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Laserlab Europe

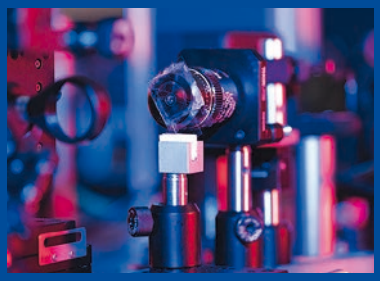
Laserlab Forum

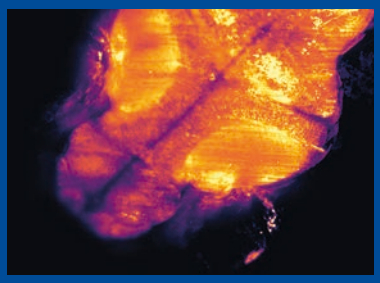
Newsletter of LASERLAB-EUROPE:
the integrated initiative of European laser infrastructures funded by the European Union's Horizon 2020 research and innovation programme

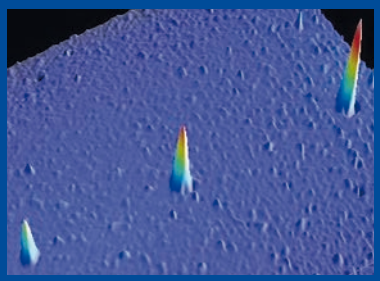
Scanning the Body: Biomedical Imaging

Image of NIH-3T3 cells
Credit: Figure from J. Reinhard et al., Microsc. Microanal.: ozad123 (2023)

In this Issue







Editorial

Sylvie Jacquemot

Following examples of how lasers are contributing to 3D biomedical imaging in health and life sciences, this Laserlab Forum is delving deeper into our anatomy, shedding light on additional methods and techniques on the researchers' quests of scanning the human body. As imaging technologies continue to advance, the resolution and depth of exploration increase. The ensuing articles highlight current studies of Laserlab-Europe's researchers contributing to the better understanding of mysteries that were once concealed beneath the surface.

I am also happy to announce that Laserlab-Europe will continue providing services to the user community: the Lasers4EU proposal has been approved by the European Union! Starting the 1st of October 2024, it will ensure that users can apply for transnational access on our laser facilities on a large variety of scientific topics during the next five years.

I wish you an interesting reading and a safe and happy New Year!s.

Sylvie Jacquemot

News

Laserlab-Europe congratulates Anne L'Huillier, Pierre Agostini and Ferenc Krausz on receiving the 2023 Physics Nobel Prize!

Erika Weiland / Ohio State University / MPQ

The Nobel Prize in Physics 2023 was awarded to Anne L'Huillier (LLC, Sweden), Pierre Agostini (Ohio State University, USA) and Ferenc Krausz (MPQ, Germany) for experimental methods that generate attosecond pulses of light for the study of electron dynamics in matter.

The three Nobel Laureates in Physics 2023 are being recognised for their experiments, which have given humanity new tools for exploring the world of electrons inside atoms and molecules. The laureates' experiments have produced pulses of light so short that they are measured in attoseconds, thus demonstrating that these pulses can be used to provide images of processes inside atoms and molecules.

Laserlab-Europe is very proud of the accomplishments of the laureates all of which have a long history of collaboration within Laserlab-Europe. Congratulations!

nanoSCAN: Revolutionising spatial biology with a cutting-edge multi-scale imaging platform

The nanoSCAN project, which started in October 2023, aims to transform tissue analysis with a novel 3D spatial biology platform that provides crucial insights into cellular and tissue functions. To address the present limitations of spatial biology imaging technologies regarding limited spatial resolution and insufficient molecular profiling, five partners, including ISMO, POLIMI, CNR IFN, Abbelight and Laserlab-Europe, joined forces to develop a new imaging platform, the SAFe-nSCAN, which combines multi-scale optical microscopy solutions, from structured illumination microscopy for rapid cell and tissue inspection and classification to single-molecule localisation microscopy techniques for deeper and higher nanoscopic 3D information over preselected regions.

The project will receive about 2.5 million euro by the European Innovation Council programme.

European Laser Science and Technology Landscape Analysis published

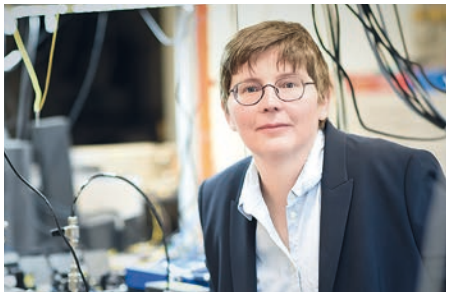
MBI / T. Merkau

Laserlab-Europe and the Extreme Light Infrastructure (ELI ERIC) have joined forces to analyse the current laser-based science landscape in Europe. The analysis, published in October 2023, aims to assess the European laser community to provide a better understanding of the services offered to users by Research Infrastructures (RIs) operating laser sources as well as the user needs and requirements.

The consolidated report gives an overview of the complex landscape of laser RIs in Europe, identifies complementarities and efforts to be aligned, and defines high-level objectives. The factual and up-to-date information provided will support discussions – with the European Commission and with national agencies – about sustainable funding for RIs. It will prove valuable to decision-makers, stakeholders and the wider laser RI community in ensuring the growth and sustainability of this critical field.

Nathalie Picqué appointed Director at the Max Born Institute

MPQ



Nathalie Picqué, an experimental physicist, has been appointed Director at the Precision-Physics Division at the Max Born Institute for Nonlinear Optics and Short-Pulse Spectroscopy in Berlin (Germany) in October 2023.

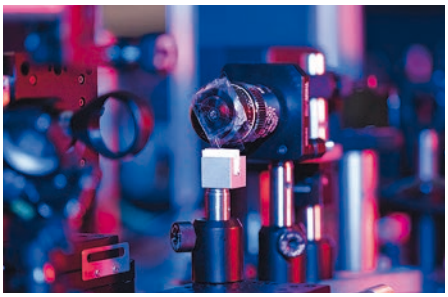
Picqué holds a joint appointment as a Professor of Experimental Physics with HU Berlin, and was previously a research group leader at the Laser Spectroscopy Division at the MPQ (Garching, Germany). She received her doctoral degree in physics from Université Paris-Saclay (France) in 1998. Her research interests are in the areas of optics and molecular physics, more particularly in interferometry, precision spectroscopy and laser technology. For over a decade, Nathalie Picqué has developed pioneering methods using frequency combs in high-resolution molecular spectroscopy. She received several prestigious awards, such as the Gentner-Kastler Prize and the Falling Walls "Breakthrough of the Year" Award. She also received an ERC Advanced Grant for exploring a new concept of precision measurements with frequency combs.

What is Laserlab-Europe?

