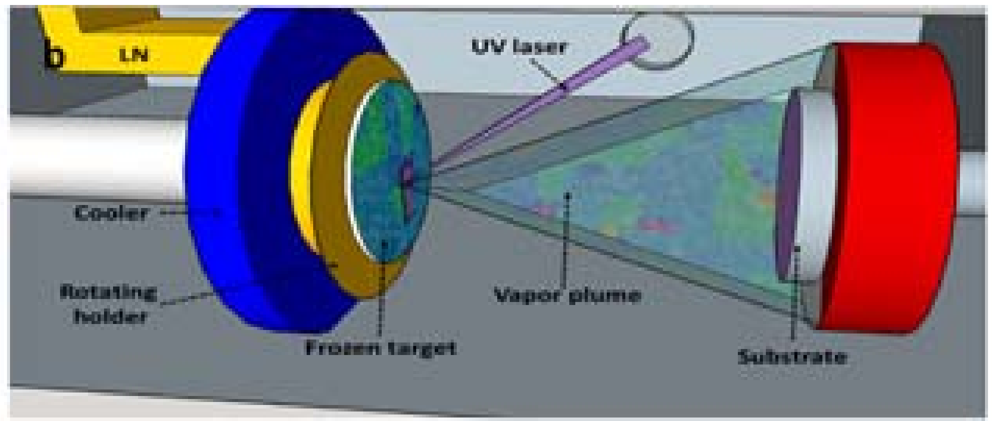


Laser deposition techniques of thin coatings

Pulsed Laser Deposition (PLD)
Inorganic thin films

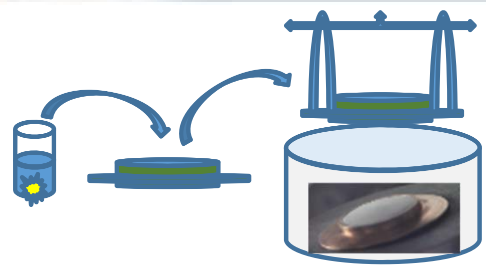
Matrix Assisted Pulsed Laser Evaporation (MAPLE)
Organic thin films





Main difference between PLD and MAPLE:

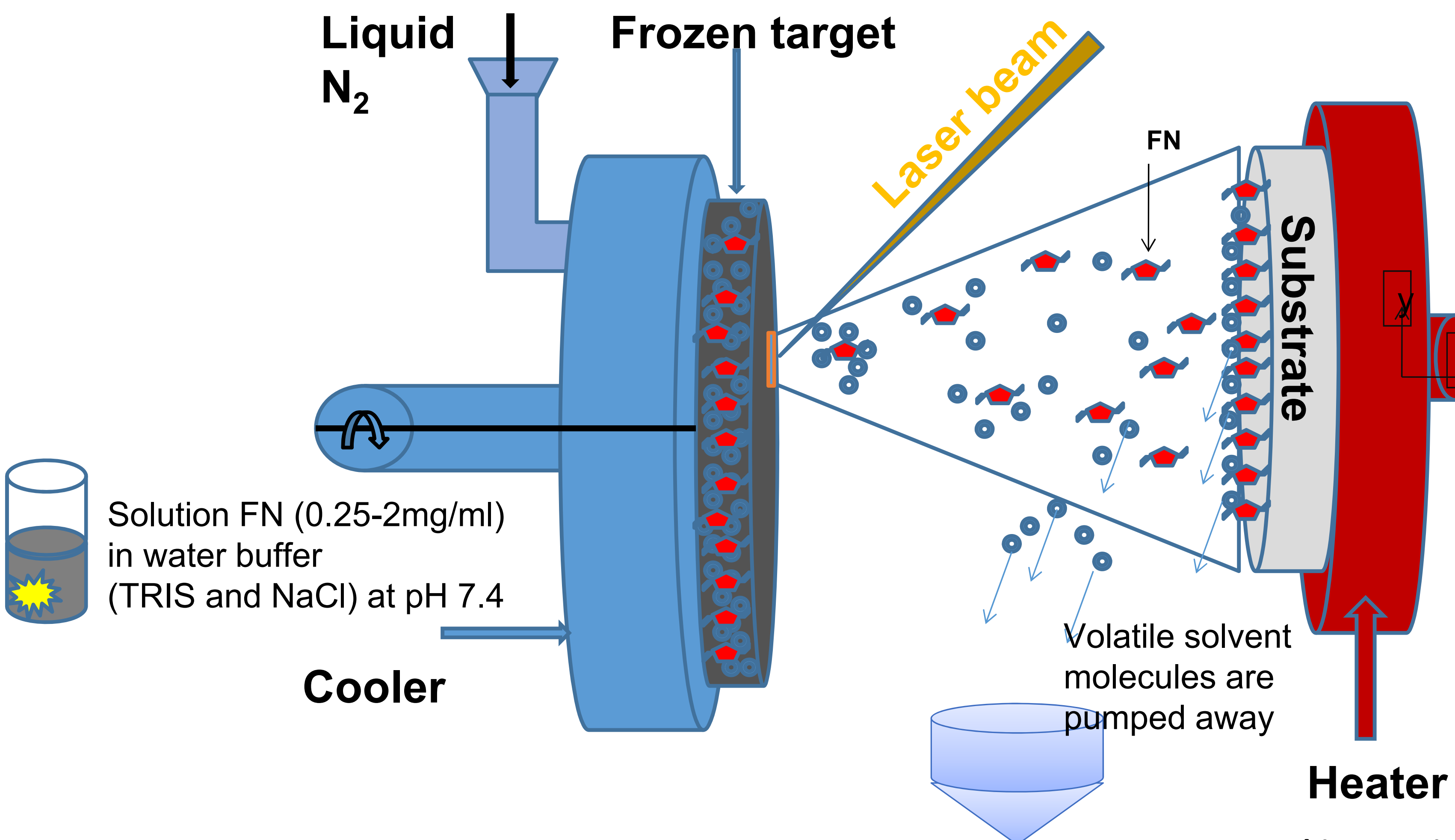
target and implicitly the mechanism of laser - material interaction



- **active material (solute) is dissolved in a solvent (matrix)**
- **the liquid mixture is transformed in solid by freezing (in LN);**
- **target kept at low temperature with a cooler during deposition (100 -200 K).**

Applied Surface Science 418, 580, 2017

MAPLE of fibronectin (FN): “dry” process?

