

leti

cea tech

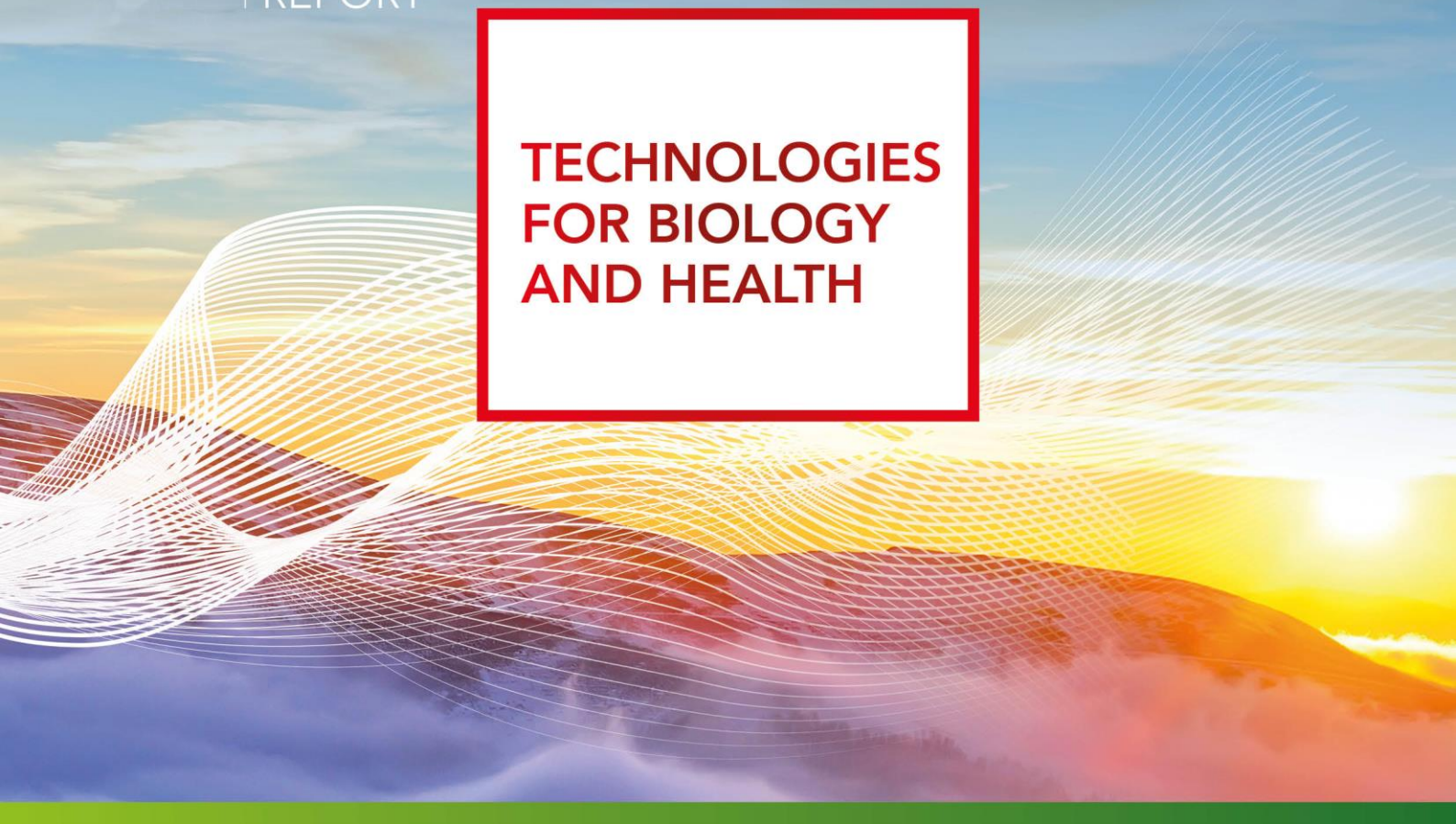
TECHNOLOGY
RESEARCH
INSTITUTE



2020

SCIENTIFIC
REPORT

**TECHNOLOGIES
FOR BIOLOGY
AND HEALTH**



COMMITTED TO INNOVATION, CEA-Leti CREATES DIFFERENTIATING SOLUTIONS WITH ITS PARTNERS

CEA-Leti is a technology research institute of France's CEA and a global leader in miniaturization technologies enabling smart, energy-efficient, and secure solutions for industry. Founded in 1967, CEA-Leti conducts pioneering micro and nanotechnology research and custom develops differentiating application-specific solutions for global companies, SMEs, and startups. CEA-Leti tackles critical challenges in healthcare, energy, and digital migration. From sensors to data processing and computing solutions, CEA-Leti's multidisciplinary teams deliver solid expertise, leveraging world-class pilot production lines to scale new technologies up. With a staff of more than 1,900, a portfolio of 3,140 patents, 10,000 sq. meters cleanrooms, and a rigorous IP policy, CEA-Leti has launched 69 startups and is a member of France's Carnot research network. based in Grenoble, France, the institute has offices in Silicon Valley and Tokyo.
Follow us at www.leti-cea.com and @CEA_Leti.

Technological expertise

CEA (the French Alternative Energies and Atomic Energy Commission) is a leading global research organization whose mission is to transfer new scientific knowledge and innovations to industry. With a focus on electronics and integrated systems from micro to nano, CEA innovations make businesses in transportation, health, safety, and telecommunications more competitive by helping them develop high-performance, differentiating products and novel solutions.

www.cea.fr/english



CEA-Leti at a glance

450
publications per year

ISO 9001
certified since 2000

114
European projects

Founded in
1967

Based in
France (Grenoble)
with offices in the

US (San Francisco) and
Japan (Tokyo)

300
industrial partners

1,904
researchers

3,140 patents
in portfolio

10,000
sq. meters of cleanrooms
100-200-300 mm wafers

69
startups created

TECHNOLOGIES FOR BIOLOGY AND HEALTH

The Biology and Health division of CEA Leti aims at playing a key role in the development of technologies for health with a “One health” strategic positioning, which takes into account human in interaction with its environment as a key issue. Its Core R&D competencies are the development, design, integration and qualification of micro- and nanotechnologies in application to health (in the broad sense as defined by WHO) and life sciences. These include sensors and actuators, imaging technologies, microfluidics, chemistry, biochemistry and electrochemistry, biology and instrumentation, including mechanics, software, information processing and electronics.

Our teams have acquired expertise in developing product prototypes with a system-development perspective.

Our facilities cover the whole value chain devoted to health technologies from the technological platform dedicated to medical device development (constituted of the “Microfluidic Integration” and “Numerical Medical Devices” platforms) to preclinical and clinical investigations.

Our facilities include cleanrooms dedicated to biochip packaging (230 m²), and to surface functionalization, (bio)probes grafting and (bio)reagent loading (100 m²), biological laboratories with L2 rooms (100 m²) dedicated to bacteria, cells and human samples handling and biological characterization equipment such as PCR, cell microscopy and FACS. We also have laboratories for synthetic chemistry, electrochemistry and characterization (430 m²) and a microfluidic platform dedicated to technologies and system validation (300 m²). These facilities are complemented with technical platforms dedicated to optics instrumentation and to electronic and integration.

With Clinathec, we placed our state-of-the-art technology and biology laboratories under one roof with a fully equipped preclinical facility hosting small and large animals and an integrated cutting-edge clinical platform operated by Grenoble University Hospital. This unit is optimal for conducting the first human medical-device clinical trials for safety and efficacy studies, as well as for hosting clinician partners for the duration of their clinical research projects.

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TECHNOLOGIES FOR BIOLOGY AND HEALTH

FOREWORD

*Patrick Chaton,
Head of Microtechnology
for Biology and Healthcare
division*



*Prof. Alim Louis
Benabid,
Chairman of the Clinatec
Board*

*Prof. Stephan
Chabardes,
Clinatec Clinical sector
Director*

2020, the year of the COVID 19 pandemic, was a special year that changed our perception of the world, trade, people and health. The latter has become a fundamental issue for our society. The progression of the pandemic, from its origin to the present day, has brought to light the multifactorial nature of health, which cannot be centered solely on the individual but have also to integrate his environment. From the primary infection by the Coronavirus, potentially via a wild animal, to its spread, by contact or via airborne particles, this pandemic has reinforced our vision of health built on a “One Health” approach taking into account man in his global environment through what he breathes, drinks or eats and the fauna he comes into contact with.

Despite the repeated confinements, this health crisis has demonstrated the ability to mobilize and the agility of our research teams, but also the relevance of our approaches.

- We were thus involved in the development of the MakAir artificial respirator in a joint effort with other CEA-Leti teams.
- In the context of "environmental" diagnostics, we proposed to the ANR, upstream of the identification of virus dissemination routes, a research project aimed at the collection and identification of airborne viral particles. Thus, our developments on the aero-collection of pathogens via electrostatic discharge were adapted to the design of a beacon (integrating microfluidic system and RT-PCR) for the detection of SARS-CoV-2 in risk areas.
- Finally, the French Research Ministry has selected our project on RNA vaccine against Covid 19, based on the use of lipid particles – Lipidots - as vaccine vectors. It is important to note that the formulation of these lipid particles, which benefit from extensive scientific experience, has been transferred to the company V-Nano in Occitania for the manufacture of GMP quality industrial batches. This approach could therefore enable us, in the event of positive pre-clinical results, to access the industrial production of a clinical batch of anti-Covid vaccine.

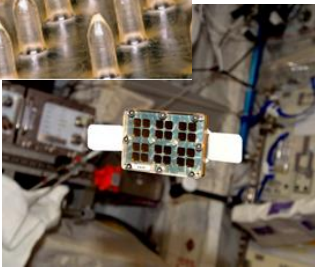
2020 was also a fertile year in terms of research. In particular, BCI Awards rewarded Alexandre Molly's work on the development of an adaptive decoder for an intracranial human-machine interface. On the other hand, to mention just one, the publication in Nature Microgravity of our work on antibacterial surfaces carried out in the international space station with the help of Thomas Pesquet.

Surely, 2020 will be an inspiration for tackling health issues in the future!

