Career path in STEM

Laser Bioprinting

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NATIONAL TECHNICAL UNIVERSITY OF ATHENS

• 8 engineering schools and 1 applied mathematical and physical sciences school.

• 540 members as academic staff.

• 8500 undergraduate students and graduate students.
Gender bias in Academia

“Gender disparity exists in higher academic positions, despite an almost equal representation across disciplines at earlier career stages”


Personal examples
- I was the **only female PhD student** when I joined the group (15 members) for the fall semester 1996, at the UC Berkeley
- My supervisor at the Max –Planck Institute admitted at the lab technician that **he thought I was a man** when he selected my cv for the post doctoral position back to 1998.
- I was the **only female engineer** (out of 70) when I did my post doct at Philips CFT in the NL
- **5 female Professors** in my School, at the NTUA. Naval Engineering school very recently elected **the first female professor since 1969**!

*On the positive side*
- The EC has a great equally opportunity policy for up to 40%
- I recently joined the EIC Women Leadership Programme 2021-2022
Laser Printing and Materials Processing Lab

1. Laser Printing Lab, for the development of flexible electronics
2. Laser Printing Lab, for tissue engineering and regenerative medicine applications
The group has > 50% gender balance
Physicists Laser Print Conducting Polymer Circuits

Solvants can cause problems in the manufacture of conducting polymer circuits. The answer is laser printing, say researchers

by Emerging Technology from the arXiv

November 16, 2012

Conducting polymers are plastics that carry current. This is an emerging technology that is beginning to have a significant impact on areas ranging from photovoltaics and printed circuit boards to batteries and biological sensors.

The advantages of plastic conductors are many. They are cheap, lightweight, and flexible. They are also simple to make and to shape into useful circuits. At least in theory.

In practice, most manufacturing techniques have subtle drawbacks that are not easy to overcome. For example, these techniques generally begin with the polymer in liquid form. It is then sprayed, spun or inkjet-coated onto a substrate.
Additive Manufacturing

“Additive Manufacturing has the potential to revolutionize the way we make almost everything” US President Barack Obama, 2013, at National Additive Manufacturing Innovation Institute (NAMII) in Youngstown, Ohio

Flexible Circuits

Stretchable sensors

Touch Screens
Why bioprinting?

“The 3D bioprinting industry is predicted to be valued at $1.82 billion USD by 2022”

Source: Grand View Research, 2021
Laser Induced Forward Transfer

- Printing in solid and liquid phase
- Spatial resolution down to 10 μm for liquid and sub-micron for solid phase
- Printing of inorganic, organic, biological materials
LIFT advantages:

- Drop-on-demand printing, non-contact printing
- Compatible with a wide range of materials
- No limitations in materials viscosity (0.4–100000 cP)
- No use of nozzles, no additives
- Receiver substrate independent (flexible, polymer materials, etc.)

Inkjet printing typically handles low viscosity inks (1-15 mPa.s) and even with piezoelectric actuation, inks up to 100 mPa.s viscosity can be processed.
LIFT for printing of Biomaterials

BioLP™

J. Barron, et. al.

I. Zergioti et. al.

Proteins arrays

I. Zergioti et. al.

DNA microarrays

Serra et al.

Virus

L.A. Fitzgerald et. al.,

Living cells

R. Devillard et. al.

Proteins

A. Palla-Papavlu et. al.
LIFT for tissue printing

From DNA and protein microarrays to 3D printing of cells for tissue engineering, in vivo printing and printing of viruses


LIFT printing @ NTUA

Printing Polymers-Chemical sensors

Direct immobilization of biomaterials on sensors

High spatial resolution bioprinting

Enzymatic Biosensors for food applications

Capacitive sensors for Pb detection

capacitive sensors for Pb detection

Label-free DNA biosensor based on resistance change of platinum nanoparticles assemblies

LIFT: Bioprinting for sensor applications
BIOSENSORS

Biosensors:
- DNA and Aptamers based environmental sensor
- Photosynthetic amperometric sensors for water monitoring
- Enzymatic sensors for food quality monitoring

Transducers:
- Capacitive sensors
- Resistivity
- Amperometric sensors
- Photonic sensors
Aptamers based environmental sensor

THE CAPACITIVE APPROACH FOR SENSOR DEVICES

Hybridization of aptamer → Membrane bending → Change of Capacitance !!