Laserlab-Europe, the Integrated Initiative of European Laser Research Infrastructures, understands itself as the central place in Europe where new developments in laser research take place in a flexible and co-ordinated fashion beyond the potential of a national scale. The Consortium currently brings together 35 leading organisations in laser-based inter-disciplinary research from 18 countries. Additional partners and countries join in the activities through the association Laserlab-Europe AISBL. Its main objectives are to maintain a sustainable inter-disciplinary network of European national laboratories; to strengthen the European leading role in laser research through Joint Research Activities; and to offer access to state-of-the-art laser research facilities to researchers from all fields of science and from any laboratory in order to perform world-class research.

Novel optical microscope sheds light on one of the still poorly understood aspects of cancer

Dario Polli (NIREOS)



A new scientific study published in "Science Advances" has investigated one of the still poorly understood aspects of cancer, therapy-induced senescence (TIS) in tumour cells. These cells, which make up a small percentage of treated tumour cells, exhibit resistance to conventional therapies, leading to tumour quiescence and ultimately, recurrence.

The study, coordinated by Dario Polli from POLIMI (Italy) uncovers the biological mechanisms behind the formation of TIS cells. One approach involved the use of ultra-short laser pulses, with an incredibly brief duration of just one millionth of a millionth of a second. This microscopy technique, called "coherent Raman spectroscopy", allowed the team to identify the chemical constituents of the different biomolecules based on their characteristic mechanical vibrations at high frequency. Another approach was based on "phase holo-tomography", an advanced imaging method that measures how laser light waves change as they pass through tumour cells. This method allowed the researchers to create three-dimensional reconstructions of the morphology of cells and visualise their inner structures.

More info: *https://doi.org/10.1126/sciadv.adg6231*

New DiPOLE 100Hz (KAZI) generates nanosecond pulses of 10 J energy at 100 Hz repetition rate

Central Laser Facility, UKRI

DiPOLE 100Hz laser system

A new generation DiPOLE laser has been developed and commissioned by the CALTA team at the Central Laser Facility (CLF, UK) as part of the Widespread collaboration with HiLASE (Czech Republic). This laser provides a tenfold increase in repetition rate compared to the Bivoj/DiPOLE100 system at HiLASE, a 100 J, 10 Hz laser developed by the CALTA team, delivered to HiLASE in 2016 and later pushed to operate at 150 J, 10 Hz as part of the Widespread collaboration. DiPOLE 100Hz targets the needs of industrial and scientific applications requiring high average power and high throughput rates. The laser generates 1 kW average power in a much more compact system compared to DiPOLE100. The 45M Euro bilateral collaboration between HiLASE and CLF called Widespread is jointly funded by the European Commission and the Czech Ministry of Education, Youth and Sports (MEYS).

ERC Grants

The European Research Council (ERC) promotes frontier research by awarding prestigious grants to outstanding researchers for projects of ground-breaking nature. Laserlab-Europe researchers have again been successful in the ERC's highly competitive selection process. Congratulations to the two scientists receiving a Starting Grant (up to 1.5 million euro)!

Maximilian Beyer (LLAMS): Helium dimer ultracold molecules – a platform for fundamental physics and ultracold chemistry


Peter Valckx

Maximilian Beyer receives an ERC Starting Grant for his research into laser cooling of molecular helium. His research is focused on precision measurements of the simplest molecules in our universe – molecular hydrogen (H_2) and its ion (H_2^+) – to test quantum electrodynamics.

Beyer will use the ERC Starting Grant to study the next slightly more complicated molecule: molecular helium (He_2). He_2 is the simplest molecule for which laser cooling to near absolute zero temperature seems feasible – which is the main goal of this project. At near zero temperature, the molecules hardly move due to the vanishing kinetic energy and therefore allow very precise measurements.

If successful, the team will deliver the fifth-ever molecule that was directly laser-cooled and trapped. Compared to all other molecules laser cooled to this day, the simplicity of He_2 makes very accurate calculations of its collision properties possible. This will allow to study ultracold collisions and chemical reactions with unprecedented accuracy. Being able to understand and control the collision properties is crucial for producing a molecular Bose-Einstein condensate. With such a system, applications range from testing quantum electrodynamics to quantum information and many-body physics.

Carl Davies (FELIX): HandShake – Research on novel technology for electively switching magnetisation


Victoria Edwards

Carl Davies, researcher in the HFML-FELIX laboratory and the Institute for Molecules and Materials at Radboud University, has been awarded an ERC Starting Grant to develop a new approach for selectively switching magnetic order based on chiral vibrations of the crystal lattice.

Specifically, the project – entitled "HandShake" – will explore how circularly polarised oscillations of the lattice can switch magnetisation. This approach could play a central role in the future of data centres, which are already struggling to cope with the enormous and exponentially growing amounts of data in the world. The project thus addresses the pressing need to develop faster approaches for reversing magnetisation with much better energy-efficiency.

"A major advantage of this approach comes from the fact that the crystal lattice – a geometrical network of many different elements – has many different frequencies of resonance, corresponding to so-called optical phonons. By driving these circularly polarised phonons at resonance, the resulting push that acts on the magnetisation may even become strong enough to switch it", Davies explains. "This approach benefits hugely from the longer lifetime of the phonons, which are usually on the order of picoseconds. Moreover, we should be able to steer the direction of the magnetic reversal via the helicity of the chiral lattice vibrations."

The project will start in January 2024.

Scanning the Body: Biomedical Imaging

In the broad field of modern medical diagnostics and scientific research, the intricate landscapes of the human body unfold through the lens of cutting-edge biological imaging technologies. The following articles present some of the ways in which Laserlab-Europe's researchers are exploring the science of biological imaging, delving into the deep intricacies of our anatomy.

Correlative fluorescence and soft X-ray microscopy in an integrated laboratory-based setup (FSU Jena and HIJ, Germany)

Correlative imaging is a very useful method for combining complementary imaging techniques with different contrast mechanisms. A good example is the correlation of the functional contrast of fluorescence microscopy (FLM) with the structural contrast of soft X-ray (SXR) microscopy in the water window (WW). The WW is a spectral region defined by the absorption edges of carbon (282 eV) and oxygen (533 eV), which offers label-free structural contrast in biological samples and a relatively high penetration depth in water.

This correlation has already been demonstrated at synchrotron sources, but a laboratory-based solution, as presented here, was needed to make the method more widely accessible. The setup used (Figure 1) combined a wide-field SXR microscope with a bright-field epi-fluorescence microscope. The required SXR radiation was generated using a laser-produced gas plasma source, based on a gas puff target (GPT) developed by the Institute of Optoelectronics at the Military University of Technology, Warsaw, with nitrogen as the working gas. The condenser optic was an ellipsoidal mirror, with a Fresnel zone plate (FZP) used for imaging. Due to the strong absorption of the SXR radiation in air, the microscope was operated in a vacuum.

The major advantage of this setup was the integrated FLM, which allowed switching between the two imaging methods without having to move the sample, ensuring identical measurement conditions and avoiding sample alteration or destruction. To facilitate this, the FLM objective was integrated into the vacuum chamber of the SXR microscope, allowing easy switching with the FZP. All other components of the FLM, except for the sample, were located outside the vacuum chamber. This approach enabled easy changing of filter sets and illumination LEDs, enabling multi-colour fluorescence imaging.