KEY FIGURES



90 Researchers
36 Post-docs and short-term contracts
23 PhDs and apprentices



56 Book chapters and journal articles
51 Conferences and workshops



31 Patents filled
417 Patents in portfolio
10 Startups created



230 m² Cleanroom for surface chemistry and biochip packaging
100 m² Biological laboratory (L1 and L2 facilities)
430 m² Chemistry laboratory
230 m² Microfluidic laboratory



6 Rooms for patients and monitoring technologies
A fully equipped surgery room with Intraoperative MRI
Multimodal investigation capabilities MEG, SPECT-CT, gait analysis

SCIENTIFIC ACTIVITY

Publications

Express, Plastic and reconstructive surgery, Analytical Chemistry, The Analyst, Journal of Biomedical Optics, Journal of Pharmaceutical and Biomedical Analysis, Biochemical and Biophysical Research Communications, ACS Sensors, American Journal of Analytical Chemistry, ACS Appl. Bio Mater, Analytical and Bioanalytical Chemistry, Lab On Chip, International Journal of Pharmaceutics, Journal of Neuro oncology, Sensors...

Experts

2 Research Directors
3 International Expert
10 Senior Experts
14 Experts
13 owning the HDR

Scientific committees

Member (Pascal Mailley) of the scientific committee of the Journeys of Electrochemistry" (International francophone congress dedicated to electrochemistry)
Convenior (Nicolas Verplanck) of the European (CEN/TC332/WG7 and International ISO/TC48/WG3 regulation groups for the normalization of microfluidic systems.

Conferences and Workshops organizations

51 Conferences and workshops

Main papers are:

"PepS: An Innovative Microfluidic Device for Bedside

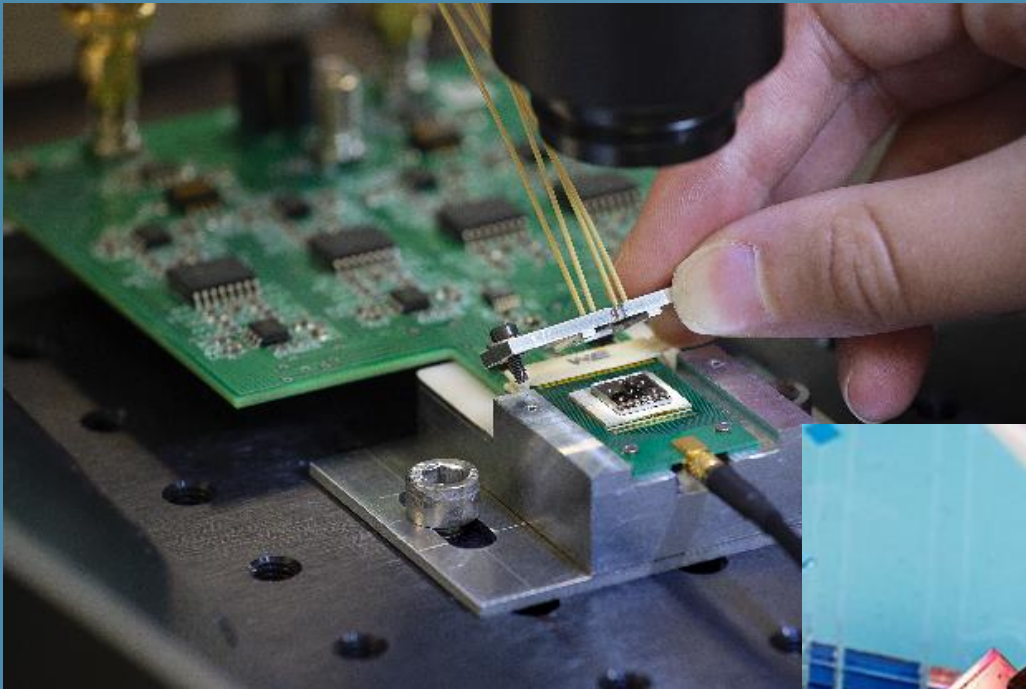
Whole Blood Processing before Plasma Proteomics Analyses", B. Gilquin, M. Cubizolles, R. Den Dulk, F. Revol-Cavalier, M. Alessio, C.E. Goujon, C. Echampard, G. Arizabalaga, A. Adrait, M. Louwagie, P. Laurent, F.P. Navarro, Y. Couté, Y. M-L. Cosnier, V. Brun, Anal. Chem. 93 (2021) 683–690, DOI: 10.1021/acs.analchem.0c02270

"Contact, high-resolution spatial diffuse reflectance imaging system for skin condition diagnosis: a first-in-human clinical trial", A. Koenig, N. Petitdidier, H. Grateau, S. Characoun, A. Ghaith, S. Verges, S. Doutreleau, S. Gharbi, R. Gerbelot, S. Gioux, J.-M. Dinten, J. of Biomedical Optics, 26 (2021) 012706, DOI: 10.1117/1.JBO.26.1.012706

"Adjuvant therapeutic potential of moderate hypothermia for glioblastoma", C. Fulbert, S. Chabardes, D. Ratel, J. Neurooncol., 152 (2021) 467-482, DOI: 10.1007/s11060-021-03704-y

International Collaborations

MIT (USA)
Politecnico di Milano, University of Pisa (Italy)
Helmoltz Association, Franhauser, Charité Berlin (Germany)
University of Twente, UMC Utrecht (Netherland)
Tyndall (Ireland)
University of Birmingham (United Kingdom)
University of Aalto (Sweden)
CIDETEC, CSIC (Spain)
University of Sidney (Australia)
CSEM, EMPA (Switzerland)
Nanomedicine European Platform (Europe)



01

BIOMARKERS, SENSORS AND BIOSENSORS

- Calibration method for NOx sensors
- Biosensing of extracellular vesicles
- Advances in cardiac biomarkers detection
- HAP preconcentration / thermodesorption device

Recent advances in cardiac biomarkers detection: from commercial devices to emerging technologies

RESEARCH TOPIC:

cardiac biomarker; troponin; natriuretic peptides; miRNA; acute myocardial infarction; point-of-care

AUTHORS:

Maud Savonnet, Tristan Rolland, Yoann Roupioz, Arnaud Buhot, Myriam Cubizolles

In cardiac pathologies, rapid patient care and management in emergencies are critical to prevent dramatic consequences. Thus, relevant biomarkers such as troponin and natriuretic peptides are currently targeted by commercialized Point-Of-Care immunoassays. Key points still to be addressed concern cost, lack of standardization, and poor specificity, which could limit the assays' reliability. Consequently, alternatives are emerging to address these issues. New probe molecules such as aptamers or molecularly imprinted polymers should allow a cost reduction of the assays and an increase in reproducibility. Moreover, the assay specificity and reliability could be improved by enabling multiplexing through the detection of several molecular targets in a single device.

Context and Challenges

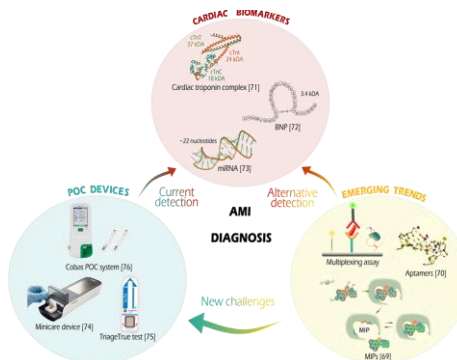
Cardiovascular diseases are the leading cause of mortality in the world. The main challenges in cardiac care include rapid intervention for at-risk patients, together with ruling-out of no-risk people as reliably as possible. Several companies have therefore developed point-of-care (POC) devices capable of performing cardiac biomarker quantification rapidly and with high sensitivity. Nevertheless, future solutions for biomarker detection are still needed in order to improve acute myocardial infarction (AMI) diagnosis.

Main Results

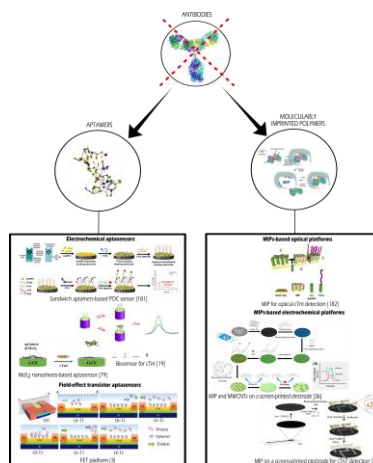
Two biomarkers for AMI, cardiac troponin I and brain natriuretic peptides, are the most widely used. Emerging biomarkers, such as miRNAs, are not yet exploited in commercial POC devices but present a huge potential. Shorter analysis time, better sensitivity, and the demand for POC devices are the requirements for a better diagnosis and an earlier triage of patients in emergency departments. Some limitations of currently available commercial products could be overcome using new advanced technologies, with alternatives to antibodies such as aptamers or MIPs. They represent a promising potential innovation in clinical care. These methods are currently under development and still at an experimental stage.

Perspectives

Multiplexed analysis of current cardiac biomarkers and implementation of novel ones are required to improve AMI diagnosis and patient care. In addition, alternative technologies are almost essential to improve methods' performance and reduce their cost.



Graphical layout of the review



Alternative technologies: aptamers & MIP

Biosensing extracellular vesicles: contribution of biomolecules in affinity-based methods for detection and isolation

RESEARCH TOPIC:

Extracellular vesicles, biomarkers, biosensors, aptamers, peptides, lab-on-chip

AUTHORS:

M. Gaillard, A. Thuair, G. Nonglaton, V. Agache, C. Raillon, Y. Roupioz

Extracellular Vesicles (EVs) are lipid vesicles secreted by cells that allow intercellular communication. They have recently attracted attention for their potential applications as biomarkers for many diseases. EVs are decorated with surface proteins that can be targeted by biochemical techniques to isolate them from background particles. We have reviewed the contribution of biomolecules used as ligands for the detection and isolation of EVs in affinity-based biosensors. Among them, antibodies are well-described biomolecules that are often used in capturing techniques, whereas DNA aptamers, which are short DNA fragments that mimic antibody action, are currently being developed. Peptides are also an exciting biochemical solution that can complement other probes.

Context and Challenges

Extracellular Vesicles (EVs) are lipid vesicles secreted by cells that allow intercellular communication. EVs are promising biomarkers which could potentially be used for diagnostic applications to detect diseases at an early stage. Their isolation and characterization however still remain challenging [1], especially when the isolation of an EV subtype is needed. Affinity-based approaches can answer to this issue as their main advantage is their specificity. Capturing biological objects like EVs with antibodies is indeed well described in the literature through different biosensing techniques. However, since handling proteins can be challenging due to stability issues, sensors using non-denaturable biomolecules, such as DNA aptamers or peptides, are emerging. This is what we report here.