Aptamers based Capacitive sensors for Pb detection

**Concept**

- **Immobilization of oligo 1**
  - AU/ GOPTS on LTO

- **Hybridization with oligo 2**
  - AU/ GOPTS on LTO

- **Cleavage of the DSaptamer, in the presence of Pb**
  - AU/ GOPTS on LTO

**Results**


LIFT conditions: 10μM on donor, 300 mJ/cm², Spot size: 50μm, 266 nm
A miniature Bio-photonics Companion Diagnostics platform for cancer treatment monitoring.

**Nano-biochemical Platform**
Antibodies selection, novel transfer and binding of antibodies, nanoparticles

**Photonic Platform**
6 Parallel asymmetric MZIs arrays for multiplex detection of the cancer biomarkers

**Microfluidics Platform**
Disposable Cartridge: Fluids handling, sample pre-treatment, filtering

**Reader Development**
System electronics, packaging and integration to PoC device
A miniature Bio-photonics Companion Diagnostics platform for cancer treatment monitoring.

Bio-photonic chip
Nanoparticle-based immunoassay

Disposable BIOCDx cartridge
LIFT & photo polymerization of hydrogels

- Improving the resolution
- Creating sophisticated structures
- Control on the thickness of the polymer film by laser pulses

* 250μg/ml DAG Cy3
Selective surface modification of Si$_3$N$_4$
Each chip contains of 6 sensing and 6 reference aMZIs
Multiplexing of the sensor by spotting of different antibodies at each aMCI

LIFT @ asymmetric Mach Zehnder Interferometers (aMZIs)

LIFT in combination with material-selective coating - Detection of spiked samples

- Selective surface modification of Si$_3$N$_4$
- Each chip contains 6 sensing and 6 reference aMZIs
- Multiplexing of the sensor by spotting of different antibodies at each aMCI
Evaluation of performance on aMZIs

Testing with spiked samples of increasing complexity

Gradual detection of 50 ng/ml, 100 ng/ml, TGFBI and 10 ng/ml, 50 ng/ml POSTN
Multiple detection of POSTN and TGFBI possible by spotting of multiple antibodies
LIFT: Lasers can tune the wettability of the surfaces
Laccase enzyme direct immobilization on graphite SPE

Reference pipette spotting ($\theta = 89.4^\circ$)

Contact angle

Shadowgraphic imaging setup

- CCD camera
- Oscilloscope
- Beam expander
- Mask
- 10x objective lens
- Photodiode
- Attenuator
- Rhodamine
- Nd:YAG laser 532 nm, 10 ns
- Nd:YAG laser 266 nm, 10 ns
- Delay generator
- Pump laser trigger
- Probe laser trigger
Laser Induced Forward Transfer Shadowgraphy study

- Printing solution: 1M phosphate buffer pH8
- Laser printing wavelength: 266 nm
- Laser illumination wavelength: 532 nm
- Laser spot size: 50 μm
- Laser pulse duration: 10 ns

<table>
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<td>260</td>
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</tr>
</tbody>
</table>

M. Chatzipetrou, K. Ellinas, E. Gogolides, A. Tserepi, I. Zergioti, submitted manuscript at APL
Wetting states transition due to high velocity impact

\[ P_d = \frac{1}{2} \cdot \rho \cdot V_{im}^2 \]

Transition from partial to complete wetting

30 μL phosphate buffer on Ti coated quartz target (60 μm thickness), 130 μm spot size

4 min oxygen plasma etched nanotexture PMMA resulting at 600 nm roughness

Wetting states

Non wetting state

- The droplets roll off

Partial wetting state

- The droplets pin on the surface
  - Air trapped between the pillars ⇒ Laplace Pressure > Impact Pressure

Complete wetting state

- The liquid touches the bottom of the surface
  - Impact Pressure > Laplace Pressure ⇒ the liquid force itself between the surface protrusions