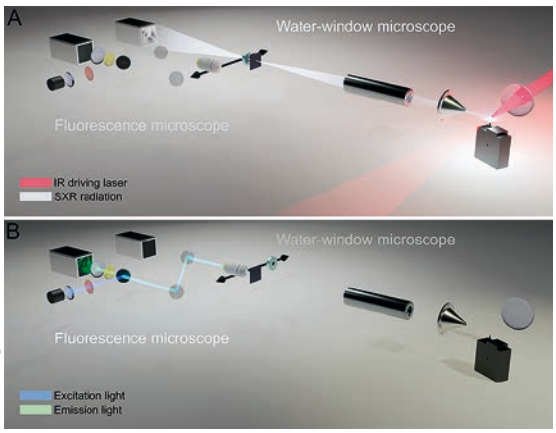


Figure from J. Reinhard et al., Microsc. Microanal.: ozad123 (2023)

Figure 1: Sketch of the setup. A) The top figure shows the operating SXR microscope with the zone plate imaging the sample structure on the detector, while the fluorescence microscope remains inactive in this mode. B) For imaging with the fluorescence microscope, the zone plate, objective and the first mirror behind it are moved sideways, while the sample remains in place. The laser and plasma source are not operating in this mode.

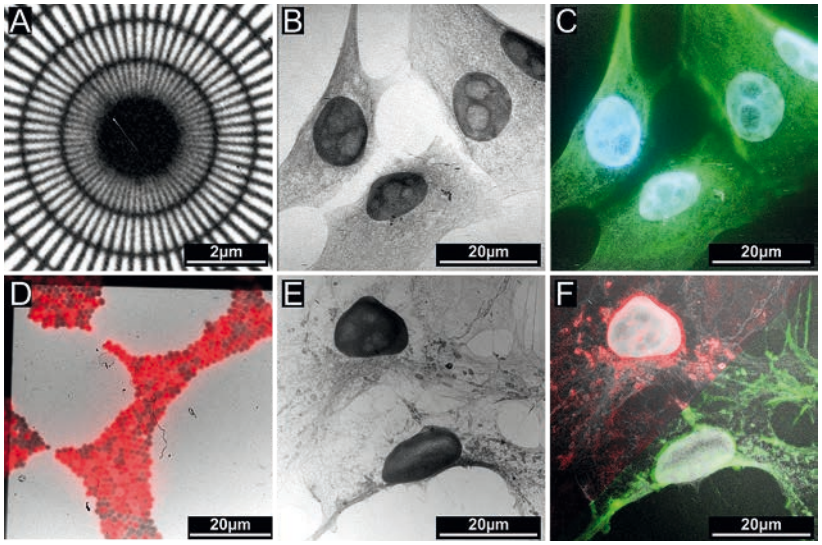


Figure from J. Reinhard et al., Microsc. Microanal.: ozad123 (2023)

Figure 2: Different samples measured with the correlative microscope. A) SXR image of a Siemens star test target, showing 50 nm half pitch resolution. B) SXR image of NIH-3T3 cells. Four nuclei and the cytoskeleton are visible. C) Composite image of SXR image B and fluorescence images, with labelled actin skeleton and nuclei. SXR contrast was inverted for better visibility of the fluorescence image. D) Correlative image of autofluorescent cyanobacteria. E) SXR image of COS-7 cells. Two nuclei, the cytoskeleton and surrounding mitochondria are presented. F) Composite image of SXR image E and fluorescence images, with labelled mitochondria (upper left) and cytoskeleton (lower right). SXR contrast was inverted for better visibility of the fluorescence image.

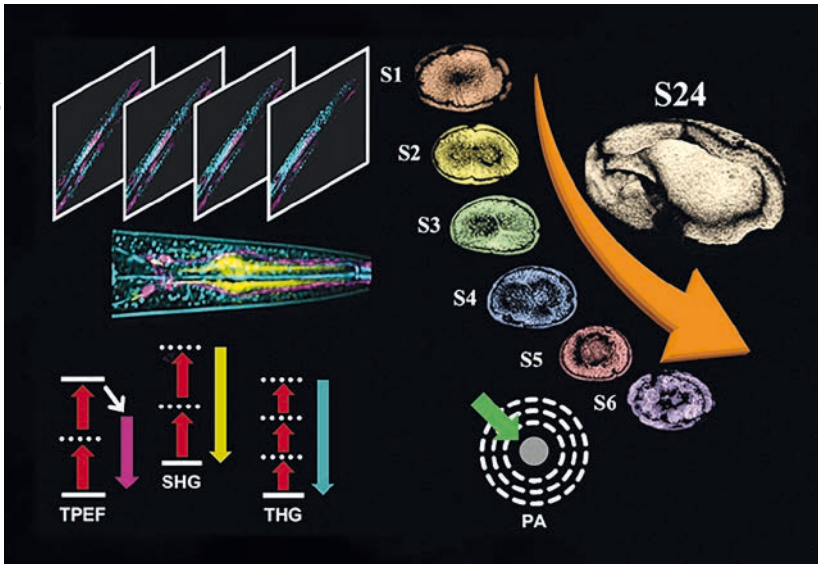
With this setup, a resolution of 50 nm half pitch was achieved, measured with a Siemens star test target, as shown in Figure 2. 3T3 cells were successfully imaged with labelled cytoskeleton and nuclei, as well as fluorescent nanobeads, autofluorescent cyanobacteria, and labelled COS-7 cells.

In addition to the imaging of biological samples, this setup also allowed the degradation of the fluorescence signal by SXR radiation to be studied, which is of particular interest for the further development of correlative experiments.

Sophia Kaleta and Julius Reinhard
(IOQ, University Jena)

J. Reinhard et al., Microsc. Microanal.: ozad123 (2023)

NLM and LBMI groups FORTH-IESL



Left side: NLOM imaging of Caenorhabditis elegans nematode. Right side: Label-free PA microscopy for monitoring the development of Parhyale hawaiensis emerging model organism.

Non-linear and photoacoustic microscopy reveal fundamental biological mechanisms in model organisms (FORTH, Greece)

The development of non-invasive microscopic techniques as new tools in biomedical research is extremely important. At IESL-FORTH, non-linear optical microscopy (NLOM) has been used for sub-cellular imaging of biological samples, and to provide new insights into fundamental biological phenomena, such as cell differentiation, embryogenesis, and fat metabolism during ageing [1] or in the context of disease [2]. Alongside label-free high resolution, high-contrast imaging, NLOM offers increased biological sample penetration depth, and permits precise quantitative analysis and testing of specific mechanisms and biological processes. IESL-FORTH has also developed novel, low-cost photoacoustic (PA) microscopy platforms [3], integrating intensity-modulated continuous wave laser sources, to provide accurate optical absorption mapping of cells and tissues with diffraction-limited spatial resolutions and excellent molecular specificity levels. As the laser-induced ultrasonic waves present significantly higher transmissivity than pure optical signals, PA imaging approaches may offer enhanced penetration depths for *in vivo* diagnostic applications without the use of labelling agents. The costs of such imaging systems are also around one third of those of conventional PA microscopes utilising Q-switched nanosecond lasers.