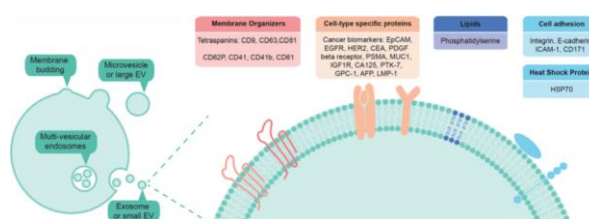
Main Results

There is a large variety of EV-targeting ligands in literature but it remains challenging to propose new probes to target EVs with high specificity. DNA aptamers, which are short DNA fragments that mimic antibody action, are currently being developed and considered as the future of antibody-like ligands. These molecules offer undeniable advantages: unparalleled ease of production, very high stability in air, similar affinity constants to antibodies, and compatibility with many organic solvents. SELEX may give access to DNA aptamers specific to any targeted protein, although this technique remains labor intensive and requires rigorous controls. To overcome these drawbacks, alternative methods are emerging. Cell-SELEX has been developed to directly target desired transmembrane proteins in living cells. The main advantage of this technique is the selection of DNA aptamers specific to membrane proteins in their native conformation. In parallel, the engineering of a novel class of proteins based on single-domain antibody fragments (nanobodies) also offers promising novel probes in the coming years. Their specificity is equal to the antibody's but they are smaller in size, more chemically stable, and therefore easier to handle. These different ligands have been used in

several types of biosensors: electrochemical, optical, microfluidic using both generic probes (targeting widely expressed membrane proteins such as tetraspanins) and specific probes (targeting disease biomarkers such as proteins overexpressed in cancer).

Perspectives

EVs have a significant potential as biomarkers for disease diagnosis and all the more as they are available in many biological fluids such as blood and urine. In parallel, the numerous detection and isolation methods available demonstrate that technology is up to the challenge of biosensing of EVs [2]. The next step towards robust and commercial biosensors will then have to deal with reliability, portability, cost and miniaturization of the devices.



Example of EVs surface biomarkers

RELATED PUBLICATIONS:

- [1] Pariset E., Agache V., Millet A., Extracellular Vesicles: Isolation Methods, *Adv. Biosyst.*, 1, 1700040, 2017
 [2] Gaillard, M., Sarrut-Rio, N., Virot, L., Boizot, F., Verplanck, N., Raillon, C., Agache, V., Roupioz, Y., Thuair, A. Deterministic lateral displacement for the sorting of extracellular vesicles from complex biological samples, *MicroTAS 2020 - 24th International Conference on Miniaturized Systems for Chemistry and Life Sciences*, 2020, 1075-1076

Reference method for off-line analysis of nitrogen oxides in cell culture media by an ozone-based chemiluminescence detector

RESEARCH TOPIC:

NO and its by-products analysis, reference method development, ozone-based chemiluminescence detector, 3D printing tissue model

AUTHORS:

A. Chmayssema, K. Monsalve-Grijalba, M. Alias, V. Mourier, S. Vignoud, L. Scomazon¹, C. Muller¹, J. Barthes¹, N. Engin-Vrana², **P. Mailley**

Nitric Oxide (NO) and its byproducts are important biological signals in human physiology and pathology particularly in the vascular and immune systems. Thus, in situ determination of the NO-related molecule (NO_x) levels using embedded sensors is of high importance particularly in the context of cellular biocompatibility testing. However, NO_x analytical reference method dedicated to the evaluation of biomaterial biocompatibility testing is lacking. Herein, we demonstrate a PAPA-NONOate based reference method for the calibration of NO_x sensors. After, the validation of this reference method, its potentialities were demonstrated for the detection of the oxidative stress related NO secretion of vascular endothelial cells in a 3D tissue issued from 3D printing.

SCIENTIFIC COLLABORATIONS: ¹ Inserm UMR 1121, 11 rue Humann, 67085 Strasbourg (FR), ² Spartha Medical, 14B Rue de la Canardière, 67100 Strasbourg (FR)

Context and Challenges

The involvement of nitric oxide (NO) in numerous physiological and pathological processes in the human body has created a need to accurately measure this free radical species in a variety of biological systems. Traditional methods dedicated to NO detection during cell culture such as "Griess method" requires samples collection and are generally based on colorimetric measurements of nitrite, the principle NO by-product. Thus, the development of an electrochemical sensor for NO and its by-product monitoring during cell-culture is of high interest. However, in order to validate the development of this electrochemical sensor, a reference method is required.

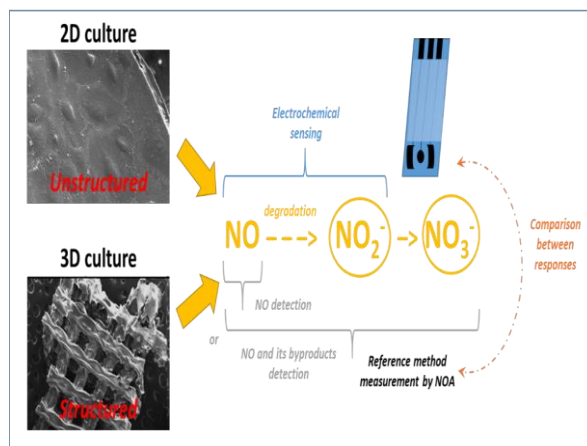
Main Results

In this study, we reported the development of a new reference method for off-line analysis of NO and its by-products using a commercial nitric oxide analyzer (NOA). This development was based on the use of PAPA-NONOate as NO-donor for the NOA system. The theoretical synthesis of NO from PAPA-NONOate degradation was studied in different experimental conditions of reaction media and temperature. In order to verify the ability of the system to measure experimentally relevant NO levels in cell culture conditions, NO released from human vascular endothelial cells from a 3D printed pre-vascularized tissue model has been quantified in comparison to conventionally produced gelatin hydrogels with endothelial cells. This demonstrates the ability of the developed method in the determination of the NO_x levels in biologically relevant configurations.

Perspectives

Real-time monitoring of key biochemical markers is an important enabling technology for on-chip organ systems. This includes the detection of NO and its by-products, which requires a reliable reference system to enable the distinguishing of the rather subtle temporal differences in NO_x levels for the assessment of artificial organ models. The herein reference method will be further used for the calibration of real-

time on-chip NO_x electrochemical sensors. The developed methodology by the NOA is of high interest. It provides the unique possibility to detect and quantify nitric oxide and/or its by-products in the cell culturing media without matrix effect. Further works are currently on the way to qualify the response of electrochemical sensors through the correlation with the results issued from NOA analysis.



Description of NO_x detection by electrochemical sensing and using the NOA

RELATED PUBLICATIONS:

- [1] Pappa, O. Parlak, G. Scheiblin, P. Mailley, A. Salleo, R.M. Owens. Organic Electronics for Point-of-Care Metabolite Monitoring. Trends in Biotechnology (2018). <https://doi.org/10.1016/j.tibtech.2017.10.022>
- [2] S. Inal, A. Hama, M. Ferro, C. Pitsalidis, J. Ozat, D. Iandolo, A. Pappa, M. Hadida, M. Huerta, D. Marchat, P. Mailley, R. M. Owens. Conducting Polymer Scaffolds for Hosting and Monitoring 3D Cell Culture. ADVANCED BIOSYSTEMS (2017). <https://doi.org/10.1002/adbi.201700052>
- [3] A. Chmaysssem, N. Verplanck, C. Edi Tanase, G. Costa, K. Monsalve-Grijalba, S. Amigues, M. Alias, M. Gougis, V. Mourier, S. Vignoud, A. M. Ghaemmaghamic, P. Mailley. Development of a multiparametric (bio)sensing platform for continuous monitoring of stress metabolites. Talanta (2021). <https://doi.org/10.1016/j.talanta.2021.122275A>

Integrated system for the rapid polycyclic aromatic hydrocarbons extraction from aqueous samples and their consecutive thermal desorption prior to gas chromatography analysis

RESEARCH TOPIC:

Portable analytical systems development

AUTHORS:

R. Pelisson, B. Fain, S. Vignoud, **F. Ricoul**

This work describes for the first time the extraction followed by thermal desorption of polycyclic aromatic hydrocarbons (PAHs) spiked water samples in a microfluidic silicon device. Thanks to the integration into an original system composed of a micropump, microvalves, and an optimized thermal management, the entire protocol is automated and combine the extraction, the drying and the desorption in less than 25 min before sending the sample to a GC-FID system. Repeatable recovery yields have been determined for 1 µg/L spiked water samples and the analysis of PAHs in a natural water spiked sample has been demonstrated without loss of performance compared to purified water samples. Compared to other extraction techniques, this system has the advantage of reduced footprint, reduced energy consumption and no solvent use.

Context and Challenges

Polycyclic aromatic hydrocarbons (PAHs) are known to present toxic, mutagenic and carcinogenic effects. They are ubiquitous present in the environment because of human activity. Real-time PAHs detection is therefore a crucial concern for environment control and human health.

Main Results

On-chip extraction and thermal desorption of PAHs prior to GC analysis is demonstrated. Thanks to the integration into an original system composed of a micropump, microvalves, and an optimized thermal management, the entire protocol is automated and combine the extraction, the drying and the desorption in less than 25 min. Repeatable recovery yields have been determined for 1 µg/L spiked samples and the analysis of PAHs in a natural water sample has been demonstrated. Our system compared to other extraction techniques has the advantage of reduced foot print, reduced process time, reduced energy consumption as well as no solvent use.

Perspectives

These results paves the way for further integration and development of a fully portable analytical system by coupling the present system to miniaturized GC system for real-time in-situ PAHs monitoring in environmental samples like natural water samples.