Laser immobilization mechanisms

High velocity due to Laser

\[ \delta = \frac{L_{\text{mean}}^2 \cdot P_{\text{Laplace}}}{16 \gamma} \]: droop of droplet

- \( H \): Height of the pillars
- \( L_{\text{mean}} \): Mean pitch between the pillars
- \( \gamma \): air-liquid interfacial tension

\[ P_{\text{im}} > P_{\text{Laplace}} \Rightarrow \delta \geq H/2 \Rightarrow \text{Direct Immobilization} \]

Direct Immobilization of thylakoid membranes on different surfaces

Rough PMMA-Height: 869±30 nm

Increasing Impact Pressure

Threshold for direct immobilization: $P_{im} = 1.2$ MPa

Rough gold SPEs- Height: 560±3 nm

Increasing Impact Pressure

Threshold for direct immobilization: $P_{im} = 0.07$ MPa

LIFT: A laser-based immobilization technique

Direct Immobilization of Biomaterials on sensor devices

- C. Boutopoulos et al. Direct laser immobilization of photosynthetic material on screen printed electrodes for amperometric biosensor. APL 98(9), 093703 (2011).

Laser can tune the wettability of the surfaces

LIFT: Printing of cells
LIFT printing of cells mixed with hydrogels

LIFT printing on glass

Cancer cells

LIFT-printed A375 melanoma cancer cell line expressing the green fluorescent protein (GFP)

Fluorescence images of Human Embryonic Stem cells mixed with alginate

LIFT technique enables,

✓ Deposition of cells at specific patterns
✓ No cells damage after printing
LIFT printing of liver cells on collagen scaffolds

Control the positions of cells on porous collagen scaffold in order to generate a co-culture element with controlling cell adhesion.

 SEM image of porous collagen scaffold structure

Using porous collagen scaffold as receiver substrate

Cell adhesion and cell culture after printing process.

In collaboration with Dimitrios Tzeranis, and Achilleas Gravanis at FORTH IMBB-Hellas

LIFT printing of liver cells on collagen scaffolds

- LIFT technique enables the deposition of cells in porous collagen scaffolds at specific patterns.
- No cells damage after printing and the viability is approximately 100%.

Fluorescence image of LIFT printed Huh7 cells 2 h after printing.

Fluorescence image of LIFT printed Huh7 cells 24 h after printing.

V. Leva, M. Chatzipetrou, L. Alexopoulos, D. S. Tzeranis and I. Zergioti, Direct laser printing of liver cells on porous collagen scaffolds, JLMN, pp.234-237, 2018
High speed visualization of different cell concentrated bioinks

Formation of 2 jets
- $2^{\text{nd}}$ jet velocity $\sim 5 \text{ m/s}$
- $2^{\text{nd}}$ jet carries the main amount of material
Influence of different cell concentrated bioinks on printed volume and droplet size as a function of laser fluence

- Droplet diameter is correlated with the concentration of the bioinks and the laser fluence.
- Both droplet diameter and volume has no systematic dependence on the cell concentration at different laser fluences.
LIFT: Printing of drugs
Laser printed drugs

- Faster dilution,
- More accurate than liquid dosage,
- Immediate absorption by the Mucous membrane
  - Bypass absorption of the API (active pharmaceutical ingredient) by the digestive system
  - Lower Dosage
  - Less side affects
- Non invasive,
- Personalized dosage
Laser printed drugs

(I) LIFT printing of Levothyroxine Sodium (thyroid diseases)

Substrate: polycarbonate membrane

Substrate: cellulose

Substrate: Orodispersible Film (ODF), 2 cm length

(II) LIFT printing of Isosorbide Mononitrate (vascular diseases)

Substrate: glass slide
Printed volume calculation

High speed imaging setup

Glass substrate

Light source (LED)

Paclitaxel solution
PEN
Quartz

Laser source

High speed camera

500um
High speed jet visualization of paclitaxel solution

- **Regime**
  - Bridging of donor-receiver substrates
  - Creation of secondary and satellite droplets

- **Threshold**
  - Large laser printing window
  - Jet front velocity ~ 25 m/s

- **Printing**
  - 500 μm

- **Splashing**

**LP-DRUGS**
Validation via HPLC

**Printed paclitaxel on wafer paper**

**Printed paclitaxel on glass**
Conclusions

LIFT can do much more than printing:

- Initiating Chemical Reactions
- Immobilization of biomolecules on the substrates
- Printing of sensors
- Printing of cells
- Printing of drugs
A Tumor-lymph node-on-chip platform composed of 3D tissue models and microfluidic chips which will connect surgically removed human primary tumors and LN tissue from the same lung cancer patient serving as a “biological twin” of the patient.