In recent interdisciplinary research, NLOM (MPEF, SHG and THG modalities) was implemented to visualise the deposition and distribution of lipid droplets (LDs) in cells of the simple nematode *Caenorhabditis elegans*. Lipid content was discovered to accumulate progressively with age in the nuclear envelope of cells in several tissues, and importantly, genetic interventions known to delay ageing reduced the number and size of nuclear LDs. NLOM measurements uncovered a molecular mechanism that preserves nuclear lipid homeostasis and organismal physiology during ageing [4], and highlighted its critical role in preventing age-associated nuclear LD build-up.

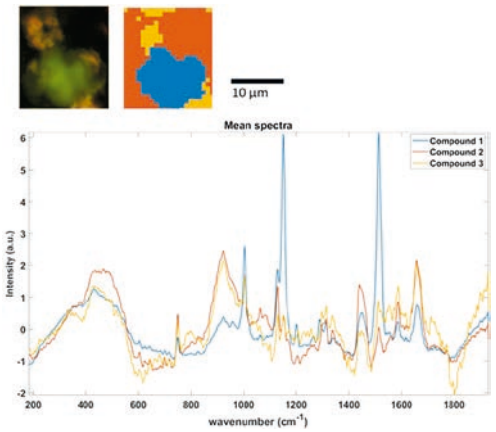
In a further collaboration, a PA microscopy system was used for label-free live imaging of developing *Parhyale hawaiensis* embryos, exploiting the intrinsic optical absorption properties of the yolk's pigments (e.g. carotenoids) [5, 6]. During the first embryogenesis stages, the yolk distribution in the cells and the membranes of the blastomeres were clearly visible. In later embryogenesis stages (soccer-ball stage – late segmentation), it was possible to observe the yolk sac location, monitor midgut development, and acquire structural information of the surrounding visceral and somatic mesoderm. At a late organogenesis stage, the spatial distribution of the signals delineated the longitudinal and circular muscles surrounding the gut and associated midgut glands. The findings of the study [7] pave the way for the broader adoption of inexpensive PA approaches for detailed investigations of developmental mechanisms in traditional model organisms, such as *Drosophila melanogaster* and zebrafish, and other emerging models without established labelling and imaging resources.

George Filippidis, George J. Tserevelakis, Meropi Mari and Giannis Zacharakis (FORTH)

[1] K. Palikaras et al., J. of Lipid Research 58: 72-80 (2017)
[2] V. Tsafas et al., J of Biophotonics 13: e202000180 (2020)
[3] G.J. Tserevelakis et al., Optics Letters 46: 4718-4721 (2021)
[4] K. Palikaras et al., Aging Cell 22: e13788 (2023)
[5] G.J. Tserevelakis et al., J. Biophotonics 15: e202200202 (2022)
[6] G.J. Tserevelakis et al., Photonics 10: 264 (2023)
[7] A collaboration among the IESL-FORTH, the Medical School of the National & Kapodistrian University of Athens (K. Palikaras) and the Institute of Molecular Biology and Biotechnology (IMBB)-FORTH (T. Pavlopoulos and N. Tavernarakis)

Carotenoid compounds identified in brain tissue from Alzheimer Disease patients (LLAMS, the Netherlands)

At LaserLaB Amsterdam, Raman imaging methodologies have been applied to post-mortem brain tissue samples from patients suffering from Alzheimer's Disease (AD). To characterise the chemical composition, these samples were only snap-frozen, with no additional fixation or staining.



Fluorescence and Raman imaging of plaque area in post-mortem brain tissue of an Alzheimer's Disease patient. The green autofluorescence (blue excitation; top left image) indicates the plaque area. Raman mapping of the same area (exc = 532 nm) followed by unsupervised spectral unmixing leads to three components (endmembers): plaque (blue), lipofuscin deposits (yellow) and regular brain tissue (orange), of which the distribution is shown in the top right image. The average Raman spectra of the three tissue types are shown in the bottom image; the plaque spectrum (blue) also shows strong carotenoid signatures. (C. Keskin MSc thesis; unpublished results)

The standard workflow [1] began with autofluorescence microscopy; the green emission of certain areas was found to be associated with so-called amyloid plaques, a hallmark of AD. Raman mapping was then carried out on those regions of interest. In addition, stimulated Raman scattering (SRS) microscopy of the same area was carried out to detect misfolded peptides (not shown). Subsequently plaque-specific staining was performed to verify the plaque location.

The top left image shows the plaque area, indicated by the green emission. The unsupervised unmixing [2] of the Raman map resulted in three endmembers, corresponding with three spectrally different compound mixtures, namely plaque, regular brain tissue, and lipofuscin. Their distribution is shown in the top right image. Interestingly, all three regions show typical Raman bands of tissue components, but the blue spectrum (plaque) also shows strong Raman bands at 1150 cm^{-1} and 1514 cm^{-1}, indicative of carotenoid compounds [3]. Their Raman signatures are enhanced due to pre-resonance excitation. The presence of carotenoids (originating from food items, such as carrots, tomatoes) had not previously been observed in AD plaques and their role is as yet unclear. Being strong anti-oxidants, they may have been invoked to fight local inflammation.

Can Keskin, Robert W. Schmidt and Freek Ariese (LaserLaB Amsterdam, the Netherlands)

[1] B. Lochocki et al., Nature Commun. Biol. 4: 474 (2021)
[2] R.W. Schmidt et al., J. Optics 24: 064011 (2022)
[3] L. Ettema et al., J. Optics 24: 054005 (2022)

