Career path in STEM

Laser Bioprinting

Ioanna Zergioti

School of Applied Mathematical and Physical Sciences, National Technical University of Athens and acting CEO PhosPrint



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"

J. Gruber et al., The Future of Women in Psychological Science, Perspect. Psychol. Sci., 16 (2020), pp. 483-516

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







Publicity

Σέββατο Ι Δεκεμβρίου 2012

ΕΠΙΣΤΗΜΗ - ΤΕΧΝΟΛΟΓΙΑ

H KAOHMEPINH - 15

Προτάσεις

ΙΕΤΟΥΓΕΝΝΑ Μταν το αστέρι

της Βηθλεέμ

тетранмеро фертівал Н «уюртії»

της Επιστήμης

«Εκτυπώνουν» ευλύγιστα φωτιστικά 🛛 🗍

Τι υπόσχεται η τεχνολογία του Μετοόβιου Πολυτεχνείου, στον κλάδο των οργανικών

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Με τη νέα μέθοδο θα κατασκευάζονται



IPEMA ÖS

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Ένα εργαστήριο μέσα σε chip για τον έλεγχο της ποιότητας τροφίμων



νθυγιεινοί κίνδυνοι σε υγιεινές τροφές...

Ο τομέας τροφίμων και ποτών, εκτός του ότι αποτελεί μια σημαντική διάσταση της ποιότητας ζωής, είναι και ένας σημαντικότατος πυλώνας της ευρωπαϊκής οικονομίας. Ζωτικής σημασίας είναι ο έλεγχος της ποιότητας των παραγόμενων τροφίμων όσον αφορά τυχόν μόλυνσή τους από τοξίνες και μικροοργανισμούς, ο οποίος αποκτά ακόμη μεγαλύτερη σπουδαιότητα όταν ο έλεγχος αφορά τρόφιμα πρώτης ανάγκης ή μεγάλης διατροφικής αξίας όπως τα γαλακτοκομικά και τα γεωργικά προϊόντα (φρούτα, λαχανικά και ξηροί καρποί).

Ειδικότερα στις σύγχρονες κοινωνίες υπάρχει μεγάλη ανάγκη για αποτελεσματικά μέσα παρακολούθησης της ποιότητας των παραγόμενων προϊόντων και της πιστοποίπσης ασφαλούς κατανάλωσής τους. Ο λόγος είναι ότι κατά τον 20ό αιώνα η επιμόλυνση των τροφών κατέστη περισσότερο συχνή και επικίνδυνη καθώς τα κρούσματα δεν αφορούν πλέον μόνο φυσικές τοξίνες και μικροοργανισμούς, αλλά και μια πλειάδα χημικών παραγόντων όπως εντομοκτόνα, φυτοφάρμακα, αντιβιοτικά και βαρέα μέταλλα.

Από τα μεγάλα εξειδικευμένα εργαστήρια, στα εργαστήρια μέσα σε chip

Το πιο σοβαρό πρόβλημα που αντιμετωπίζει ο έλεγχος της ποιότπτας των παραγόμενων τροφίμων έγκειται στο ότι οι μεθοδολογίες που υιοθετούνται για την ανίχνευση μολυσματικών παραγόντων σήμερα βασίζονται σε αναλυτικά MIT Technology Review

Featured Topics Newsletters Events Podcasts

Uncategorized

Physicists Laser Print Conducting Polymer Circuits

Solvents 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, flexible and light. They are also simple to make and to shape into useful circuits. At least in theory.

In practice, most manufacturing

ns laser mask clipicitive quartz carrier-

techniques have subtle drawbacks that are subtrate 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.

2 free stories remaining



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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?

Man-made organs could reshape life sciences

Bioprinting enables the creation of living biological tissue and organs through the layering of living cells and supportive biomolecules.

Forbes



Source: CBINSIGHTS

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Laser Induced Forward Transfer

	Chemical sensors	Laser	Wire bonding
Ma	Quartz aterial to be dep Substrate	osited	

- 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





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LIFT advantages

- Drop-on-demand printing, non-contact printing
- **Compatible** with a wide range of materials
- □ No limitations in materials viscosity (0.4–100000cP)
- □ 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

BioLPTM



J. Barron, et. al. **Biosensors & Bioelectronics**, 20(2), 246–52, 2004.



I. Zergioti et. al. Applied Physics Letters 86(16), 163902, 2005.

DNA microarrays





300 µm



Virus

L.A. Fitzgerald et. al., J. Virological Methods, 167(2), 223, 2010.

Living cells



R. Devillard et. al. Methods in Cell Biology, 119, 159, 2014.

Proteins

A. Palla-Papavlu et. al. Sensors Actuators B Chem., 192, 369, 2014





LIFT for tissue printing

BONE-PRINTING



V. Keriquel et. al. Biofabrication, 2 (1), 014101, 2010.

R. X. et. al. **Biofabrication**, 7 (4), 45011, 2015.



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



L. Koch et. al. **Biotechnology and Bioengineering**, 109 (7), 1855–63, 2012.



LIFT printing @ NTUA

Printing Polymers-Chemical sensors

Percentage of coverage

C. Boutopoulos, V. Tsouti, D. Goustouridis, S. Chatzandroulis I. Zergioti, *Appl. Phys. Lett.* 93 (19), 191109, **2008**.



70 % of coverage High spatial resolution bioprinting

Capacitive sensors for Pb detection



G. Tsekenis, M. K. Filippidou, M. Chatzipetrou, V. Tsouti, I. Zergioti, S. Chatzandroulis, *Sensors Actuators B Chem.*, 208, 628–635, **2015**.