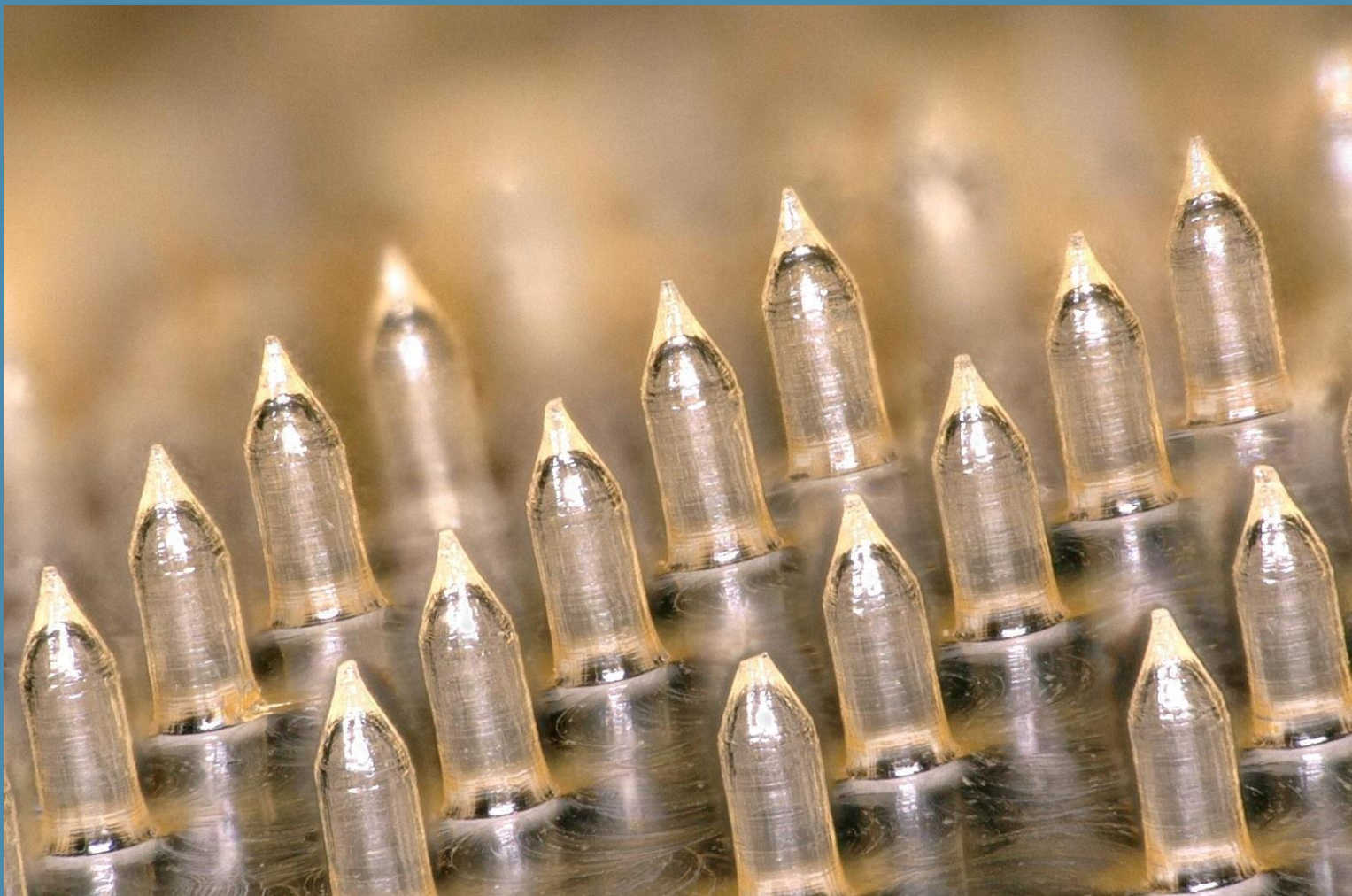
Picture of the glass/silicon chips



Picture of the prototype

RELATED PUBLICATIONS:

[1] Ricoul, F., et al., Integrated System for the Rapid Polycyclic Aromatic Hydrocarbons Extraction from Aqueous Samples and Their Consecutive Thermal Desorption Prior to Gas Chromatography Analysis. American Journal of Analytical Chemistry, 2020. 11(2): p. 75-89.



O2

BIOMATERIALS AND SURFACES

- **Bioresorbable microneedles**
- **Encapsulation of human islets**
- **Fighting against biocontamination in ISS**

A facile fabrication of dissolving microneedles containing 5-aminolevulinic acid

RESEARCH TOPIC:

5-Aminolevulinic acid, Hyaluronic acid, Dissolving microneedles, solvent casting molding method, photodynamic therapy

AUTHORS:

M. Champeau¹, D. Jary, L. Mortier¹, S. Mordon¹, **S. Vignoud**

Photodynamic therapy induced by protoporphyrin IX (PpIX) is widely used to treat precancerous skin lesions. The penetration depth of the prodrug 5-aminolevulinic acid (5-ALA) using topical application is currently limited, which hampers the production of PpIX in deep seated lesions. To enhance 5-ALA delivery in deep skin layers, a soluble microneedles patch (MN-patch) containing 5-ALA has been successfully developed by using a fast solvent casting molding method. The shape, number and height of the needles have been designed according to the medical application and the mechanical strain necessary for skin insertion. Overall, the MN-patch can be a promising technique to enhance 5-ALA penetration and produce PpIX in deeper skin lesions.

SCIENTIFIC COLLABORATIONS: ¹ Univ. Lille, Inserm, CHU Lille, U1189 – ONCO-THAI – Assisted Laser Therapy for Oncology, F-59000 Lille, France

Context and Challenges

Photodynamic therapy (PDT) induced by protoporphyrin IX (PpIX) has been widely used in dermatological practices such as treatment of skin cancers. Clearance rate depends on different factors such as light irradiation, skin oxygenation and drug penetration. The poor penetration of 5-aminolevulinic acid (5-ALA) with topical application restrains the production of PpIX which could restrict PDT outcomes⁽¹⁾. In this study, we aimed at exploring the properties of Hyaluronic acid (HA) based microneedles with 5-ALA embedded in the polymer matrix. Since 5-ALA and HA were mixed together, interactions between the polymer and the drug was carefully studied and 5-ALA stability was investigated for different time periods. Furthermore, microneedles shape was designed as a “pencil-tip” to confer them strong mechanical properties to penetrate the cutaneous lesions. As all basal cell carcinomas do not extend to the same depth, different heights of needles were processed and characterized. Moreover MN-patch could be loaded with large different amounts of 5-ALA which offer different dosages. The characteristics of the MN-patch (compression, insertion tests, dissolution rate) have been conducted to evaluate whether this strategy is a promising approach to enhance PDT outcomes on deep skin cancers.

Main Results

Microneedle patches were fabricated by a single step of solvent casting molding method⁽²⁾. The manufacture way very easy, low cost and relevant which would allow a facile translation of this technology. Two heights were developed: 400 μm and 750 μm. Each microneedle patch could contain 20 mg or 100 mg of 5-ALA consequently a single application is sufficient to deliver the amount required for PDT treatment. 5-ALA stability was followed to ensure that there was no dimerization in PY and exhibited a very good stability in the MN-patch even 5 months after manufacturing. Mechanical compression tests were performed and the different kinds of MN-patches do not present a significant deformation when the force applied was not superior to 0.3 N/needle. These results suggested that MN-

patch had efficient strength to pierce the stratum corneum. Indeed, ex vivo rat skin was well perforated due to microneedle application since micropores were created. A clear penetration profile was distinguishable on phantom skin with depth penetration of 374 10 μm ± m and 594 61 μm ± m for respectively MNI and MNt. Dissolution rate was estimated on ex vivo rat skin demonstrating that the microneedles were dissolved within 45 min and the support started to dissolve after 60 min.

Perspectives

According to the previous results, MN-patches might be a promising technique to enhance 5-ALA penetration and produce PpIX in deeper skin lesions. In the very near future, in vivo tests on rats suffering from precancerous skin lesions will be carried out to show microneedle possible benefit during PDT treatment.



Digital microscopy images of MN-patch fabricated by solvent casting molding method

RELATED PUBLICATIONS:

- [1] M. Champeau, S. Vignoud, L. Mortier and S. Mordon, Photodynamic therapy for skin cancer: how to enhance drug penetration ?, Journal of photochemistry and photobiology b-biology, 197, DOI: 10.1016/j.jphotobiol.2019.111544, (2019)
- [2] M. Champeau, J. Dorothee, L. Mortier, S. Mordon and S. Vignoud, A facile fabrication of dissolving microneedles containing 5-aminolevulinic acid. International journal of pharmaceutics. Vol 586, DOI: 10.1016/j.ijpharm.2020.119554 (2020)
- [3] M. Seon-lutz, AC Couffin, S. Vignoud, G. Schlatter and A. Hebraud, Electrospinning in water and in situ crosslinking of hyaluronic acid/cyclodextrin nanofibers: Towards wound dressing with controlled drug release. Carbohydrates, Polymers, 207, DOI: 10.1016/j.carbpol.2018.11.085 (2019)

Improved human islets' viability and functionality with mesenchymal stem cells and arg-gly-asp tripeptides supplementation of alginate micro-encapsulated islets in vitro

RESEARCH TOPIC:

Diabetes mellitus type 1; Islets of Langerhans transplantation; Mesenchymal stem cells; Microfluidics; Micro -Capsules; Bioprocessing

AUTHORS:

C. Laporte¹, E. Tubbs¹, M. Pierron, A. Gallego², A. Moisan³, F. Lamarche¹, T. Lozano², A. Hernandez², C. Cottet-Rousselle¹, A.S. Gauchez⁴, V. Persoons³, C. Fontelaye, F. Boizot, S. Lablanche^{1,5}, F. Rivera, **F. Bottausci**

Islets transplantation for a wide number of type 1 diabetes patients is compromised by severe adverse events related to the immunosuppressant therapy required for allogenic islet transplantation. In this context, microencapsulation offers the prospects of immunosuppressive-free therapy by physically isolating islets from the immune system. However, current biomaterials need to be optimized to: improve biocompatibility, guaranty viability and functionality of the graft, and prevent fibrosis overgrowth around the capsule in vivo. Here, in the frame of the European project BIOCAPAN, we investigated the effect of supplementing reference M-rich alginate microcapsules with MSCs and RGD-G rich alginate on human pancreatic islets viability and functionality.

SCIENTIFIC COLLABORATIONS: ¹LBFA (INSERM U1055) and BEeSy), UGA, Grenoble, France, ²Nanoimmunotech S.L, Vigo, Pontevedra, Spain, ³Cell Therapy and Engineering Unit, EFS Saint Ismier, France, Grenoble Alps University Hospital, Grenoble, France

Context and Challenges

Pancreatic islet transplantation is a promising cell therapy for the treatment of type 1 diabetes. To date, according to the Collaborative Islets Transplant Registry more than 1000 patients have been transplanted within clinical trials around the world [1]. The main challenge is to avoid immunosuppressive treatments [2]. To address this challenge, islets' immuno-isolation in alginate microcapsules is a promising approach [3] allowing to reduce or even to avoid the use of immunosuppressants [4].