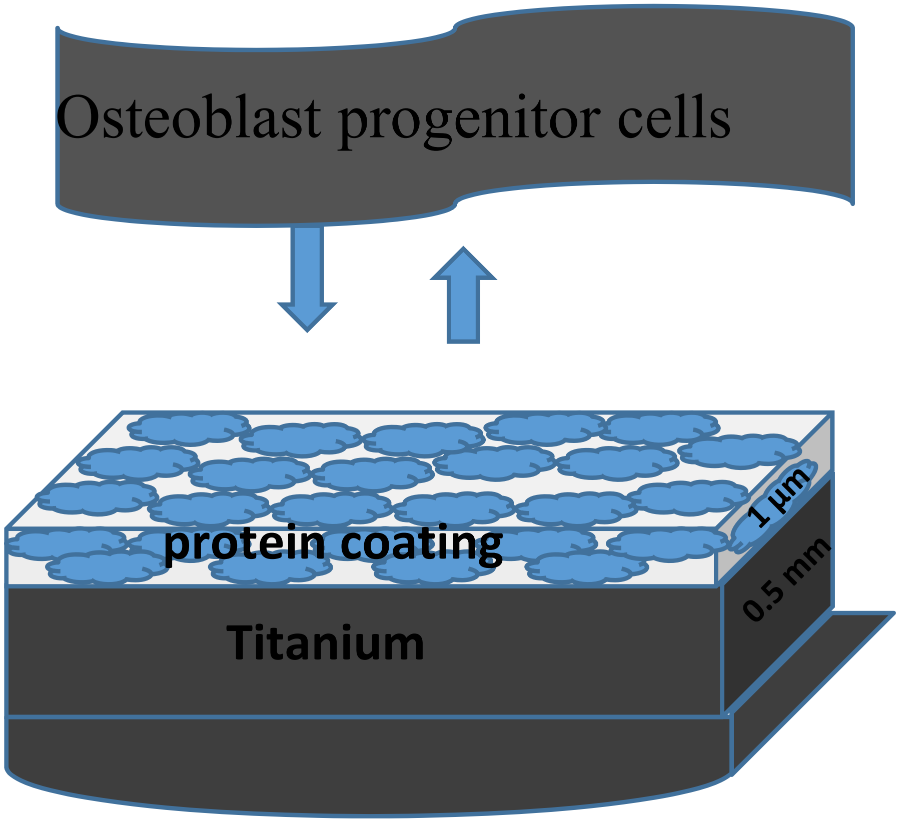


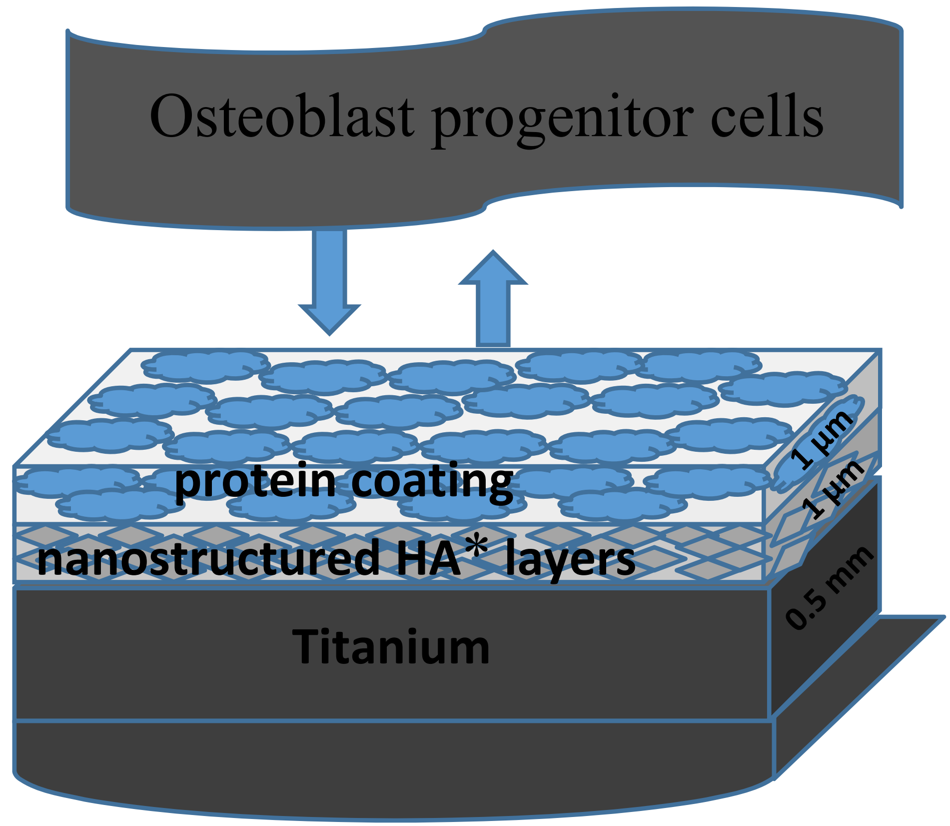
Laser:
λ=248 nm, 25 ns;
15 Hz
E=140 mJ;
Spot A: 18 mm^2;
N. pulses: 20000

Other parameters:
Dist. T-S = 30 mm;
Temperature 30 ºC;
Pressure: 10^{-1} mbarr
Substrates:
Si, Ti, HA* coated Ti;

Acta biomaterialia 7 (10), 3780, 2011

Fibronectin coatings on Ti and HA coated Ti: why using HA as interlayer?

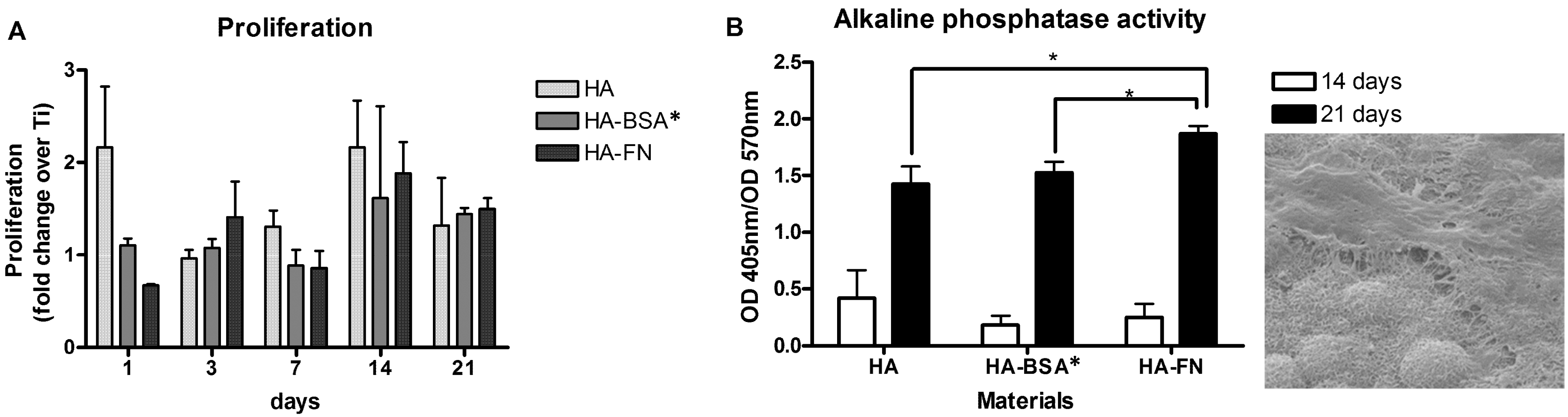




$Ca_{10}(PO_4)_6(OH)_2$ - HA* - hydroxyapatite, main inorganic component of the bone; Ca^{2+} ions of HA are binding sites for FN molecules

Applied Physics A 105 (3), 611, 2011

In vitro evaluation: osteoprogenitor cells



For all coatings, the number of cells is more or less constant in the first few days, until day 7 after which the number of cells increases. Between day 14 and 21 a relative decline in proliferation is observed which in the case of osteoprogenitor cells is quite systematic behavior.

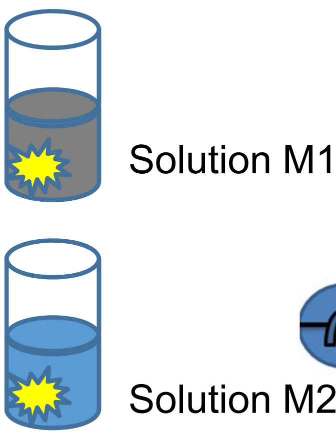
FN induces a higher degree of differentiation at day 21 in comparison with the cells grown on HA or HA-BSA structures. Less than 7 μg of FN per cm^2 deposited by MAPLE on HA coatings improve cell differentiation compared to HA coatings alone.

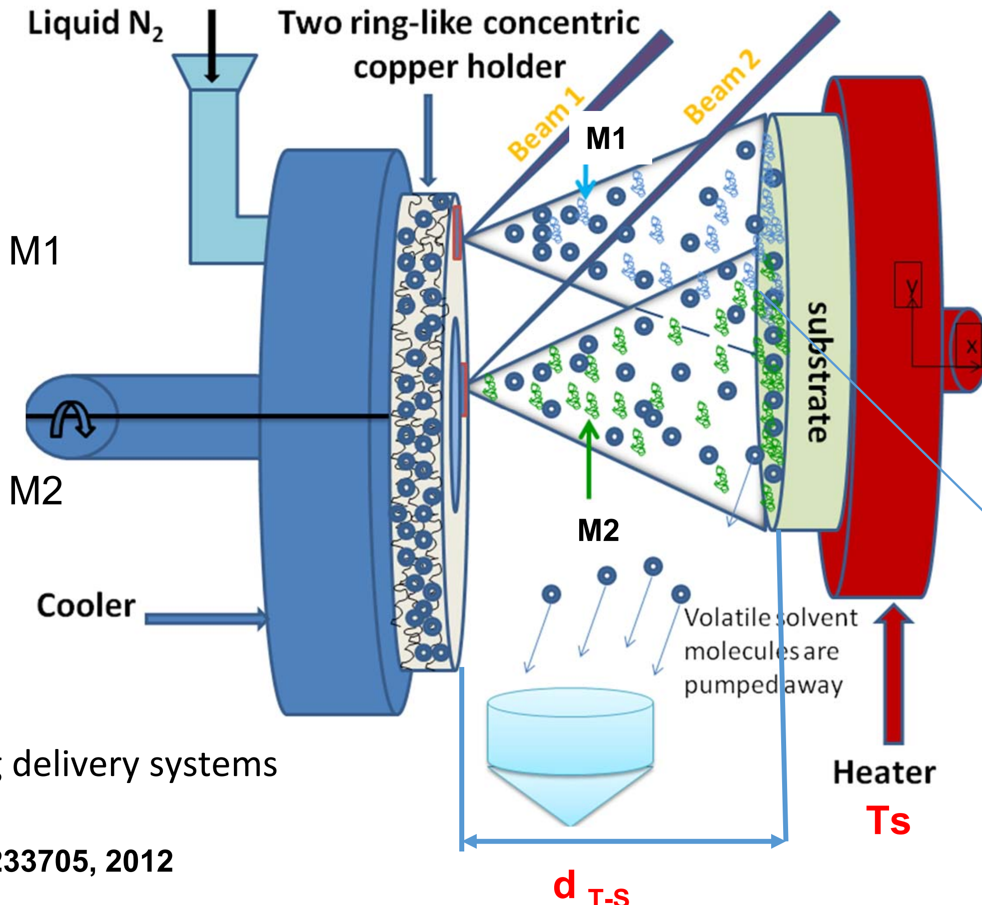
ACS applied materials & interfaces 7 (1), 911, 2015