Label-free DNA biosensor based on resistance change of platinum nanoparticles assemblies E. Skotadis, K. Voutyras, M. Chatzipetrou, G. Tsekenis, L. Patsiouras, L. Madianos, S. Chatzandroulis, I. Zergioti, D. Tsoukalas, Biosensors & Bioelectronics 81, 388–394

(2016) https://doi.org/10.1016/j.bios.2016.03.028

Direct immobilization of biomaterials on sensors



M Chatzipetrou, F Milano, L Giotta, D Chirizzi, M Trotta, M Massaouti, M.R. Guascito, I. Zergioti, Electrochemistry Communications 64, 46-50 (2016)

Enzymatic Biosensors for food applications

E. Touloupakis, M. Chatzipetrou, C. Boutopoulos, A. Gkouzou, I. Zergioti, *Sensors Actuators B Chem.*, 193, 301–305, **2014.**





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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







Aptamers based Capacitive sensors for Pb detection



BRFAA





BIOCD_X A miniature Bio-photonics Companion Diagnostics platform for cancer treatment monitoring.

Bio-photonic chip





LIFT & photo polymerization of hydrogels





* 250µg/ml DAG Cy3

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





BIOCD_XLIFT @ asymmetric Mach Zehnder Interferometers (aMZIs)



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

- Selective surface modification of Si₃N₄
- Each chip contains of 6 sensing and 6 reference aMZIs
- Multiplexing of the senor by spotting of different antibodies at each aMZI



BIOCDx Evaluation of performance on aMZIs

Testing with spiked samples of increasing complexity

Binding of recombinant TGFBI

Binding of recombinant POSTN

Multiplexing of the same chip





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







Shadowgraphic imaging setup





Laser Induced Forward Transfer Shadowgraphy study



- Laser printing wavelength: 266 nm
- Laser illumination wavelength: 532 nm
- Laser spot size: 50 µm
- Laser pulse duration:10 ns

J (mJ/cm ²)	u (m/s)	P=1/2*pu ² (MPa)
180	33	0,61
260	47	1
450	70	3
900	255	36
1400	260	39

M. Chatzipetrou, K. Ellinas, E. Gogolides, A. Tserepi, I. Zergioti, submitted manuscript at APL



Wetting states transition due to high velocity impact





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

Wetting states





C. Boutopoulos, M. Chatzipetrou, A. G. Papathanasiou, and I. Zergioti, Laser Phys. Lett., vol. 11, no. 10, p. 105603, 2014.

Laser immobilization mechanisms



Direct Immobilization of thylakoid membranes on different surfaces



Biomaterial deposition method



LIFT: A laser-based immobilization technique

Direct Immobilization of Biomaterials on sensor devices



- E. Touloupakis et al. A photosynthetic biosensor with enhanced electron transfer generation realized by laser printing technology. Anal. Bioanal. Chem 402 (10), 3237 (2012).
- C. Boutopoulos et al. Direct laser immobilization of photosynthetic material on screen printed electrodes for amperometric biosensor. APL 98(9), 093703 (2011).
- E. Touloupakis et al. A polyphenol biosensor realized by laser printing technology. Sens. Actuators B-Chem193: 301 (2014).

1000 us

150 µs

200

Laser can tune the wettability of the surfaces



- C. Boutopoulos, et al. (2013). "Sticking of droplets on slippery superhydrophobic surfaces by laser induced forward transfer." APL 103(2): 024104 (2013).
- C. Boutopoulos, et al. "Time resolved imaging and Immobilization study of bioliquids on hydrophobic and suberhydrophobic surfaces by means of Laser-Induced Forward Transfer." Laser Physics Letters (2014).





Quartz

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LIFT: Printing of cells



LIFT printing of cells mixed with hydrogels



LIFT printing on glass

Matrigel





Fluorescence images of Human Embryonic Stem cells mixed with alginate

Cancer cells





after LIFT printing

OF ATHENS

RFAA

1hour after LIFT 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.

Donor substrate

Jet formation



SEM image of porous collagen scaffold structure

In collaboration with Dimitrios Tzeranis, and Achilleas Gravanis at FORTH IMBB-Hellas Using porous collagen scaffold as receiver substrate

Cell adhesion and cell culture after printing process.

> "Direct Laser Printing of Liver Cells on Porous Collagen Scaffolds", V. Leva, M. Chatzipetrou, L. Alexopoulos, D. S. Tzeranis, I. Zergioti, JLMN-Journal of Laser Micro/Nanoengineering **13** (3), (2018)

FORTH IMBB



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



7.8 μs 31.2 μs 78 μs 117 μs 156 μs 195 μs 273 μs 327 μs 405 μs 500 μs 624 μs 780 μs

- Formation of 2 jets
- 2^{nd} jet velocity ~ 5 m/s
- 2nd 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: Orodispersible Film (ODF), 2 cm length

Substrate: polycarbonate membrane

Substrate: cellulose

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



Substrate: glass slide



40

LP-DRUGS



Printed volume calculation





High speed jet visualization of paclitaxel solution





Validation via HPLC





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





Tumor-LN-oC's overall Goal

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 a s 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 Call: H2020-FETOPEN-2018-2019-2020-01 Start: 01/09/2021-31/08/2025 Duration: 48 months Topic: FETOPEN-RIA-2019-01





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PhosPrint

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 IP (PCT/EP2017/084740, 28/12/2017 and US Provisional Patent 63062176, 6/8/2020).
- 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.

PhosPrint

Team



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

Advisors



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



PhosPrint

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



Dr Dimitri Papaioannou



Spin off from ICCS

Our story so far

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

2017/2018: Pre-industrial prototype, IP Protection (PCT/EP2017/084740)

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

PhosPrint

2021 September – 4 years: European Funded Project "UroPrint" under the H2020-FETOPEN-2018-2020



Group and collaborators

- 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