Main Results

We designed an innovative microfluidic cartridge and developed a barium-free biocompatible composite microcapsule of RGD G-rich alginate in combination with MSCs. It is the first time that such high viscosity mix of biomaterials (12.7 Pa s) is processed within microfluidic devices for cell encapsulation [5] without deleterious effect on cells.

This study demonstrates that compared to encapsulation using conventional alginate, supplementing the capsule composition with both MSCs and RGD-G-rich alginate is beneficial for human pancreatic islets viability and functionality (cf Fig 1&2).

This microcapsule composition improves the islets encapsulation outcomes in vitro and appears as a new promising cell therapy product for type I diabetes.

Perspectives

In vitro results suggest that RGD-G rich alginate and MSCs improve the outcome of islets encapsulation. The final validation of this combination of biomaterials will have to be further confirmed in vivo.

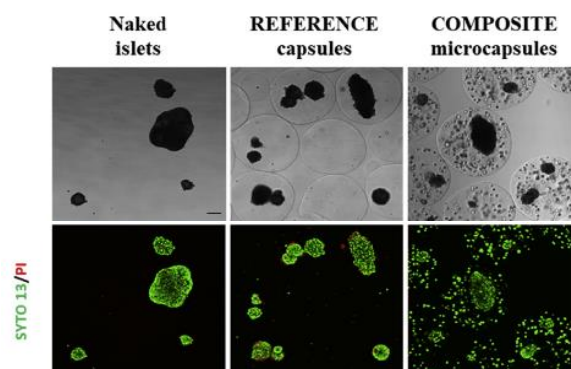


Fig1:Compared analysis of microcapsules.

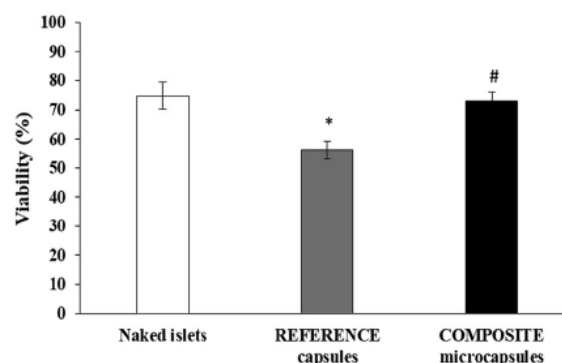


Fig2:Encapsulated human islets viability.

RELATED PUBLICATIONS:

- [1] T.C.C.C.a. Investigators, CITR 0th Annual Report, Internet, The CITR Coordinating Center and Investigators, 2016.
- [2] M.M. Hafiz et al., Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation, *Transplantation* 80 (2005) 1718e1728.
- [3] S. Darquy, G. Reach, Immunoisolation of pancreatic B cells by microencapsulation. An in vitro study, *Diabetologia* 28 (1985) 776e780.
- [4] R.B. Elliott et al., Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation, *Xenotransplantation* 14 (2007) 157e161.
- [5] U. Průše et al., Comparison of different technologies for alginate beads production, *Chem. Pap.- Slovak Acad. Sci.* 62 (2008) 364e374

Fighting against biocontamination in the International Space Station (ISS): a surface chemistry approach studied during the MATISS experiment of the Proxima Mission

RESEARCH TOPIC:

Surface Chemistry ; Hydrophobic coatings ; Biocontamination ; Microbiology ; Long-duration human spaceflight

AUTHORS:

Pierre Marcoux, Laurence Lemelle¹, Christophe Placé¹, Lucie Campagnolo², Cécile Thévenot², Sébastien Barde³, Jérémie Teisseire⁴, **Guillaume Nonglaton**

MATISS is one of the 7 experiments selected by CNES for the PROXIMA mission carried out by Thomas Pesquet in the ISS from November 2016 to May 2017. From a perspective of long-duration human spaceflights, biological contamination could have significant impacts for the health of the crew and the biodegradation of the equipment. Currently, to fight against this biocontamination, astronauts spend 8 to 10% of their time cleaning. In this context, the MATISS project aims to demonstrate that surfaces with hydrophobic properties could be a possible and applicable response at the scale of a spacecraft. The object of this study was to develop a rack that could allow exposure of functionalized glass coverslips for more than 6 months in the ISS's atmosphere.

SCIENTIFIC COLLABORATIONS: ¹ENS de Lyon, CNRS, Lyon (FR), ²MEDES-IMPS for CADMOS, Toulouse (FR), ³CNES, Toulouse (FR), ⁴Surface du Verre et Interface, UMR CNRS/Saint-Gobain, Aubervilliers (FR)

Context and Challenges

From the perspective of long-duration manned flights, biological contamination could have significant impacts on the health of the crew and the biodegradation of equipment. Currently, to fight against this biocontamination, astronauts spend 8 to 10% of their time cleaning. The main source of pathogens comes from humans and micro-organisms are most of the time expelled through the mouth or nose in the form of an aerosol. In this context, the aim of the MATISS project, one of the seven experiments selected by CNES for the PROXIMA mission of Thomas Pesquet, in the ISS from November 2016 to May 2017, was to study the effect of hydrophobic surfaces to limit the adhesion of bacteria by preventing these small water droplets from attaching to surfaces. This strategy has several advantages: it does not use toxic products, it is compatible with a large number of materials and is easily transferable to industry. Thus this solution could be a possible and applicable response at the scale of a spacecraft.

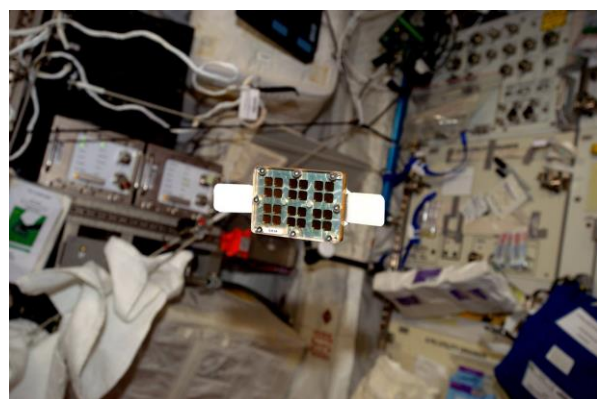
Main Results

To verify this hypothesis, glass substrates were functionalized by LETI using standardized and scalable methods for depositing FOTS, SiOCH and Parylene hydrophobic materials. An innovative spatializable sample holder was designed by ENS de Lyon to protect glass surfaces from breakage during take-off and landing and to sample the atmosphere of the ISS. Four holders containing the substrates were exposed for six months, in three different places of the Columbus module. Back on Earth, a systematic analysis by microscope image processing was carried out under confinement so as not to contaminate the surfaces with terrestrial flora. First, the average density was estimated to be 2 particles per mm², leading to a significant coverage-rate (>2% in 20 years). In this contamination, we can distinguish large particles corresponding to textile fibers, dust and many small particles similar to bacteria. When we analyze these small particles (<10 μm²) in detail, we see a clear impact of the FOTS coating on their number. Microbiological samples were analyzed at LETI. Four

species of bacteria have been identified, all from the skin or dust microbiota.

Perspectives

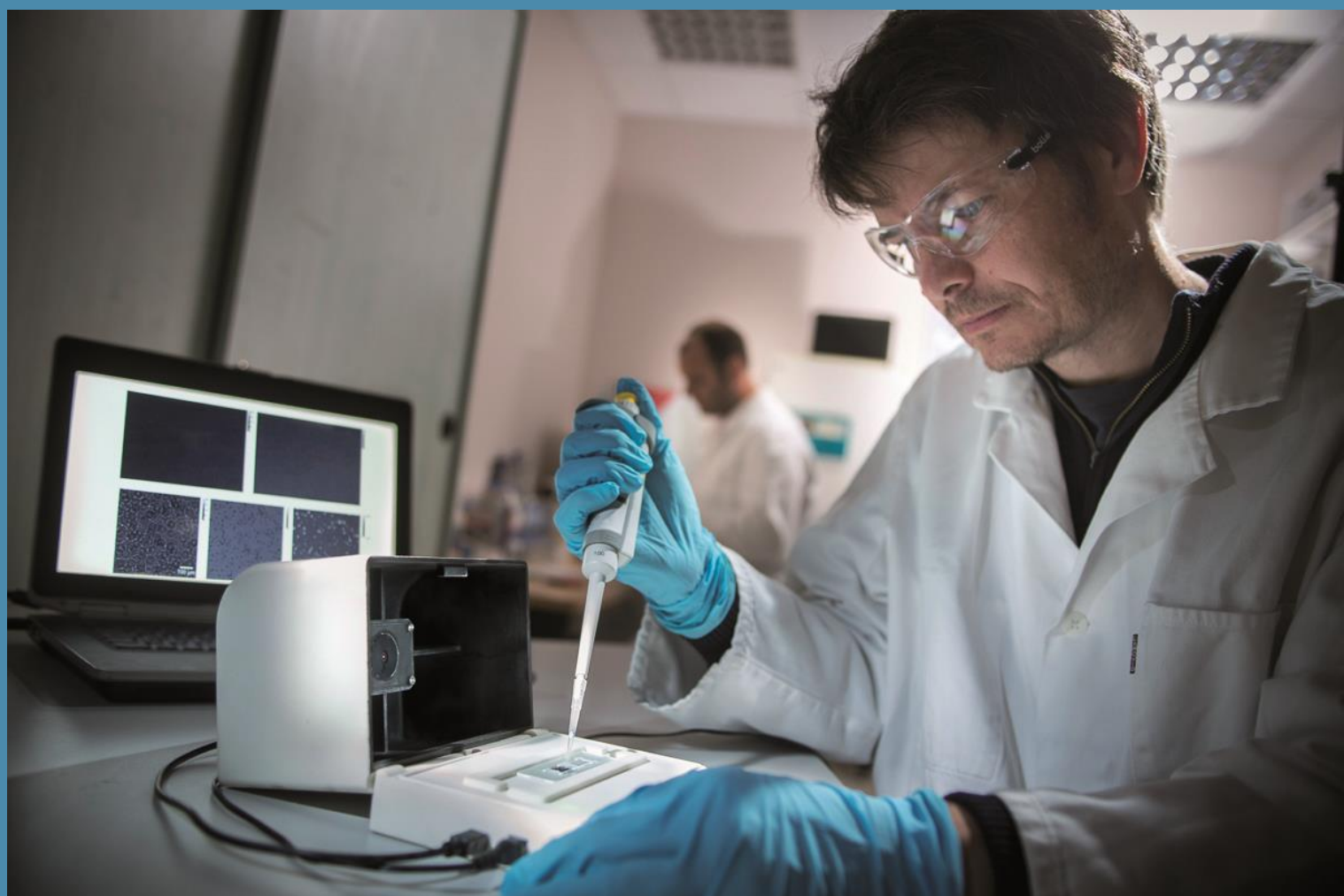
After these encouraging results, CNES wished to continue funding studies on this subject. Since then, additional experiments have been spatialized: in 2018 to study the adsorption kinetics of particles and in 2019 to study the hydrophobic-hydrophilic gradient effect. The results are being analyzed. In 2021, we will test a support compatible with other characterization techniques, for example Raman to test other innovative coating limiting microbial spread (superhydrophilic or bacteriostatic). Before then testing a smarter holder incorporating sensors that would allow monitoring of biocontamination directly in the ISS.