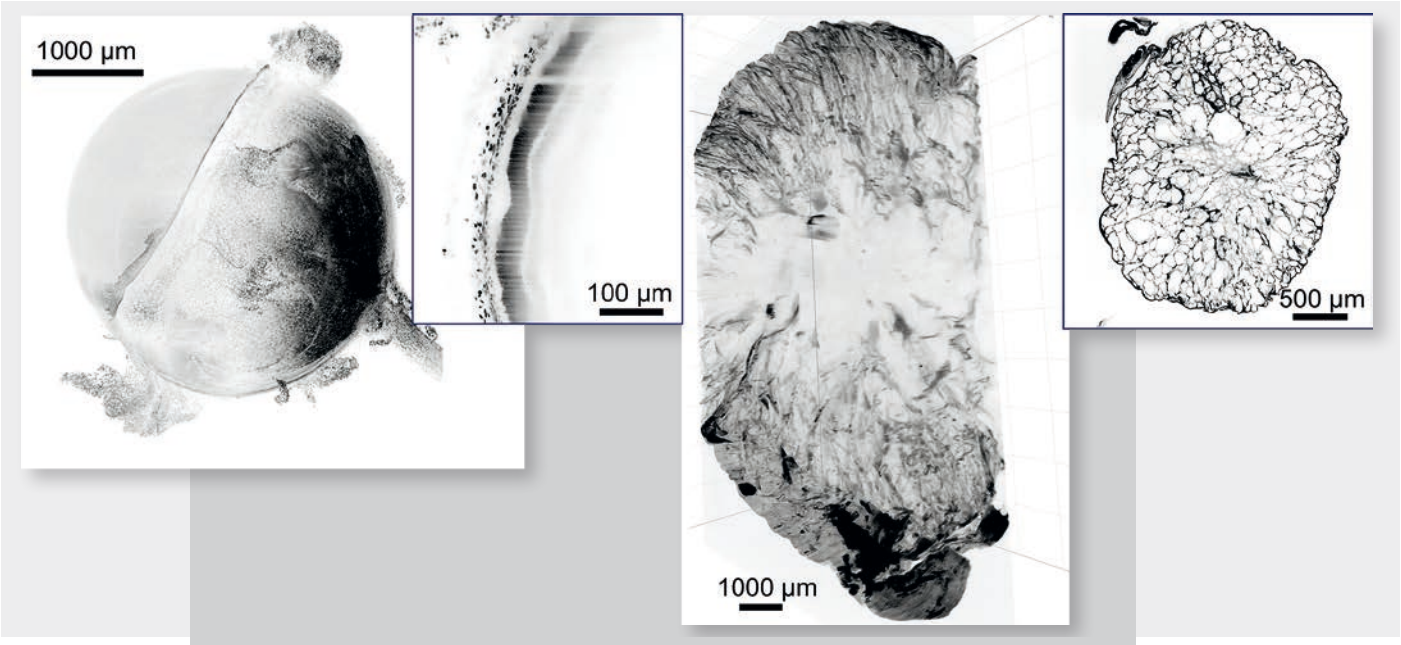
Translational biomedical imaging of whole organs using lightsheet microscopy (Central Laser Facility, United Kingdom)

Lightsheet microscopy is growing in popularity and proving to be a fast, efficient, and informative method of obtaining three-dimensional data of whole mouse organs, and for biopsies of human organs. Key biomedical issues, such as the differences between healthy and diseased tissue, are traditionally addressed through histology, which is the process of cutting thin slices of tissue and imaging each slice as a two-dimensional image. Sometimes only a few slices are chosen and used to represent the entire sample (using a process known as stereology to derive three-dimensional data from two-dimensional samples). However, after the tissue has been made transparent in a process called 'clearing,' lightsheet microscopy is capable of optically sectioning tissue without destroying it, using a sheet of light orthogonal to the plane of the camera. The light illuminates only a very thin plane of the transparent sample and can be scanned to collect multiple planes, building a three-dimensional representation of the sample for comprehensive analysis.

The Central Laser Facility has been collaborating with scientists from University College London, UK, to apply this technique, using a 3i Cleared Tissue Lightsheet microscope to acquire images. The ultimate aim of the work is to create a digital database (or atlas) of organs, or biopsies of organs, from different genetic backgrounds and disease states, and to preserve this data online in a central location. In early work, images have been acquired of whole mouse hearts, lungs, kidney, eyeballs, and sections of gut at a resolution of <1 µm. Knowledge gained from these early studies will be used to develop on-the-fly analysis of images as they are acquired by the microscope; for example, extracting cell positions that will reduce the burden of data storage and handling. Images and data collected are contributing to several projects, including: determining the causes of kidney rejection in human kidney transplant; understanding how the immune system develops in the eye and brain during disease; and understanding the structure of the lymphatic vasculature in organs.

Robert Lees (Central Laser Facility)

Renderings of three-dimensional data from a mouse eyeball (left two panels) and a mouse cystic kidney (right two panels), with an inset (blue outline) of a single optical section for each dataset. The insets show single-cell resolution features across the layer of the eye (left), and the abundance of large cysts in the kidney (right)



Central Laser Facility and University College London

Illuminating the brain: Unveiling the YWHAZ gene with lightsheet fluorescence microscopy (ICFO, Spain)

An international team of scientists employed cutting-edge techniques, specifically lightsheet microscopy in whole-brain imaging, to investigate the role of the YWHAZ gene in brain development and function. This novel approach delivered unprecedented insights into neural activity and connectivity in zebrafish, a powerful model organism for studying brain disorders.

Whole-brain imaging using lightsheet microscopy enables non-invasive investigation of neuronal activity and connectivity in living organisms. Using an electrically tunable lens, modulated in synchrony with the axial displacement of the illuminating light sheet, enabled high-resolution, three-dimensional images of the entire zebrafish brain to be captured.

By combining the power of whole-brain imaging with genetic engineering techniques, researchers aimed to unravel the intricate neural mechanisms underlying human brain disorders associated with YWHAZ dysfunction. The ability to observe neuronal activity and connectivity in live zebrafish embryos and adults enabled the impact of YWHAZ on neurotransmission, and behaviour across different developmental stages, to be assessed.

This innovative approach sought to bridge the gap between molecular studies of YWHAZ and the complex behavioural phenotypes observed in neurodevelopmental and psychiatric disorders. By visualising and analysing neural dynamics in a living organism, the studies aimed to uncover the functional consequences of YWHAZ alterations, providing valuable insights into the pathogenesis and potential therapeutic targets for these disorders.