BSA* - Bovine Serum Albumin – inert protein

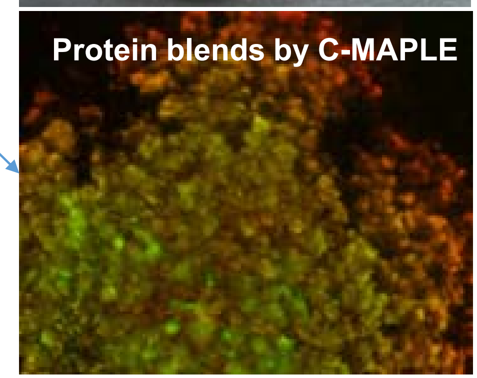
Gradient M1-M2 by Combinatorial MAPLE

Innovative design: ring-like concentric holder

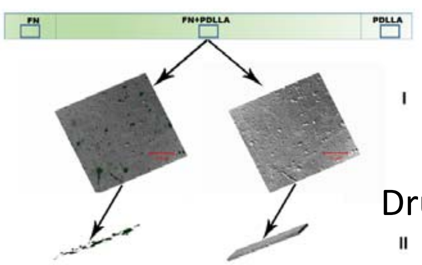




substrate area



Surface activation



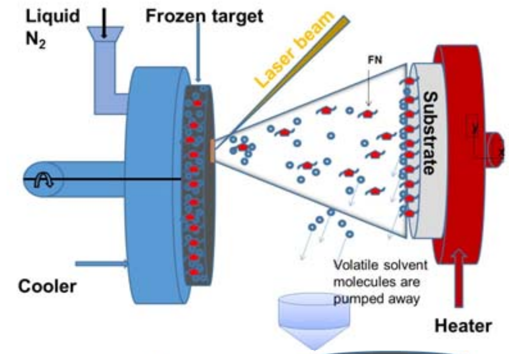
Drug delivery systems

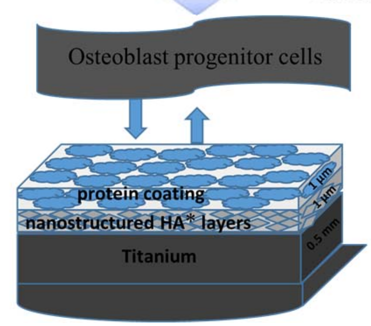
Applied Physics Letters 101 (23), 233705, 2012

Applied Surface Science 306, 75, 2014

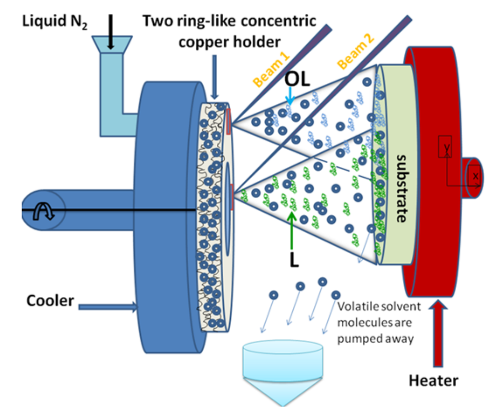
Conclusions:

1. The safe laser transfer of a large molecular mass protein – intact and functional;





2. Introducing a hybrid biomimetic inorganic –organic system;



3. Development of a combinatorial laser approach (C-MAPLE) for growth of gradient organic thin films with variable composition or for active material release from polymeric matrix.

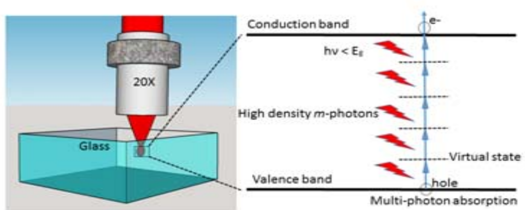
Part II

Biomimetic 3D environments fabricated by pulsed laser technologies

Ultrashort laser pulses – high enough peak intensities (around 10^{13} W/cm^2)

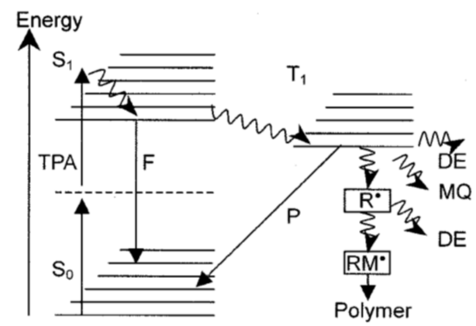
3D micro and nano processing based on multiphoton absorption using ultrashort lasers

Subtractive manufacturing



$$nE_{ph} > E_g$$

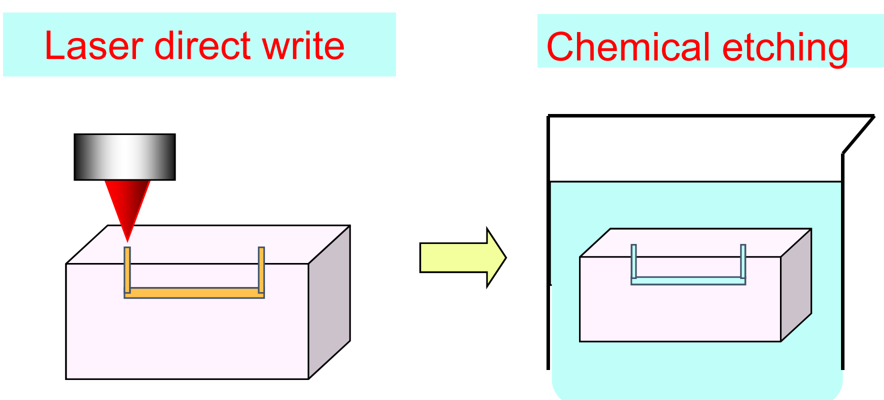
Additive manufacturing



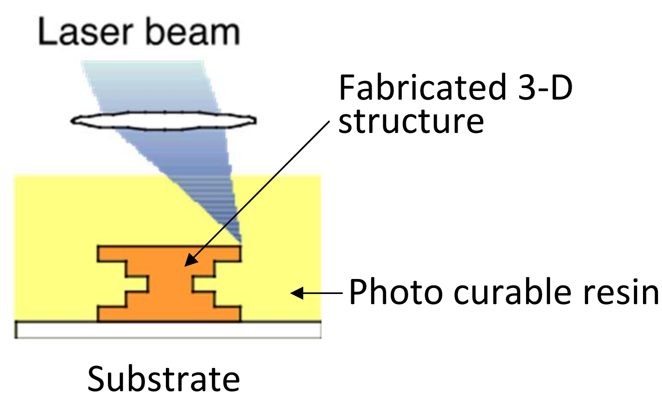
Initiation: $I + 2h\nu \rightarrow 2R\bullet \Rightarrow R\bullet + M \rightarrow RM\bullet$,