Photograph of the sample holder.

RELATED PUBLICATIONS:

[1] Lemelle, L.; Campagnolo, L.; Mottin, E.; Le Tourneau, D.; Garre, E.; Marcoux, P.; Thévenot, C.; Maillot, A.; Barde, S.; Teisseire, J.; Nonglaton, G.; Place, C., Towards a passive limitation of particle surface contamination in the Columbus module (ISS) during the MATISS experiment of the Proxima Mission. *npj Microgravity* 2020, 6 (1), 29.



O3

HOLOGRAPHIC IMAGING

- **Deep-learning in holographic imaging**
- **Phage susceptibility testing**
- **Ultradian rhythm observation**
- **Fluorescent lensfree smart microscope**

Alternation of inverse problem approach and deep learning for lens-free microscopy image Reconstruction

RESEARCH TOPIC:

Lensfree microscopy, diffraction, reconstruction, inverse problem, Deep-Learning

AUTHORS:

L. Hervé, D. Kraemer, O. Cioni, O. Mandula, M. Menneteau, S. Morales, **C. Allier**

A lens-free microscope is a simple imaging device performing in-line holographic measurements. In the absence of focusing optics, results are computed via a reconstruction algorithm which solves the inverse problem. However, unsatisfactory results were obtained especially when phase wrapping errors occur for thick samples as cells in suspension and cells undergoing mitosis. Such problems limits the application of lens-free microscopy. We address this issue by a novel approach with alternations between optimization and deep learning steps. We demonstrate the applicability of this approach by treating the challenging case of cell in suspension at large densities that cannot be tackled by conventional means without phase wrapping errors.

Context and Challenges

A lens-free microscope is a minimalist setup for in-line holography. It allows to observe unstained and transparent samples by recording the intensity of its far diffraction. Image of the sample is retrieved through computation; the conventional computational methods is based on an inverse problem approaches but if the optical thickness of the sample exceeds $\lambda/2$, phase wrapping errors occur that lead to largely incorrect reconstructed images (see Fig.1, left column). This is the case for cells in suspension and cells undergoing mitosis. This problem considerably limits the application of lens-free microscopy in live cell imaging.

Main Results

We tackle wrapping error by the use of a convolutional neural network (CNN) specifically trained to the task of unwrapping results of a traditional reconstruction. The neural network used is very simple by its architectures, 20 inner layers with 32 features, 3x3 convolution kernel and basic ReLU activation functions and is trained on realistic simulations so as the ground truth is known exactly. These simulations account for diffraction modelling, partial source coherence and noise corruption.

Since deep-learning is a data-driven approach, it can deliver an outcome very close to the reality, however with an intrinsic lack of confidence due to known problems of hallucination, generalization and adversarial fragility. To address those issues, we propose an approach that alternates between the deep learning and inverse problem approach, namely a convolution approach (see results on Fig.1-left), then the application of the CNN and a second inverse problem approach which returns the final result (see Fig.1-right). We demonstrate the applicability of the proposed alternation approach in solving the phase wrapping problem that occurs in lens-free holographic reconstruction on the difficult case of observations of cells in suspension for various density of cells (See Fig.1, top row for low density and Fig.1, bottom row for high density). It has first been developed and assessed on simulations and next

validated on experimental acquisitions. Quantification of results were obtained on simulations with good agreement for samples with density as high as 60 million cells/mL. Excellent quality of reconstruction was validated by comparison with fluorescence microscope modality on a stained sample for samples with various cell density.

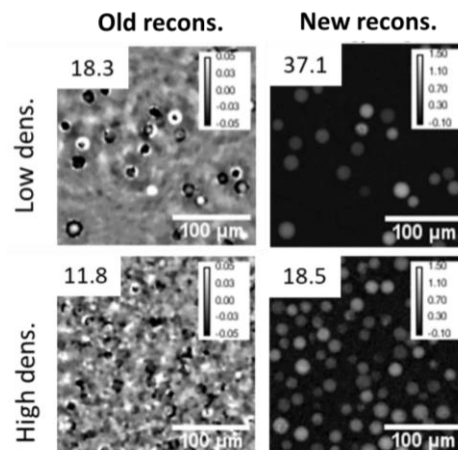


Fig 1: old (left) vs new (right) reconstruction

Perspectives

Introduction of deep-learning strategy in the lens-free data processing flow opens new perspectives. We demonstrated its use for improving reconstruction on the unsolved case of cells in suspension. It is envisioned that it can be also used to accelerate the reconstruction process, to pre-process data and to perform post-processing tasks such as segmentation, quantification or to foresee future of time-lapse acquisitions.

RELATED PUBLICATIONS:

[1] L. Hervé, D. C. A. Kraemer, O. Cioni, O. Mandula, M. Menneteau, S. Morales and C. Allier, Alternation of inverse problem approach and deep learning for lens-free microscopy image reconstruction, Nature Science Report 10(1), 2020, 20207 doi: 10.1038/s41598-020-76411-9.

Phage susceptibility testing with lensless imaging technique

RESEARCH TOPIC:

Phage therapy uses viruses, called phages, which specifically infect and destroy bacteria without impact on human cells

AUTHORS:

Prisca Perlemaire, Emmanuel Picard, Emmanuel Hadji, Marc Zelsmann¹, Alexis Maire¹, Eric Lacot², **Pierre R. Marcoux**

Based on the use of a personalized cocktail composed of highly specific bacterial viruses, phage therapy relies on a range of tests on agar media to determine the most active phages on a given bacterial target (phage susceptibility testing), or to isolate new lytic phages from an environmental sample (enrichment of phage banks). However, these culture-based techniques are still solely interpreted through direct visual detection of plaques. Our main objective is to investigate computer-assisted methods in order to ease and accelerate diagnosis in phage therapy, but also to study phage plaque growth kinetics. For this purpose, we designed a custom wide-field lensless imaging device, which allows continuous monitoring over a very large area sensor (3.3 cm²).

SCIENTIFIC COLLABORATIONS: ¹ University Grenoble Alpes, CNRS, LTM - Micro and Nanotechnologies for Health, Grenoble (FR), ² University Grenoble Alpes, CNRS UMR 5588, Laboratoire Interdisciplinaire de Physique, St-Martin d'Hères (FR)

Context and Challenges

The lensless imaging approach has already been implemented to detect and count viral plaques and study eukaryote cells death dynamics in plaques using eukaryotic viruses with a 24-mm² FoV. However, the authors do not provide results on the plaque growth kinetics. We therefore suggest using a similar approach but with a wider 3.3-cm² FoV CMOS sensor to study phage plaques. Here we report computer-assisted detection and counting of phage plaques as well as simultaneous measurement of the growth kinetics of nineteen plaques from the same sample. Finally, we propose a lensless imaging technique to monitor phage-resistance selection through imaging of the interior of phage plaques.

Main Results

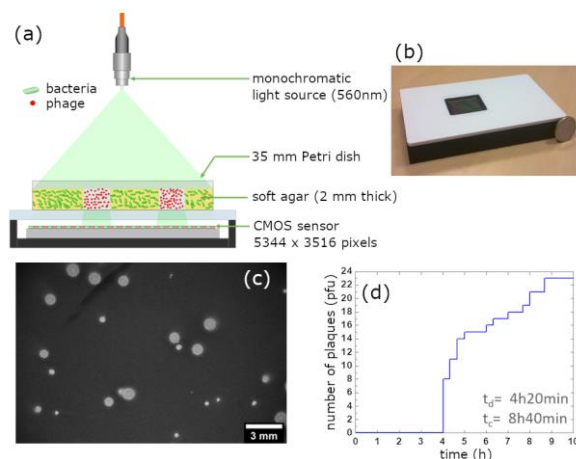
In this work, [1,2] we report the use of a custom lensless device, over a 3.3 cm² area sensor (figures a and b), for the continuous monitoring of phage lysis plaques (figure c). Leveraging wide-field lensless imaging, we performed a computer-assisted detection and counting of plaques that allowed detection of bacterial susceptibility to phages within 4 hr 20 and accurate estimation of infectious titer in only 8 hr 40 min (figure d).

Moreover, by investigating the growth rates of 19 isolated plaques we confirmed the previously observed correlation between bacterial density and phage diffusion in the soft-agar layer. However, lensless imaging allowed us to identify three very distinct phases during the growth of a plaque independent of the size of the final plaque. Moreover, we demonstrated that plaques harboring the smaller sizes at the end of the experiment were always associated with a delayed onset of growth in phase I as well as lower growth rates during the three phases.

In addition, we showed that lensless imaging could be a powerful tool to screen the emergence of phage-resistant bacteria by imaging bacterial microcolonies within plaques. Additional experiments are needed to evaluate whether this technique could lead to an estimation of the phage-resistance frequency.

Perspectives

Future work will also focus on the development of an algorithm allowing morphological classification of plaques (and therefore phages) according to their plaque growth rates but also their morphotypes (though we did not discuss this aspect here). Moreover, we think that lensless imaging could reveal a very important approach to study phage-antibiotic synergy (PAS). Indeed, it has for instance been reported that the inclusion of an antibiotic in the agar layer leads to significantly larger plaques, making visible plaques that would otherwise be invisible to the naked eye. Finally, a setup based on multiple image sensors working simultaneously is currently under development, which will increase the field of view and consequently the amount of data acquired at the same time.