ICFO

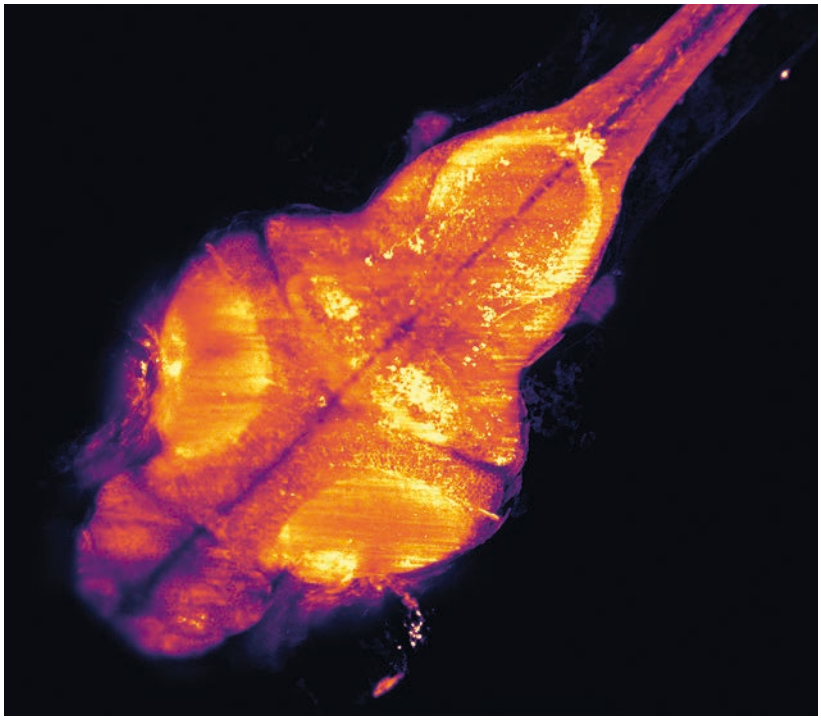


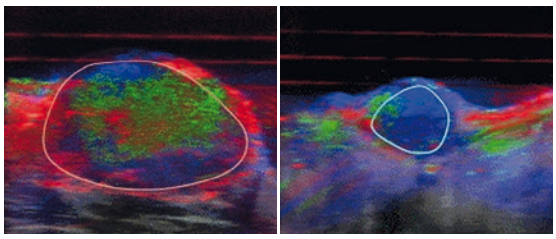
Image of zebra fish (ZF) whole brain showing neuronal activity. In the experiment we used LSFM to record the calcium activity of ~30 wild type ZF whose activity was compared with ~30 mutant ZF with a deficiency in the YWHAZ gene. A deficiency in the YWHAZ gene is involved in neurodevelopmental disorders.

Overall, the use of advanced techniques, such as whole-brain imaging through lightsheet microscopy, will provide a crucial step in investigations into the role of the YWHAZ gene in brain development and function. This approach has allowed the intricate neural processes involved in neurodevelopmental disorders to be explored, and enabled researchers to gain a deeper understanding of the underlying mechanisms at a comprehensive, whole-brain level.

Gustavo Castro and Pablo Loza-Alvarez (ICFO)

E. Antón-Galindo et al., Mol. Psychiatry 27: 3739–3748 (2022)

Drug biodistribution and pharmacokinetics by photoacoustic tomography (CLL, Portugal)



CLL/University of Coimbra

Photoacoustic tomography signals showing redaporfin levels in CT26 tumours (left) and 4T1 tumours (right)

The most basic principle in pharmacology is that a drug must reach its target to elicit a therapeutic effect. Following the biodistribution of drugs in an organism is very important to understanding the pharmacological action, but is very challenging to achieve using non-invasive methods. Researchers from the University of Coimbra made use of photoacoustic tomography, a non-invasive method, to follow the biodistribution of the drug redaporfin in clinical trials for advanced head and neck cancer. The organisms investigated were BALB/c mice with subcutaneous colon carcinoma (CT26) or with orthotopic breast (4T1) tumours.

The locations of the tumours in the mice were identified by ultrasound, and are shown in the photographs by continuous lines encircling the tumour regions where the tumours diameters attained their maximum values. Redaporfin was injected intravenously before the acquisition of the photoacoustic tomography signals. The colours refer to the intensities of the signals, where red is oxyhaemoglobin, blue is deoxyhaemoglobin and green is redaporfin. Redaporfin is particularly suited to photoacoustic tomography, because its absorption maximum occurs at 750 nm and its phototoacoustic spectrum is distinct from those of both forms of haemoglobin.

Imaging revealed that CT26 tumours accumulate a higher amount of redaporfin and have more oxyhaemoglobin, which explains why these tumours respond better to treatment than 4T1 tumours. The poor oxygenation and limited redaporfin infiltration in orthotopic 4T1 tumours may be attributed to high solid stress and elevated interstitial fluid pressure.

Luis Arnaut, Fabio Schaberle, Maria Inês Mendes and Catarina Lobo (Coimbra Laserlab (CLL); University of Coimbra)

C.S. Lobo et al., Sci. Rep. 13: 11667 (2023)

Advancements in HILO microscopy for optimal single-molecule microscopy performances (LENS, Italy)

Highly inclined and laminated optical sheet (HILO) microscopy, originally introduced by Tokunaga *et al.* in 2008, has emerged as a revolutionary strategy for imaging samples with increased contrast.

HILO microscopy employs a single-objective inclined lightsheet illumination method that exploits refraction at the glass/water interface to produce a thin, focused sheet of light, targeting specific regions of the sample volume. This ingenious approach significantly minimises unwanted background fluorescence from out-of-focus planes, a prevalent issue in conventional microscopy.

Despite its widespread adoption and popularity, HILO's underlying beam characteristics and mechanisms have remained poorly understood, leaving researchers without established procedures for optimal control and customisation. A recent paper, published in Optics Express [1], presented a theoretical model that precisely describes the propagation of the inclined beam. Through meticulous far-field and near-field experiments, the model has been successfully validated, providing researchers with the essential tools to predict the beam's geometrical features at the sample level.

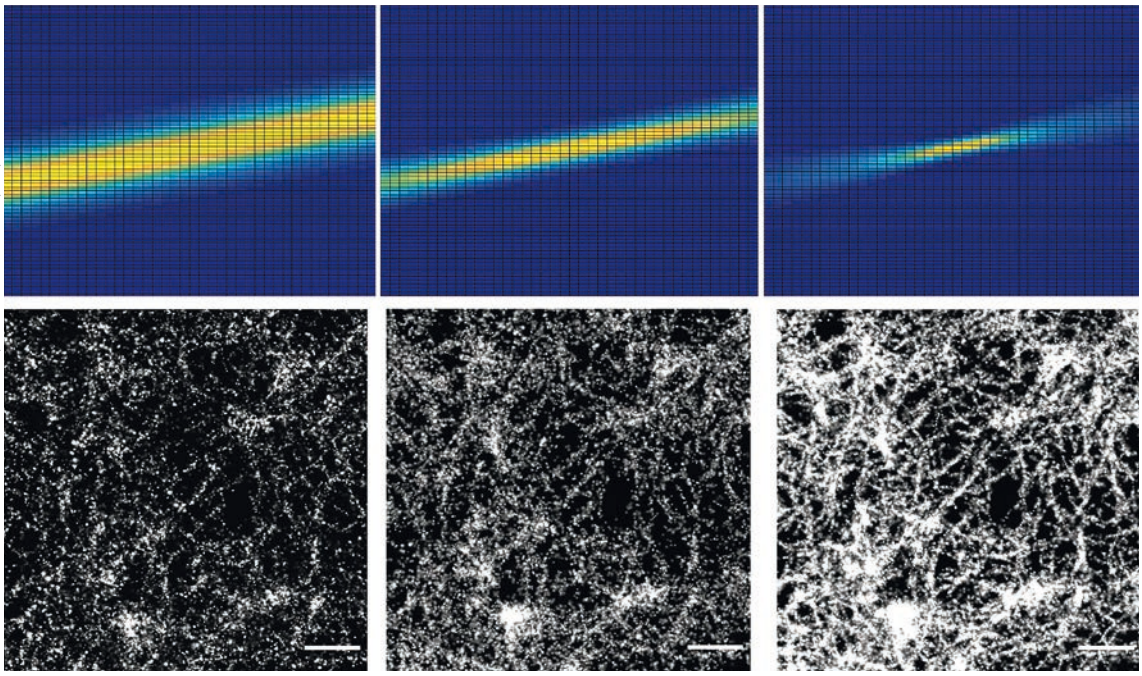
The paper also introduced an efficient alignment and beam shaping procedure, which will allow users to tailor the beam to their specific experimental needs with ease. By progressively reducing the inclined beam thickness, the team demonstrated a remarkable impact on image quality, both in conventional fluorescence microscopy and, for the first time, in localisation-based super-resolution microscopy. Through optimisation of the optical adaptation of the HILO configuration, it was possible to shrink the inclined beam thickness to less than three micrometres while maintaining a suitable field-of-view for cell imaging. This innovative approach resulted in a remarkable doubling of the number of single molecule localisations, effectively extending the resolution capabilities and significantly reducing the acquisition times in super-resolution PALM/STORM imaging.