GA No: 953234
Call: H2020-NMBP-TR-IND
Start: 01/05/2021-30/4/2025
Duration: 48 months
Topic: DT-NMBP-23-2020 (LS)
UroPrint’s overall Goal

Urinary bladder bioprinting for autologous transplantation

The ultimate goal of UroPrint relies on a radically new concept: the anatomical structural and functional transdifferentiation at the tissue level (tissue transdifferentiation).

GA No: 964883
Start: 01/09/2021-31/08/2025
Duration: 48 months
Topic: FETOPEN-RIA-2019-01
3D Laser Bioprinter Solutions & Services

Aspiring to be a key player in the tissue regeneration and biotechnology field

IOANNA ZERGIOTI, CO-FOUNDER AND CTO
Our product: PhosDB·I

Meet PhosDB·I

A high-resolution, user-friendly laser bioprinter for demanding cell-printing applications

Read more
Executive summary

- We are a high tech spin off, established as a PC (IKE) at the Attica Technology Park “Lefkippos”
- We aspire to make laser bioprinting technology attractive to researchers and developers primarily in the Biomedical sector with our compact laser bioprinter.
- We have a fully functional pre-industrial prototype and we are in the process of developing our product.
- We are also focusing on testing applications on tissue regeneration, such as bladder, esophagus and cartilage.
Team

Dr Ioanna Zergioti
Co-founder, CEO co-inventor
First Scientist worldwide to apply LIFT for solid phase DNA printing

Dr Apostolos Klinakis
Co-founder, Clinical Director
25-year background on cancer biology mouse genetics and stem cell biology

Dr Symeon Papazoglou
Co-founder, CTO, co-inventor
Researcher with >5 years experience in laser printing systems

Maria Pallidou
Co-founder, CFO
20 years experience Health Care Industry in EMEA Region

Advisors

Dr Achilleas Gravanis
Professor of Pharmacology Medical School University of Crete. Researcher IMBB FORTH, Affiliated Research Professor Center of Drug Discovery Northeastern University Boston

Dr Ioannis Viniotis
Professor at the Department of Electrical and Computer Engineering at North Carolina State University

Dr Dimitri Papaioannou
Managing Director of Exelon Partners a specialty management consulting firm based in Athens & Silicon Valley.

Spin off from ICCS

PhosPrint
Our story so far

2016: Initiation of dual beam Laser bioprinting process by I. Zergioti/ICCS


2019: PhosPrint PC incorporated-spin off ICCS/NTUA

2019: Grant from Bodossaki Foundation in Greece to support our IP costs

2019: EU Seal of Excellence/SME instruments phase I

2020: Winner of an accelerator Science Park (50k€)

2020: Filing of US Provisional Patent Application on bladder regeneration including also cartilage and esophagus application cases

2021 May - 4 years: European Funded Project “Tumor-LN-oC” under the H2020 NMBP-23-2020 call

2021 September – 4 years: European Funded Project “UroPrint” under the H2020-FETOPEN-2018-2020
1. E. Elezoglou, MSc student
2. H. Cheliotis, MSc student
3. K. Magoula, MSc student
4. M. Logotheti, MSc
5. C. Kryou, PhD student
6. M. Chliara, PhD student
7. Ch. Katopodis, PhD student
8. S. Kananakis, Meng, MSc
9. D. Mandala, MSc
10. Dr. S. Papazoglou
11. Dr. M. Makrygianni
12. Dr. F. Zacharatos
13. Dr. M. Chatzipetrou
14. Dr. C. Chandrinou
15. Dr. I. Theodorakos

A. Klinakis
P. Karakaidos
C. Tamvakopoulos
M. Orfanou
G. Tsekenis