Propagation: $R-(Mn)_x\bullet + M \rightarrow R-(Mn)_{x+1}\bullet$,

Termination: $-M\bullet + \bullet M \longrightarrow -M-M-$



Femtosecond laser assisted etching (FLAE) of glass

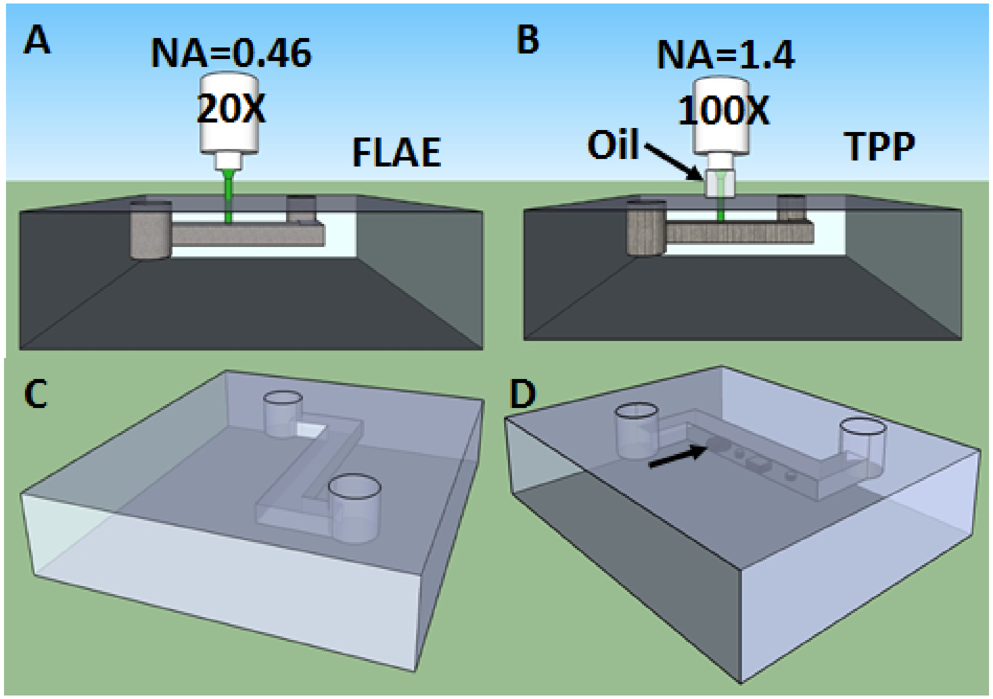


Two-photon polymerization (TPP)

Hybrid subtractive and additive manufacturing will further enhance performance of femtosecond 3D microprocessing.

Hybrid FLAE-TPP to integrate polymeric patterns inside glass: photosensitive glass - negative photoresist

Scale up scale down aspects



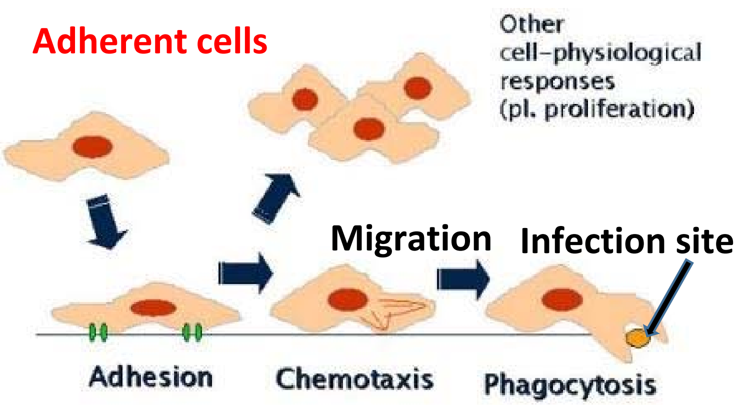
sub-diffraction-limit spatial resolution is possible in a threshold system in which material responds to light excitation with a pronounced threshold behavior.

The hybrid process (FLAE-TPP) allows lowering the size limit inside channels to smaller details, improve the structure stability in the same time as it offers the required robustness for assembling a concrete LoC device.

Micromachines 8 (2), 40, 2017; Nanophotonics 7 (3), 613, 2018

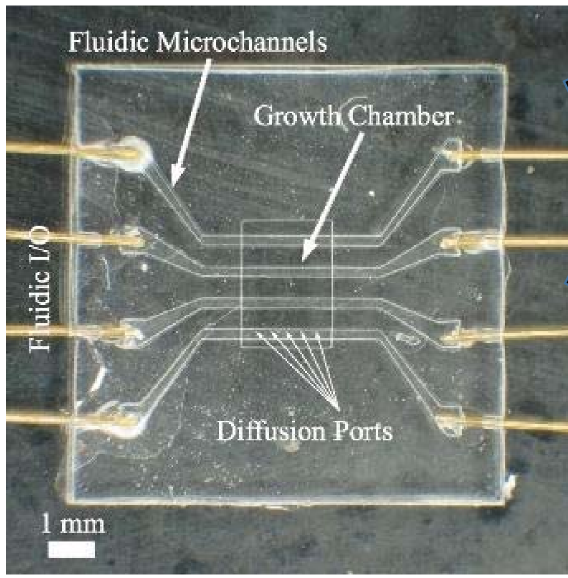
Aim: reducing size in glass, fabrication of very narrow channels bioplatform for cell chemotaxis

Cell response to external stimuli (organism)

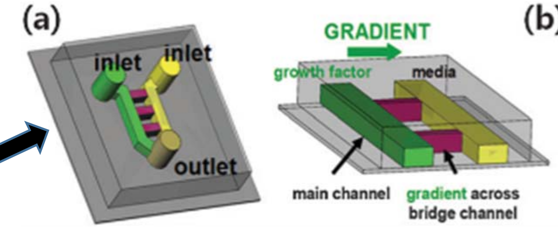


http://www.chemotaxis.usn.hu

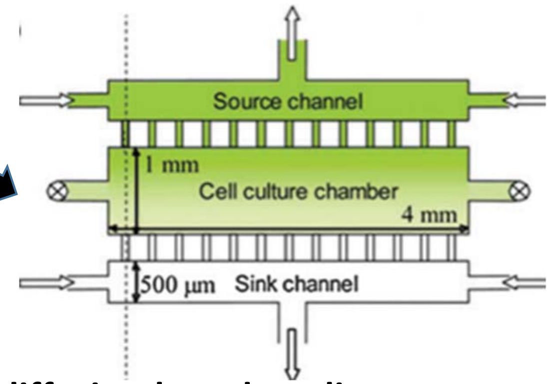
Microfluidic gradient generators for chemotaxis in research laboratory



design-site.net



flow-based gradient generators

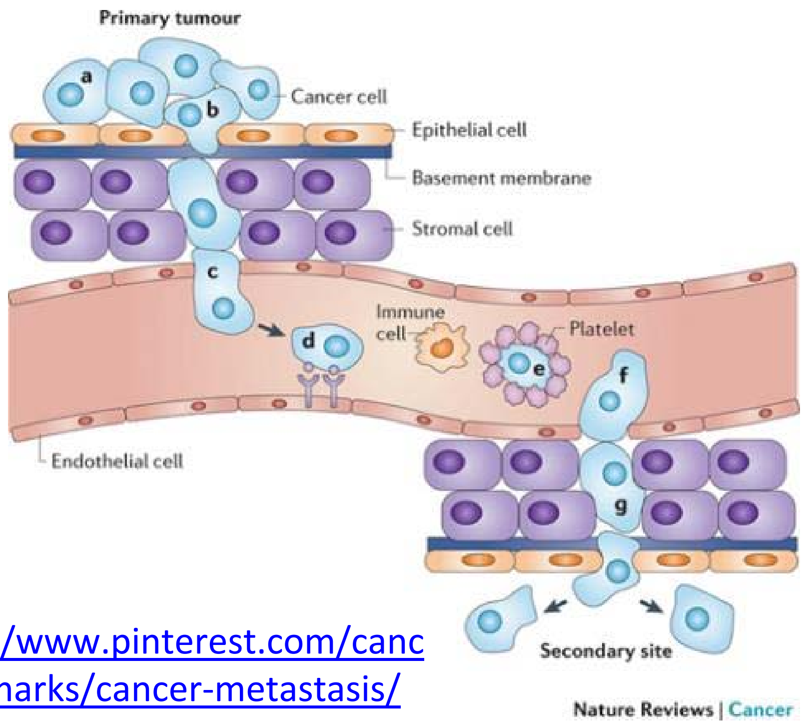


diffusion-based gradient generators

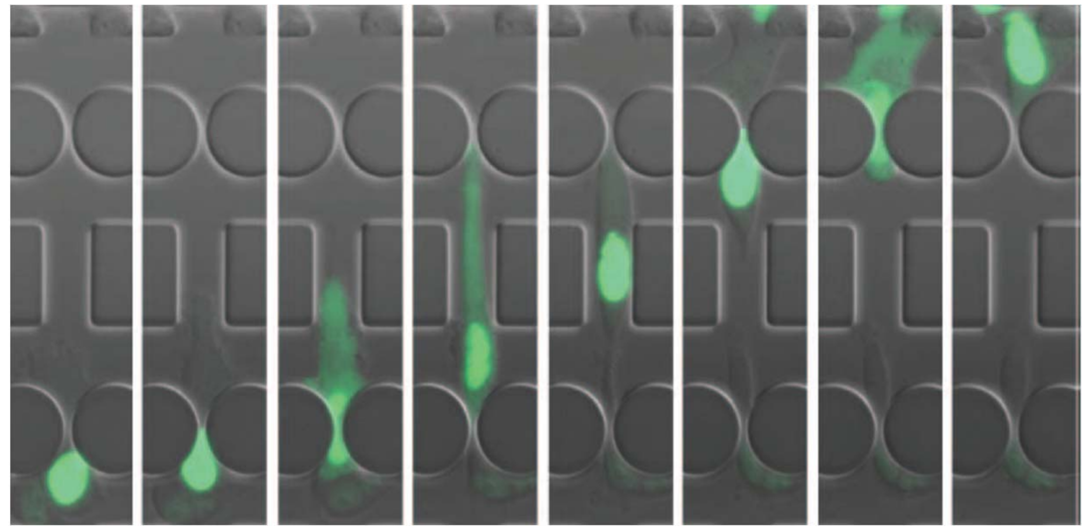
Integr. Biol., 2010, **2**, 584–603

Geometric design of a gradient forming region, concentration of a source and a sink and relative degree of molecular influx and outflux determine the gradient profile.
Understanding cellular behavior such as orientation and migration as a whole population.