The research team believes that their work addresses a widespread need for a better understanding of this powerful microscopy technique. It provides all the tools needed to achieve full control and shape the beam according to the user's experimental need, with a simple solution that can be implemented with minimal effort on any inverted fluorescence microscope.

The implications and the possible applications of this research are broad, covering various scientific fields, including biology, medicine, and materials science.

Lucia Gardini (LENS)

[1] L. Gardini et al., Opt. Express 31, 26208-26225 (2023)



Adapted from L. Gardini et al., Opt. Express 31, 26208-26225 (2023)

Upper panels: simulations of inclined Gaussian beam propagating at an angle of 77 degrees with decreasing thicknesses (from left to right). Lower panels: super-resolved images of actin cytoskeleton reconstructed from 10 thousand frames with decreasing inclined beam thickness (from left to right).

Successful laser printing of innovative materials with properties that change with voltage

Many telecommunications devices use materials with tuneable optical properties, to enable optical switching or multiplexing, but the current process for manufacturing such devices is both long and expensive. To provide a cheaper, faster process, researchers have been exploring an innovative printing technique that exploits the capability of pulsed lasers to transfer pixels of matter with a high level of precision and controllability.

Within the framework of the Laserlab-Europe transnational access programme, Professor Daniel Puerto of the *Holography and Optical Processing Group* (GHPO) at the University of Alicante, Spain, recently led a collaborative project to demonstrate the laser printing of innovative materials that can be activated through external voltage application. A six-member research group from the University worked with Dr Camilo Florian from Princeton University, USA, and Dr Catalin Constantinescu and Dr Patricia Alloncle of the CNRS-LP3 laboratory in Marseille, France, where the experimental demonstration of laser-induced printing took place.

The laser printing technique that was used is known as LIFT (Laser-induced Forward Transfer), and was first demonstrated some 30 years ago. The technique (see Figures a and b) uses short or ultra-short laser pulses (10^{-12} to 10^{-15} seconds) for digital transfer, and can be scaled down to sub-micrometre scale when high spatial resolution is required [1]. Over the past two decades the technique has been further developed in LP3 [2], and has successfully been applied to print functional organic transistors, microcapacitors and conductive lines, with high resolution on various substrates (flexible PET and polyimide, glass, silicon etc.). LIFT offers a fast, high-precision, chemical-free and environmentally friendly approach to the digital printing of optical (micro) devices and electronic components, opening up a wide range of possibilities in photonics and microelectronics, as well as tissue engineering applications.

Through their Laserlab-Europe access project, Daniel Puerto's research consortium succeeded in demonstrating the printing of tuneable materials for the first time using the LIFT technique. This innovative work was carried out on the high-speed, digitally-controlled setup at the LP3 laboratory (see Figure b), and proves that it is possible to accurately print a polymer doped with liquid crystals [3].

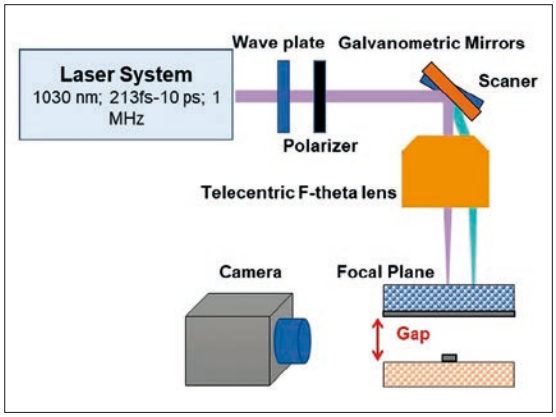
The main challenge involved developing a method to print viscous (600 mPa.s) tuneable photopolymer microdroplets at high velocity, without any contaminants arising from the absorption of the laser beam. Processes that use LIFT to print liquid materials typically use a thin layer of absorbent material between the donor substrate and the donor print layer, to induce strong absorption of the beam at this interface and generate a cavitation bubble inside the material to be printed. The dynamics of this cavitation bubble creates a liquid jet that breaks away from the donor substrate and transfers print material to the receiver substrate. However, under irradiation by the laser beam, the absorbing layer can be vaporised, resulting in some residual contaminants (nano-/micro-particles) that affect the optical quality of the transferred material.

To be able to print without using an absorption layer, researchers exploited the optical properties of a focused femtosecond laser beam to generate multiphoton absorption within the donor print layer. A very narrow energy window was found that delivered reproducible printing of 10 µm diameter, 15.7 fL polymer droplets on BK7 glass with indium tin oxide (ITO) substrates (see Figures c and d).

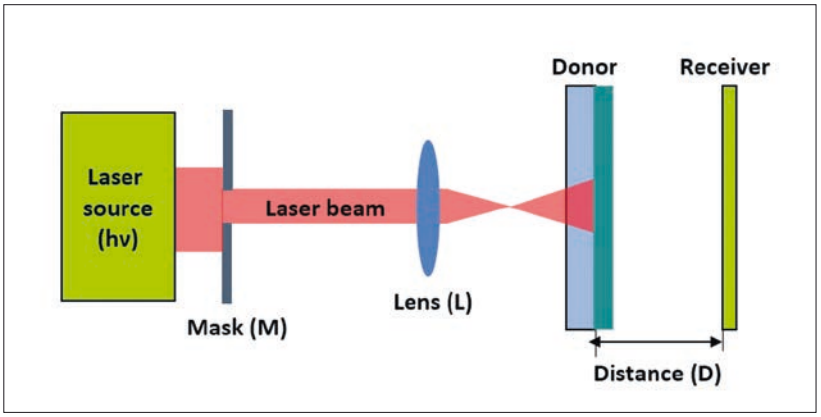
Comparisons between droplets printed with and without a metallic absorption layer confirmed that the new laser-based method is capable of printing clean micro-droplets at high speed in a reproducible manner. The simpler, cleaner direct transfer process can be used to manufacture innovative lenses in a time- and cost-efficient manner. Moreover, LIFT printing of polymer-based materials doped with liquid crystals will enable quicker production of versatile and innovative devices such as foldable mobiles and tablets, suitable for fast and easy integration into industrial systems.