Lensless monitoring of a soft agar assay

RELATED PUBLICATIONS:

- [1] Phage susceptibility testing and infectious titer determination through wide-field lensless monitoring of phage plaque growth, Perlemaire P., Marcoux P. R., Picard E., Hadji E., Zelsmann M., Mugnier G., Marchet A., Resch G., Lacot E., PLOS ONE, 2021, doi: 10.1371/journal.pone.0248917
 [2] Phage susceptibility testing with lensless imaging technique (Conference Presentation), Perlemaire P., Picard E., Hadji E., Zelsmann M., Maire A., Lacot E., Marcoux P. R. Proc. SPIE 11223, Photonic Diagnosis, Monitoring, Prevention, and Treatment of Infections and Inflammatory Diseases 2020, 112230D (10 March 2020); doi: 10.1117/12.2543785

A new ultradian rhythm in mammalian cell dry mass observed by holography

RESEARCH TOPIC:

Fundamental biology, quantitative phase imaging, lens-free microscopy

AUTHORS:

Lamya Ghenim, Cédric Allier, Patricia Obeid, Lionel Hervé, Jean-Yves Fortin¹, Maxim Balakirev & **XavierGidrol**

We have discovered a new 4 h ultradian rhythm that occurs during the interphase of the cell cycle in a wide range of individual mammalian cells, including both primary and transformed cells. The rhythm was detected by holographic lens-free microscopy that follows the histories of the dry mass of thousands of single live cells simultaneously, each at a resolution of five minutes. It was vital that the rhythm was observed in inherently heterogeneous cell populations, thus eliminating synchronization and labeling bias. We show that the amplitude of the fundamental frequency provides a way to quantify the effects of, chemical reagents on cells, thus shedding light on its mechanism. The rhythm is suppressed by proteostasis disruptors and is detected only in proliferating cells, suggesting that it represents a massive degradation and re-synthesis of protein every 4 h in growing cells.

SCIENTIFIC COLLABORATIONS: ¹ Laboratoire de Physique et Chimie Théoriques CNRS UMR7019, Université de Lorraine, 1 Boulevard des Aiguillettes, BP 70239, 54506 Vandoeuvre-lès-Nancy Cedex, France

Context and Challenges

To detect periodicity in cell behavior, the expression of specific genes is usually measured by biochemical sampling or by using specific luminescent markers. Because the individual rhythms in a cell population are out of phase, they need to be synchronized by using an external stimulus. As a result, the most common approaches to studying biological clocks have a poor time resolution and may suffer from artifacts introduced by labeling or synchronization. On the other hand, our ability to study single-cell dynamics in asynchronous culture has been limited by the availability of quantitative phase imaging techniques for simultaneous, time-resolved, single-cell data acquisition from thousands of cells in parallel. Large population sampling is required to overcome the complex "noisy" behavior of a single cell and determine the standard characteristics of cell dynamics.

Main Results

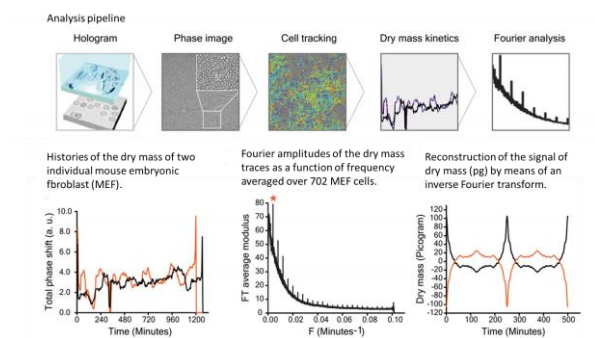
Recently, we have described a lens-free microscopy technique that allows real-time measurements of dry mass with a precision of approximately 35 pg [1,2]. The dry mass of the cell is calculated by integrating the measured phase shift of the diffracted light over the whole area of the cell. Compared to conventional optical methods, lens-free microscopy provides a unique way to track thousands of live cells in real time and with a large field of view (30 mm²) without any labeling or synchronization. In the present work, we use this technique to demonstrate periodic oscillations in the cell dry mass.

We measured dry mass history in mouse embryonic fibroblasts (MEFs) revealing a dominant frequency of 0.004 min⁻¹, and higher harmonics, superimposed on 1/f noise. Reconstruction of the signal by applying an inverse Fourier transform to exclude the noise, reveals periodic symmetric spikes, 30 min wide, every 4.17 h. In HeLa, U2OS, MEF, CHO-K1, NHEK and NHDF cells we also observed the same principal frequency of 0.004 min⁻¹. The amplitude of the dry mass change was approximately 100 pg in MEF cells; three times the method

precision. Examination of experimental conditions and analysis of a random dataset ruled out artifacts

Perspectives

It should be noted that our analysis cannot determine whether the dry mass is rising during the pulses as a result of increased synthesis or is dropping because of accelerated degradation. Even though both possibilities remain, the second hypothesis seems more thermodynamically likely. Further studies are thus needed to draw a conclusion. Another aspect of the 4 h rhythm is that it is linked to the cell cycle. The 4 h rhythm we described may reflect the same time keeping mechanism that regulates cell growth during interphase and dictates cell cycle duration. Further studies are thus needed to correlate the rhythm measurement with cell cycle measurement, e.g. using FUCCI fluorescent markers.



RELATED PUBLICATIONS:

- [1] Allier, C., Morel, S., Vincent, R., Ghenim, L., Navarro, F., Menneteau, M., ... & Usson, Y. (2017). Imaging of dense cell cultures by multiwavelength lens-free video microscopy. *Cytometry Part A*, 91(5), 433-442.
 [2] Allier, C., Hervé, L., Mandula, O., Blandin, P., Usson, Y., Savatier, J., ... & Morales, S. (2019). Quantitative phase imaging of adherent mammalian cells: a comparative study. *Biomedical optics express*, 10(6), 2768-2783.

Smart Microscopy: Automated lens-free, fluorescent microscopy to study cell populations

RESEARCH TOPIC:

Smart bi-modal imaging (fluorescence and phase) allowing automated single cell analysis of rare events while studying the entire cell population

AUTHORS:

D.C. Kraemer, C. Allier, L. Hervé, K. Padmanabhan¹, A. Foudi², S. Morales

Obtaining large quantities of imaging data no longer poses a limitation to scientific research. We are however faced with a challenge to obtain specific data, in particular of infrequently occurring events in large cell populations. We therefore propose the concept of a smart microscope, which performs automatic detection of the occurrence of an event and thereupon directs the imaging to the exact position. We focused on performing automatized detection of mitosis in large cell populations. Mitosis detection is based on an AI prediction algorithm, which detects mitosis on reconstructed lens-free images? allowing us to start the imaging process at the respective positions more than one hour in advance of mitosis occurrence.

SCIENTIFIC COLLABORATIONS: ¹ ENS, Institut Génomique Fonctionnelle de Lyon, UMR 5242 IGFL, France, ² INSERM CR1, ATIP-Avenir group of CNRS and Inserm, UMR-S 935, Villejuif, France

Context and Challenges

Quantitative lens-free microscopy is a minimalist technique where diffraction patterns are acquired and reconstructed with a numerical algorithm [1]. This label-free imaging approach allows to measure cell parameters, such as the cell dry mass over a large field of view. To automatize the image acquisition process, we designed a Convolutional Neural Network based decision algorithm that automatically detects the mitosis events and guides the fluorescent module to the respective position.

Main Results

We combine lens-free quantitative phase imaging and fluorescence microscopy (fig. 1.a/b). Lens-free allows imaging the entire cell-population (fig 1.d). With fluorescence, we image specific events at higher resolution (fig. 1.e). Location of the fluorescence acquisition is determined by the detection of mitosis events through a CNN based decision algorithm applied on lens-free OPD images. X and Y positions of the cells that are predicted to undergo mitosis followed in time-lapse fluorescence acquisitions, between two lens-free images.

Perspectives

The algorithm - applied to different cell types - is used to perform in advance predictions: mitosis is detected 1h before it occurs (fig.2) [2]. By combining both modalities, we can obtain population/single cell measurements. The imaging acquisition at two different length scales is automatically performed by the microscope. This new smart microscope will facilitate image collection and analysis.

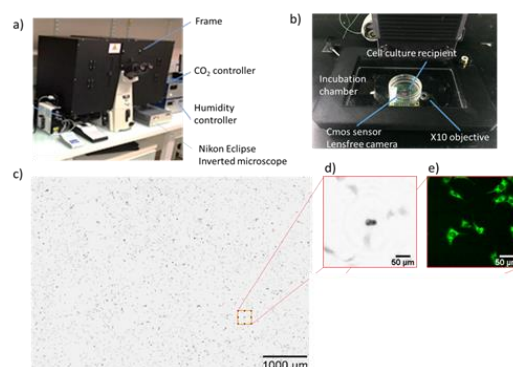


Fig. 1: Smart microscope (phase and fluorescence)

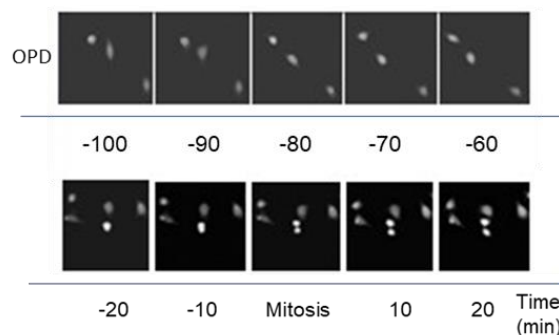


Fig. 2: Mitosis predicted 1h before cell division

RELATED PUBLICATIONS:

[1] Alternation of inverse problem approach and deep learning for lens-free microscopy image reconstruction - L. Hervé, D. C. A. Kraemer, O. Cioni, O. Mandula, M. Menneteau, S. Morales & C. Allier - Nature Scientific Reports - <https://doi.org/10.1038/s41598-020-76411-9>.