Use of narrow channels – cancer cell invasiveness and migration study



https://www.pinterest.com/cancerhallmarks/cancer-metastasis/



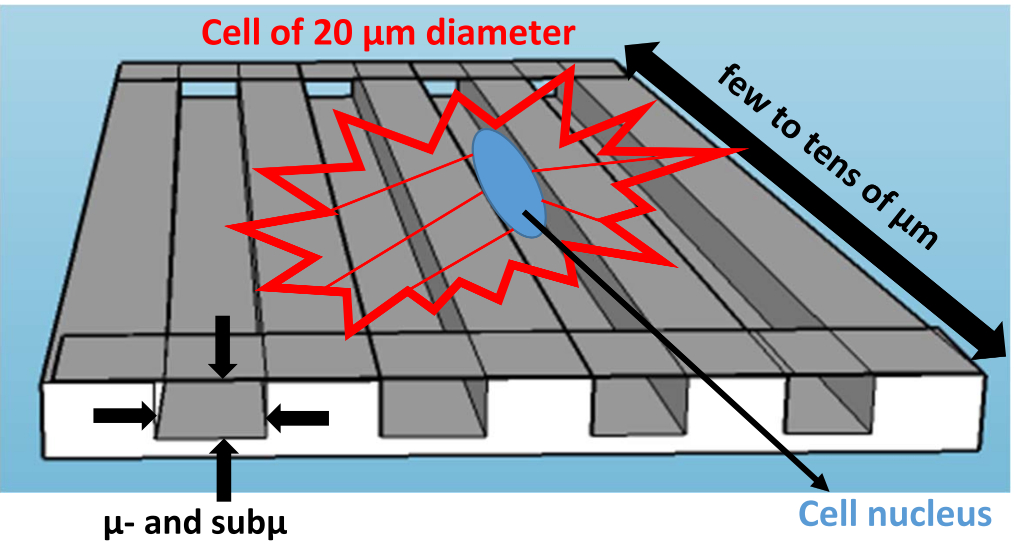
Science, 2016; 352(6283):353

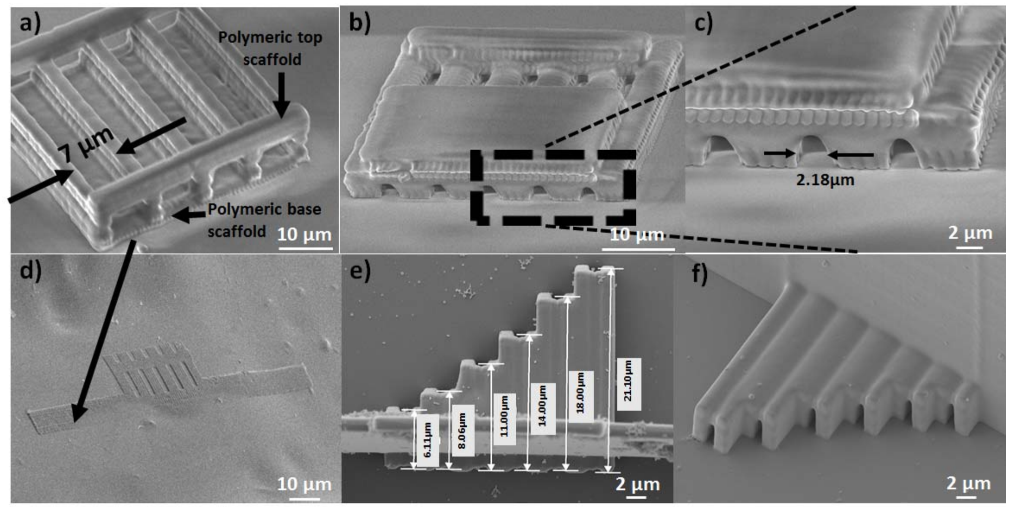
Cell migration incurs substantial physical stress on the nuclear envelope and its content and requires damage repair for cell survival.

Early stages of metastasis formation and cancer cell invasion. During migration and invasion, cells must undergo large morphological changes in order to cross the basement membrane and move through connective tissue.

Understanding cancer cell invasion and migration in 3D closed μ-environments

Size **reduction** challenging in glass µ-channels – **innovative scaffold** for **single** cell manipulation and analysis

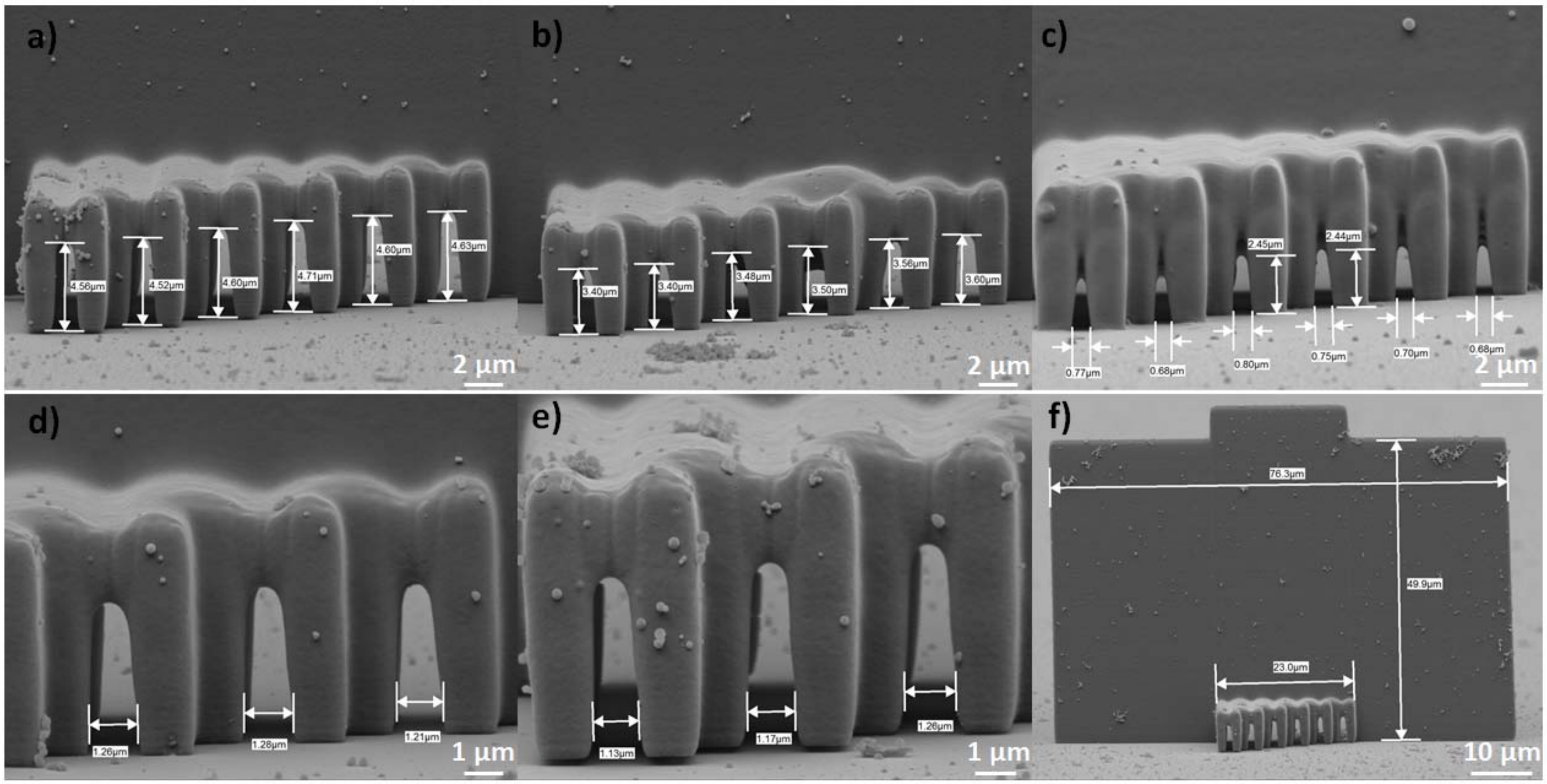




improvement of TPP resolution

ACS Applied BioMaterials, 2018

Size reduction challenging in glass µ-channels – innovative scaffold for single cell manipulation and analysis