Daniel Puerto, Sergi Gallego, Manuel Ortuno, Andrés Marquez, Jorge Francés, Inmaculada Pascual, Augusto Belendez (University of Alicante, Spain), Camilo Florian (Princeton University, USA), Catalin Constantinescu and Patricia Alloncle (LP3, CNRS, France)

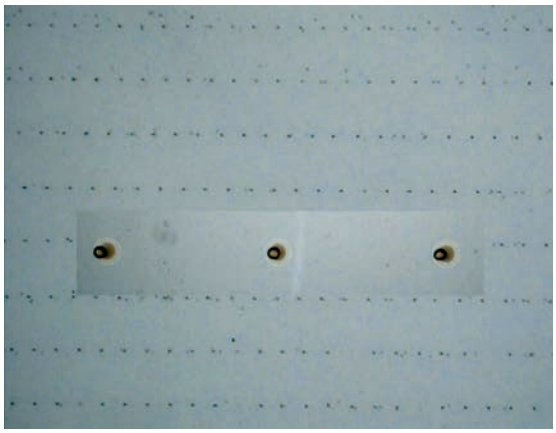
[1] Q. Li et al., Appl. Surf. Sci 471: 627-632 (2019)
[2] Ph. Delaporte and A.-P. Alloncle, Opt. Laser Technol. 78: 33-41 (2016)
[3] D. Puerto et al., Opt. Express 31: 17619-17628 (2023)



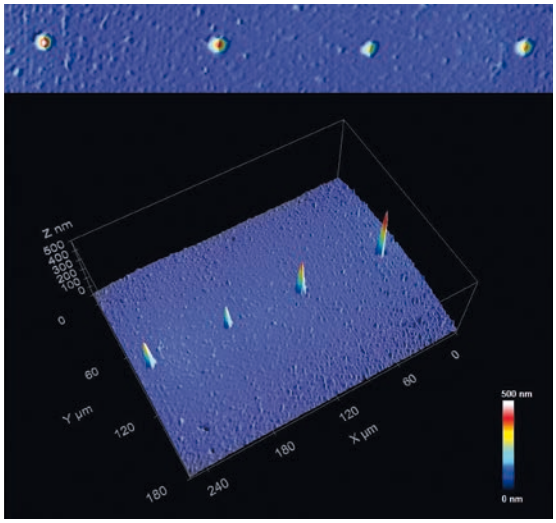
a) *Schematic of the experimental system used for polymer printing. It includes: a 1030 nm laser with a variable pulse duration between 210 fs and 10 ps, and a repetition frequency of up to 1 MHz; a scanner; and a F-theta lens to move the laser focus position along a 5 × 5 cm² surface.*



b) *Schematic of the LIFT printing process.*



c) *Optical microscopy image of an array of droplets deposited on a BK7 glass slide with ITO substrates, printed with 1.5 µJ femtosecond laser pulses and a gap of 60 µm.*



d) *3D image of the polymer droplets deposited on a glass sample holder with a femtosecond laser under conditions of a 75 µm gap and an energy of 1.5 µJ.*

Advances in joint ELI User Programme and strategic integration of ELI ALPS

The Joint User Programme of the Extreme Light Infrastructure (ELI ERIC) continued to evolve in 2023 with the implementation of user experiments accepted in the 2nd Call and launch of the 3rd Call in September. A total of 227 research proposals involving over 500 scientists from 28 different countries have been submitted across the three calls for proposals at all three ELI facilities. As a core event to engage with the user community, the Joint ELI User Meeting was held at ELI ALPS in Szeged, Hungary, in early December. The aim of the annual User Meeting is to update the scientific community about research opportunities at ELI with news about recent developments at the facilities and to review and discuss current and upcoming user campaigns.

ELI ERIC

ELI ERIC

Another significant milestone was achieved in November with the signing of the agreement between ELI ERIC and the University of Szeged to aquire ELI ALPS. The agreement provides a framework for ELI ERIC to take over the responsibility for the long-term operation and financing of ELI ALPS from 1 January 2024. The integration of ELI ALPS follows a year after the incorporation of ELI Beamlines and realises the original vision for ELI ERIC to jointly operate the two research facilities under a unified governance and single management structure. The ELI ERIC, a European consortium with member and observer countries, now paves the way for further international collaboration.

ELI ERIC

How to gain access to tools and techniques at more than 50 analytical facilities

If you are interested in or working on challenges in circular materials, don't miss your chance to take part in ReMade@ARI's calls for proposals: More than 50 research infrastructures provide transnational access to a broad range of instrumentation and techniques to international scientists. The third call runs 28 February to 10 April 2024.

Want to find out what you can do? To plan your proposal, the "Catalogue of Techniques" at Remade@ARI's website gives you an overview of all available techniques and where to find them. In addition, in a series of recorded webinars, Laserlab-Europe members present their techniques available to academic and industrial users in the field of recyclable materials. You can find it on Laserlab-Europe's YouTube channel.

If you are planning your proposal, ReMade@ARI's Smart Science Cluster is there to help you. About 20 Junior Scientists will support you all over Europe: From the design of your experiment to the data collection and evaluation, they are happy to share their expertise matching the experimental techniques

offered within ReMade@ARI. For your requests and to learn more, contact sciencesupport@remade-project.eu

You can also submit a pre-proposal to discuss suitable techniques. Pre-proposals can be submitted at any time.

https://remade-project.eu

How to apply for access

Interested researchers are invited to contact the Laserlab-Europe website at www.laserlab-europe.eu/transnational-access, where they find relevant information about the participating facilities and local contact points as well as details about the submission procedure. Applicants are encouraged to contact any of the facilities directly to obtain additional information and assistance in preparing a proposal.

Proposal submission is done fully electronically, using the Laserlab-Europe Proposal Management System. Your proposal should contain a brief description of the scientific background and rationale of your project, of its objectives and of the added value of the expected results as well as the experimental set-up, methods and diagnostics that will be used.

Incoming proposals will be examined by the infrastructure you have indicated as host institution for technical feasibility and for formal compliance with the EU regulations, and then forwarded to the Access Selection Panel (ASP) of Laserlab-Europe. The ASP sends the proposal to external referees, who will judge the scientific content of the project and report their judgement to the ASP. The ASP will then take a final decision. In case the proposal is accepted, the host institution will instruct the applicant about further procedures.

Laserlab Forum Contact

Sylvie Jacquemot
Coordinator – Laserlab-Europe
Email: *jacquemot@laserlab-europe.eu*

Laserlab-Europe Office
Daniela Stozno
Max Born Institute
Max-Born-Str. 2A | 12489 Berlin | Germany
Phone: +49 30 6392 1508
Email: *stozno@laserlab-europe.eu*

Editorial Team:
Tracey Burge, Marina Fischer,
Julia Michel, Daniela Stozno
Email: *office@laserlab-europe.eu*
Layout: unicom Werbeagentur GmbH

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