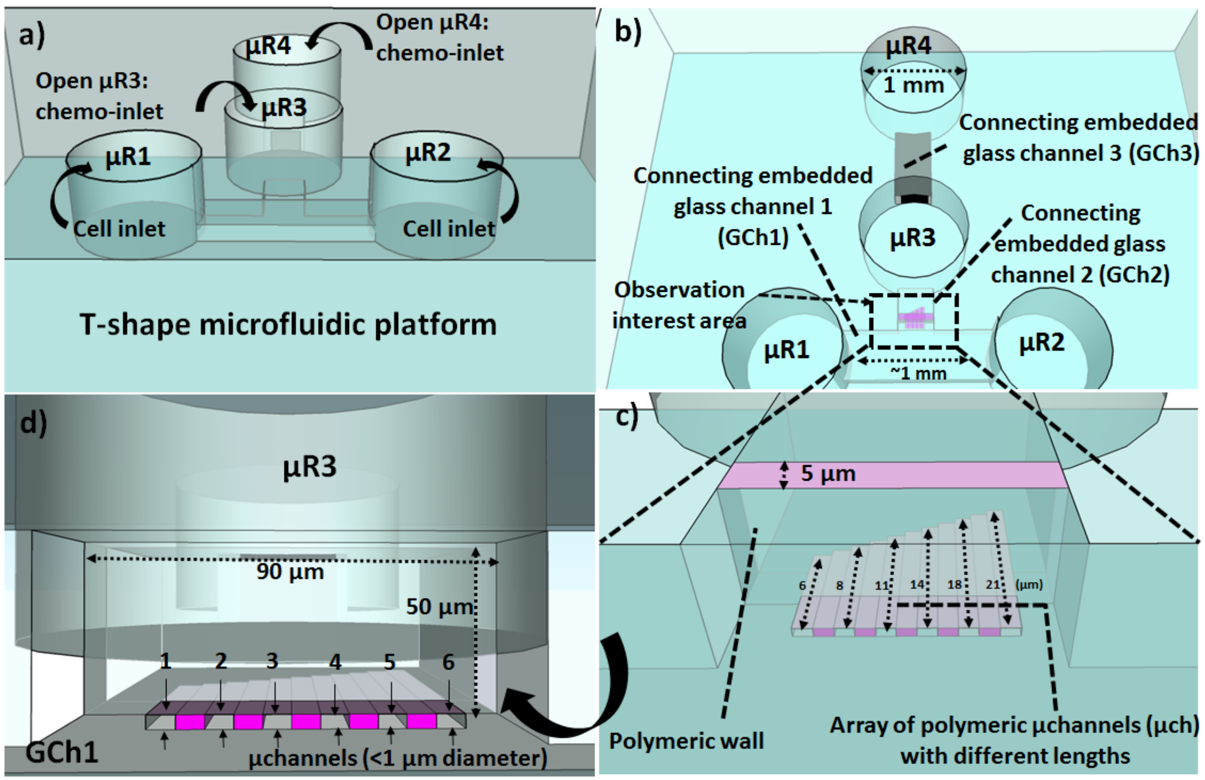


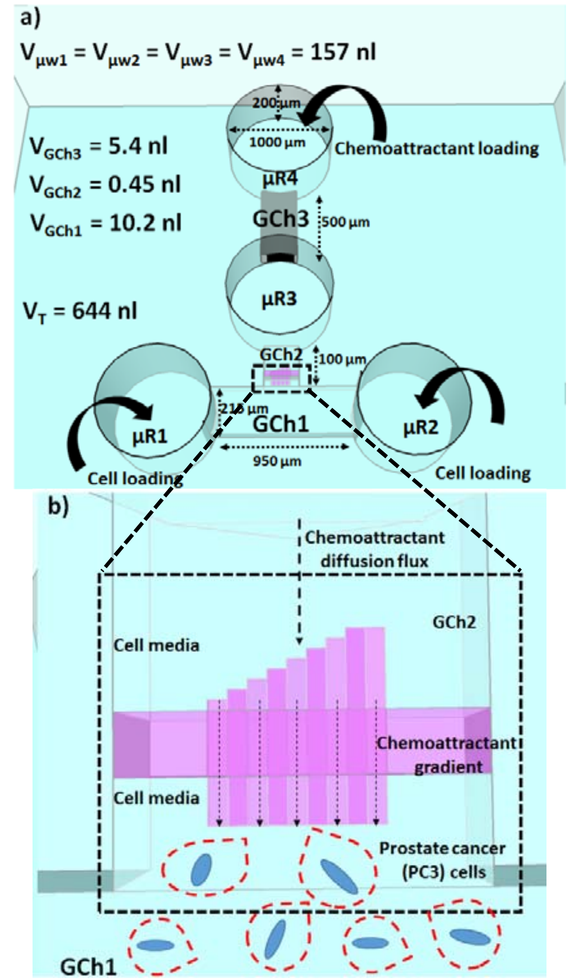
3D confined spaces fabricated using TPP: laser tailoring downsizing

ACS Applied BioMaterials, 2018

Size reduction challenging in glass μ-channels for single cell manipulation and analysis – design proposal

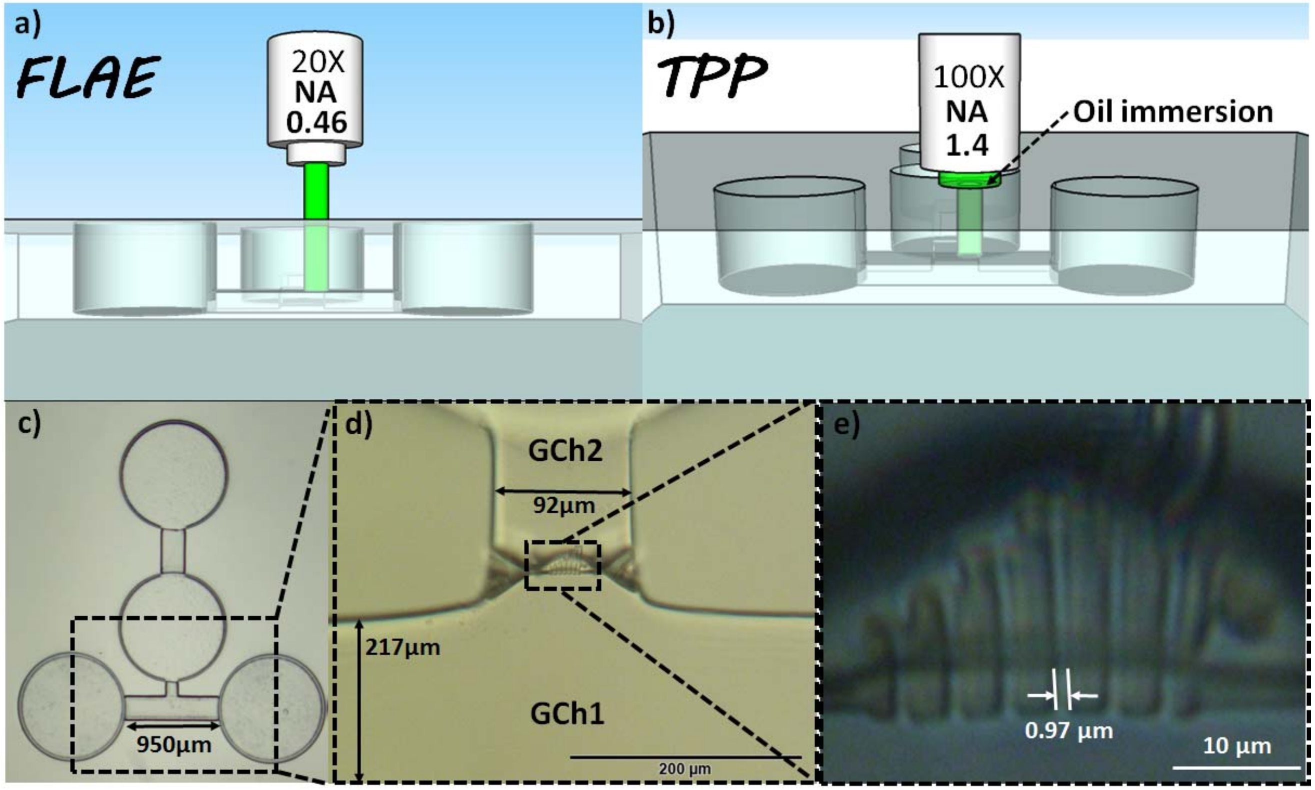
Hierarchical biochip design and concept





Substractive-additive processes
observation area and scheme for in vitro chemoattractant gradient generation for experimental testing of cancer cell migration potential in confined spaces.

Size reduction challenging in glass μ-channels for single cell manipulation and analysis



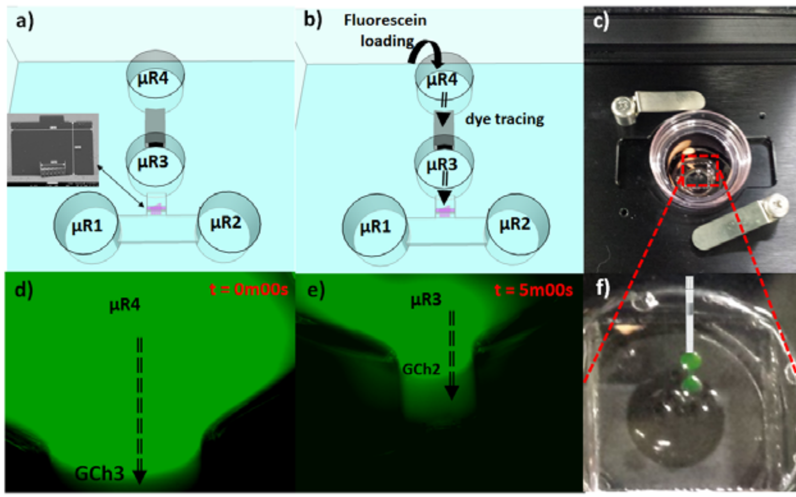
For a better observation and channel size evaluation inside glass, the microfluidic system was filled with SU-8 photoresist to compensate refractive index mismatch

Gradient formation: fluorescein test without and with scaffold

Funnel effect

1. **Measure**
2. **Analyze**
3. **Improve**
4. **Control**

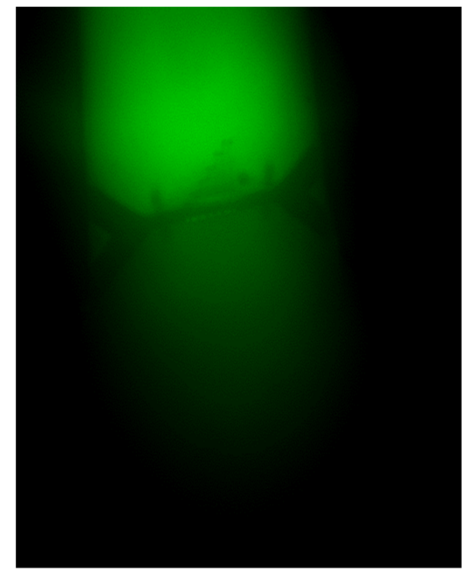
Scale up - scale down



Funnel effect + filter

1. **Measure**
2. **Analyze**
3. **Improve**
4. **Control**

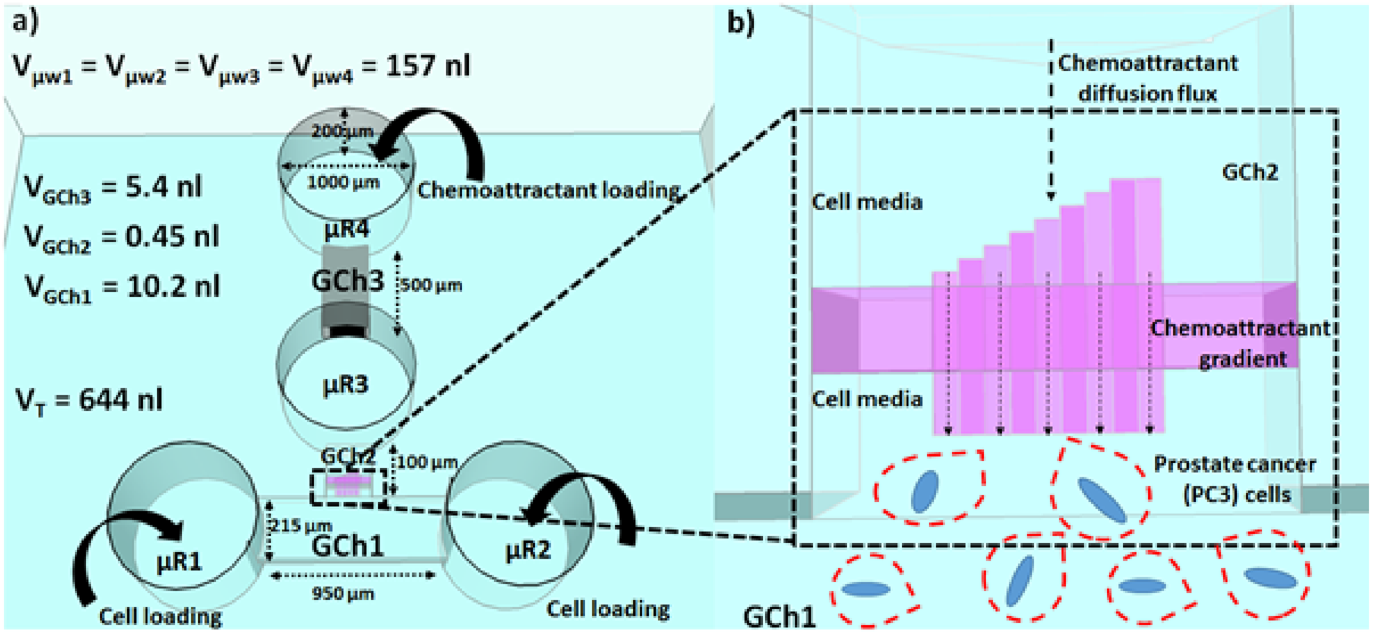
5. Slow release



Movie

Fluorescein has an absorption maximum at 494 nm and emission maximum of 512 nm (in water)

Time lapse imagining of cancer cells (PC3): area of observation

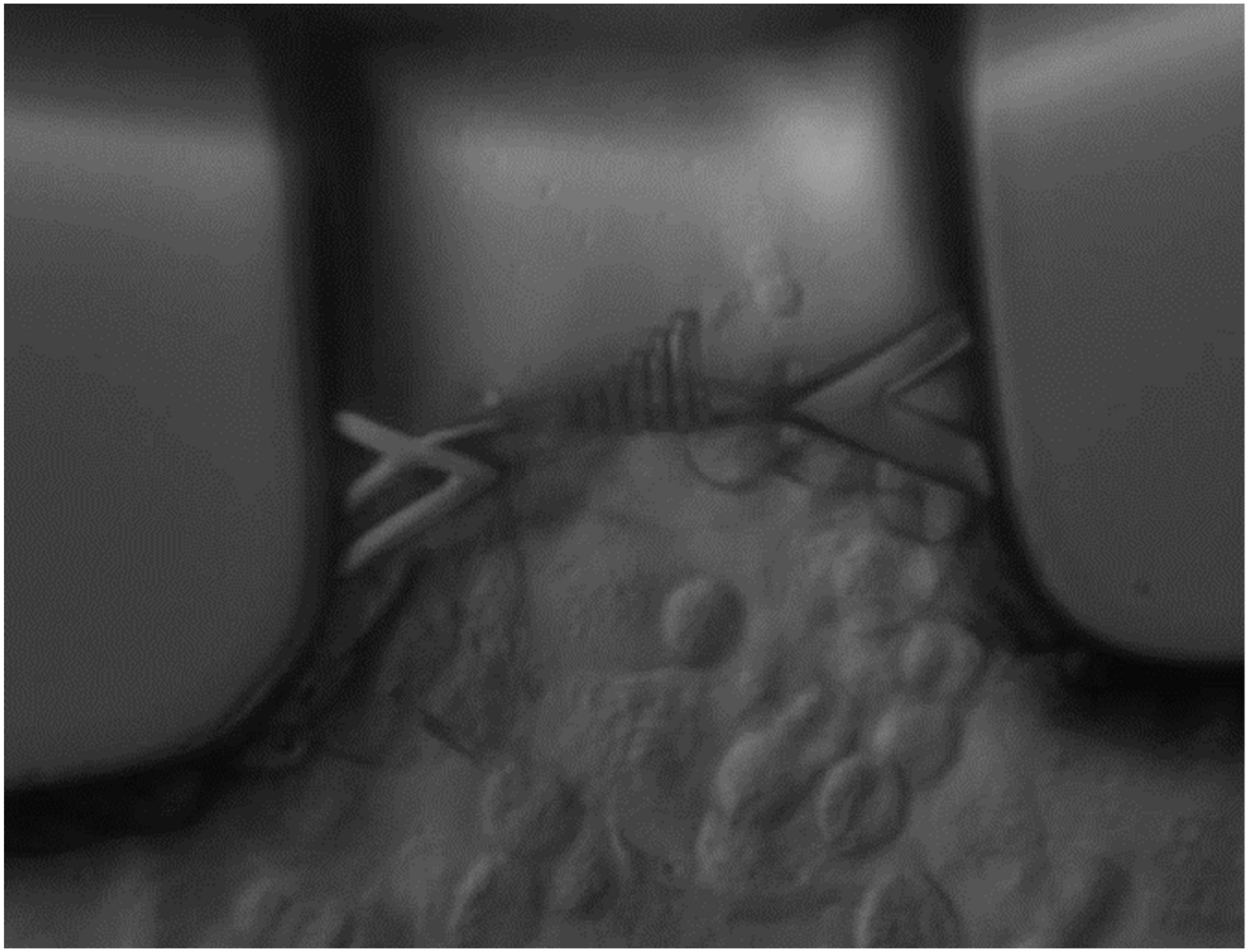


μ-channel array scaffold (area of observation)

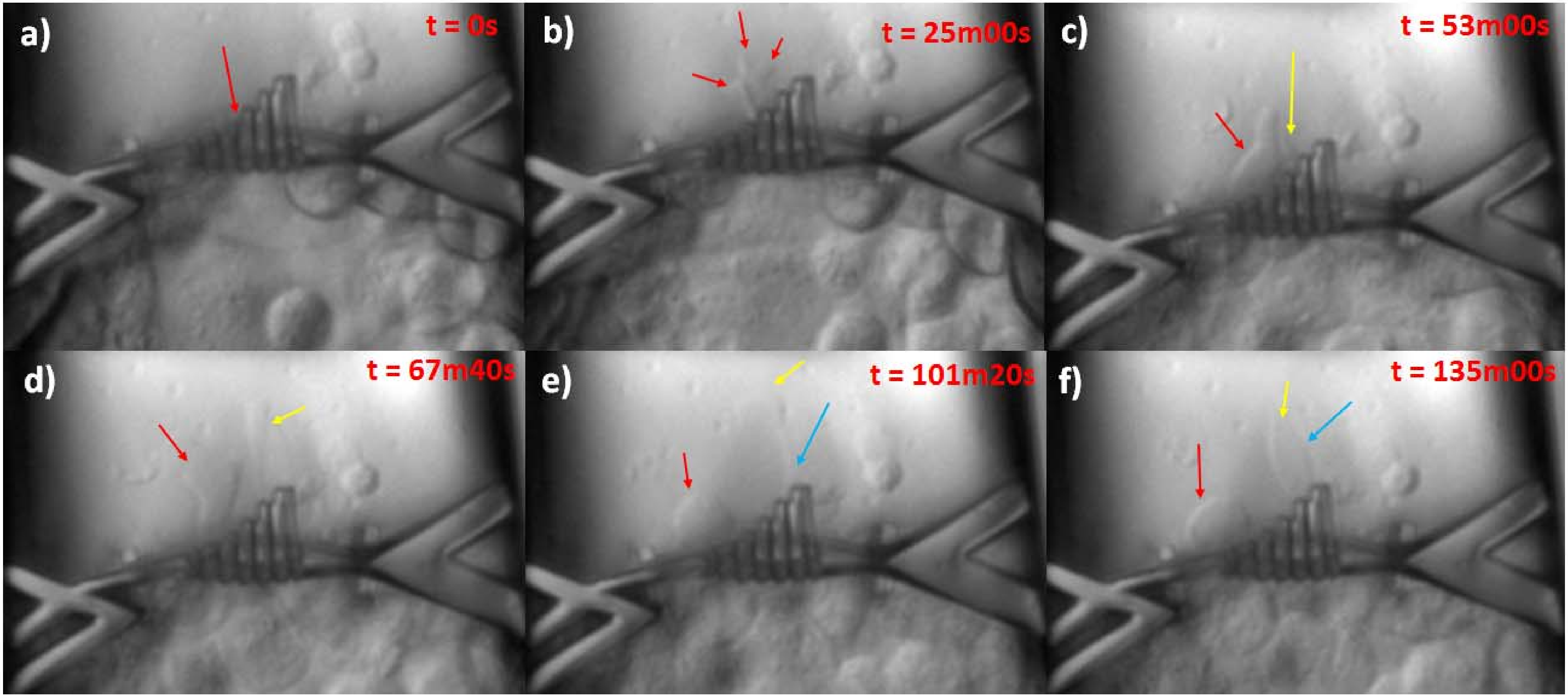
1. **Cells loaded and grown inside channels for few days to increase their density;**
2. **Cells were starved over night (by using cellular media without FBS);**
3. **EGF* was added next day and orientation and migration monitored for 3 h.**

*epidermal growth factor

Time lapse imagining of cancer cells (PC3) responding to chemo-gradient



Time lapse imagining of cancer cells (PC3) responding to chemo-gradient



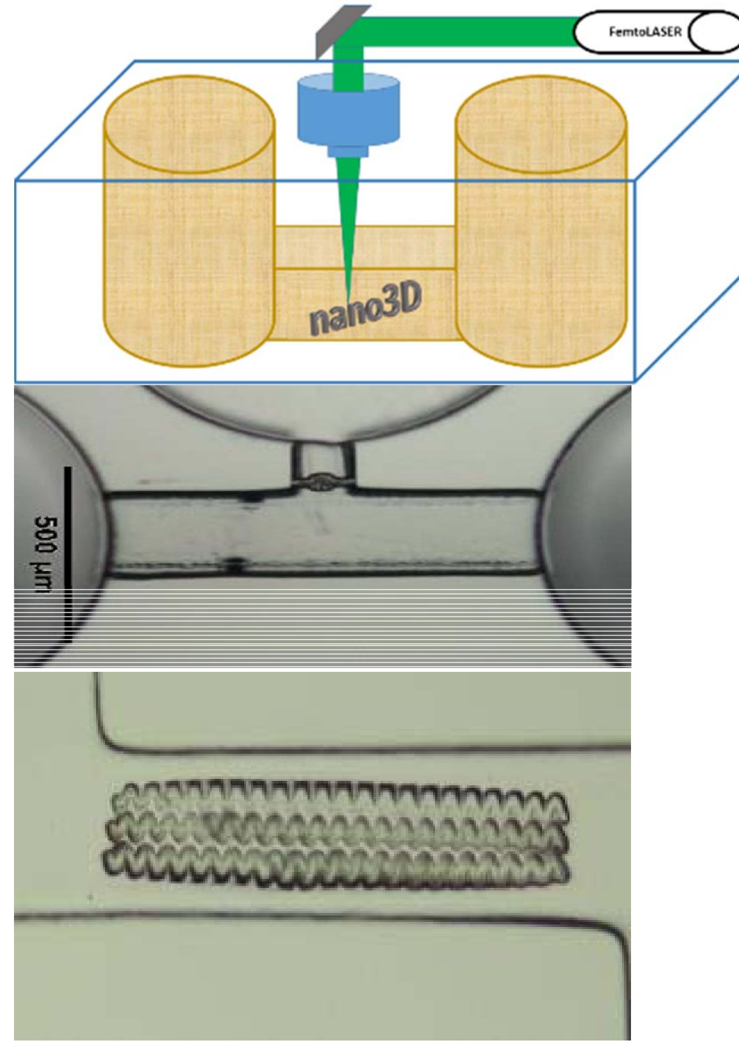
PC3 cell migration through 3D sub-micron-scale confined spaces: a) red arrow indicates the exit of the 3rd channel from the left from which the first cell will appear; b) the first PC3 cell is disintegrating after migration; c) first PC3 cell (red arrow) is reintegrating after migration while a second cell (yellow arrow) appears at the exit of the 4th channel; d) first PC3 cell (red arrow) is still reintegrating after migration while the second cell (yellow arrow) is spreading and disintegrating; e) first PC3 cell (red arrow) is reducing motility, second cell (yellow arrow) is reintegrating after migration while the third cell (blue arrow) appears at the exit of the 6th channel; f) the second PC3 cell (yellow arrow) fusing with third cell (blue arrow).

Conclusions

1. Ultrashort laser hybrid method allows both photosensitive glass 3D processing and TPP additive polymer integration inside glass microchannels for true 3D "ship-in-a-bottle" biochips.

2. Capability of 3D micro- and nanofabrication of fluidic systems by combining the advantages of individual specific characteristics and compensating the drawbacks (e.g. structure stability and functional device assembling).

3. Polymeric micro- and nanostructure pattern integration inside microfluidic systems covers the scale-down - scale-up aspects of a multi-functional microfluidic device, manipulation on both 2D and 3D environments and optical visualization, increase in sensitivity and eventually in the performance of assembled devices.



Thank you for your attention!