[2] Automated lens-free, fluorescent microscopy to study cell populations - Kraemer, D. C., Allier, C., Hervé, L., Padmanabhan, K., Foudi, A., Morales, S. - SPIE Photonics Europe, Mars 2020



04

**IN VIVO AND
WEARABLE MEDICAL
DEVICES**

- **Closed-loop adaptive BCI**
- **Wearable capnometer**
- **Noninvasive Monitoring of Deep Tissue Oxygenation**
- **Motor cortex localization using MEG**
- **Hypothermia for glioblastome therapy**
- **Clinical trial of CMOS-based DRS system**

Evaluation criteria for closed-loop adaptive dynamic discrete-continuous brain-computer interfaces: clinical study case with tetraplegic patient

RESEARCH TOPIC:

Brain-computer interface performance metrics

AUTHORS:

F. Martel, T. Dupuy, A. Moly, S. Chabardès¹, T. Aksenova

Quantifying and evaluating properly the performances is a critical issue in BCI experiments. The choice of the most adapted metrics can be difficult because they are specific to the experimental paradigm, task control, and data. In the current study evaluation criteria for closed-loop adaptive dynamic and hierarchical discrete-continuous brain-computer interfaces are examined. The challenges such as imbalanced multi-class bias for discrete decoding (classification), online computing cost and dynamic analysis of results in time are considered. There are two main objectives of the study: identifying the best suited performances metrics according to the requirements and combining several levels of evaluation in the whole BCI system (decoder and patient oriented). The main novelty of the study is the combination of several common metrics with new temporal metrics and a time dynamic approach, which allows adequately reflect the performance of adaptive dynamic and hierarchical discrete-continuous brain-computer interfaces BCI systems. Additional response time and blocking error patterns reveal complementary information for BCI system performance evaluation. Developed criteria are applied to performance evaluation of 8-dimensional exoskeleton control by tetraplegic patient for both decoder and user-centered metrics.

SCIENTIFIC COLLABORATIONS: ¹ Centre Hospitalier Universitaire, Grenoble Alpes F-38700, La Tronche

Context and Challenges

Brain-Computer Interface (BCI) is a system that aims to establish a direct communication between brain and external world, by translating brain neuronal activity signals into control commands sent to external effectors. It was successfully demonstrated that it is possible for severely motor-impaired patients to control by mind such complex effectors, as an exoskeleton. To do this, the brain neuronal activity decoder first needs to be trained using machine learning approaches. More efficient adaptive decoders allow being trained while BCI system being used, and are well suited to overcome the data non-stationarity issue. A critical question for BCI technology is to be able to quantify properly BCI performance. In training stage, it plays a critical role in achieving the optimal decoding by identifying the optimal model parameters. For adaptive decoders, BCI performance should be evaluated online and in real time. BCI decoder may include the discrete decoding (binary or multiclass classification of mental tasks) and continuous decoding (regression to predict the direction or the movement trajectory). Various performance criteria were proposed for discrete and continuous decoding in BCI studies and other studies. The goal of the article is to propose the online performance measurement system for the hybrid BCIs (combination of discrete and continuous decoding) and, in the same time, dynamic BCIs, which take into account temporal dependences in the data. The context of the article is the clinical protocol "BCI and tetraplegia" in progress.

Main Results

The state of the art performance measurement indexes are analyzed considering the particular requirements and the restrictions of adaptive learning of the BCI discrete/continuous dynamic decoders. Performance measurement system for online adaptive hybrid (discrete/continuous) dynamic BCI decoder is developed. This system combines different levels of evaluation to obtain a complementary and complete performance assessment, meeting the requirements fixed by

the experimental setup. The novelty is the addition of an error dynamics analysis to the discrete decoder evaluation. Performance measurement system was tested offline using a unique data base collected during the ongoing clinical trial "BCI and tetraplegia" conducted at CLINATEC.

Perspectives

The proposed BCI performance measurement system will be completed. In particular, the measure of distinctiveness and stability is at the moment limited to the discrete part. Adapting it to continuous movements requires supplementary study, but could lead to interesting results on the differentiation of the directions of movements for a given limb. Additionally, the decoder convergence indexes will be added to the evaluation system. The next step is the integrating of the performance evaluation system to BCI clinical platform to be used in real time BCI experiments. Online performance and convergence evaluation during real-time BCI experiments while the model is trained will allow developing an automated experiment supervisor (an adaptive task designer) capable of estimating the learning potential of the model, proposing an optimal adaptive training protocol and thus automatically triggering and stopping the model update according to the current measured performances.

RELATED PUBLICATIONS:

[1] F. Martel, T. Dupuy, A. Moly, S. Chabardès and T. Aksenova, "Evaluation criteria for closed-loop adaptive dynamic discrete-continuous brain-computer interfaces: clinical study case with tetraplegic patient.," 2020 International Joint Conference on Neural Networks (IJCNN), 2020, pp. 1-10, doi: 10.1109/IJCNN48605.2020.9207243.

[2] AL Benabid et al. "An exoskeleton controlled by an epidural wireless brain-machine interface in a tetraplegic patient: a proof-of-concept demonstration". *Lancet Neurol* 2019 Dec;18(12):1112-1122. doi: 10.1016/S1474-4422(19)30321-7

Evaluation in Healthy Subjects of a Transcutaneous Carbon Dioxide Monitoring Wristband during Hypo and Hypercapnia Conditions

RESEARCH TOPIC:

Wearable medical device, Capnometry, Signal processing, Respiratory disease, Health monitoring, Homecare, Clinical evaluation

AUTHORS:

S. Gharbi, A. Koenig, M.-P. Comşa, M. Accensi, H. Grateau, A. Ghaith¹, S. Chacaroun¹, S. Doutreleau¹, S. Vergès¹, **P. Grangeat**

The development of wearable devices for healthcare monitoring is of primary interest, in particular for homecare applications. But it is challenging to develop an evaluation framework to test and optimize such a device by following a non-invasive protocol. As well established reference devices do exist for capnometry, we propose a protocol to evaluate and compare the performance of the transcutaneous carbon dioxide monitoring wristband that we develop. We present this protocol, the signal processing pipeline, the data analysis based on signal alignment and intercorrelation study, and the first results on a cohort of 13 healthy subjects. This test allows demonstrating the influence of the device response time and of the carbon dioxide content in the ambient air.

SCIENTIFIC COLLABORATIONS: ¹ Univ. Grenoble Alpes, Laboratoire HP2 (U 1042), CHU Grenoble Alpes, INSERM, Hôpital Sud, F-38434 Echirolles, France

Context and Challenges

Measuring the carbon dioxide pressure in the blood is of primary interest for several clinical applications, in particular for monitoring acute and chronic respiratory failure. We are investigating a wearable technology based on a wristband using a dual wavelength Non-Dispersive InfraRed sensing [1] for a transcutaneous measurement at the forearm level.

Main Results

We propose here a non-invasive protocol combining hypocapnia and hypercapnia conditions on a subject at rest (Fig.1). This follows a protocol developed in the team of S. Vergès [2] using a facial mask connected to a gas-mixing device (Altitrainer®). The CAPNO device was placed at the forearm level and compared with reference devices, a SENTEC electrochemical device placed nearby, and a CORTEX MetaMax 3B device in the exhaled air.

The signal processing pipeline defines how to compute the carbon dioxide pressure [1,3]. We present on Fig.2 **Erreur ! Source du renvoi introuvable.** the aligned centered normalized signals for CAPNO (blue), SENTEC (green) and CORTEX (red), and the P_{CO_2} template (black). A strong intercorrelation greater than 0.8 has been observed in 5 healthy subjects out of 13 and factors influencing the intercorrelation have been suggested.

Perspectives

This clinical evaluation has established the proof-of-concept of this innovative wristband for monitoring respiratory gas in a homecare environment and the main improvements needed in terms of sensitivity, response time, and interferences from other gas sources.



Fig. 1: The clinical experimental set-up

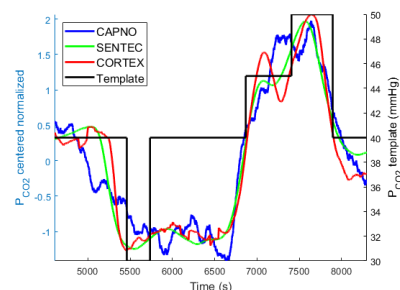


Fig. 2: Signals comparison after alignment

RELATED PUBLICATIONS:

- [1] P. Grangeat, S. Gharbi, M. Accensi, et H. Grateau, « First Evaluation of a Transcutaneous Carbon Dioxide Monitoring Wristband Device during a Cardiopulmonary Exercise Test* », in *2019 41st Ann. Intern. Conf. of the IEEE Engineering in Medicine and Biology Society (EMBC)*, 2019, p. 3352-3355.
- [2] T. Rupp *et al.*, « Cerebral Hemodynamic and Ventilatory Responses to Hypoxia, Hypercapnia, and Hypocapnia during 5 Days at 4,350 m », *J. Cereb. Blood Flow Metab.*, vol. 34, n° 1, p. 52-60, janv. 2014.
- [3] P. Grangeat, S. Gharbi, M. Accensi, et H. Grateau, « Etude d'un modèle linéaire quadratique appliqué à la mesure optique infrarouge du gaz carbonique sanguin émis par la peau », *XXVII^{ème} Colloque GRETSI*, 26-29 août 2019, Lille, France.

Noninvasive Monitoring of Deep Tissue Oxygenation in Buried Flaps by Time-Resolved Near-Infrared Spectroscopy in Pigs

RESEARCH TOPIC:

Tissue oxygenation monitoring in depth thanks to Time-Resolved Near Infrared Spectroscopy

AUTHORS:

Rodolphe Lartizien¹, Maxime Henry², Jean-Luc Coll², Audrey Dot², Georges Bettega^{1,2}, Michel Berger, **Anne Planat-Chrétien**

For the first time, we demonstrate the relevance of an optical Time-Resolved probe to non-invasively assess deep buried flaps viability. Our device detects and identifies arterial and venous occlusions in depth (>1cm) on a pig experimental model. Monitoring deep events under a superficial diffusive layer is an issue in various fields of application that can be addressed with such a technology (head traumas, stroke monitoring...). Next step consists in giving a large diffusion in clinics of this innovation, i.e. bring the current prototype to the clinic and prepare the conditions for bringing this technical innovation to the European market.

SCIENTIFIC COLLABORATIONS: ¹ Centre Hospitalier Annecy Genevois (CHANGE), 1 avenue de l'hôpital, Metz Tassy, BP 90074 -74374

Context and Challenges

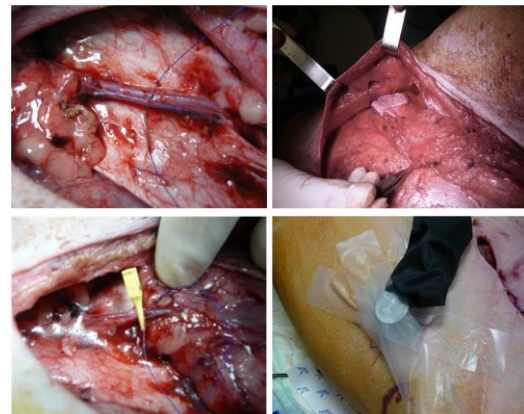
Flap monitoring in reconstructive surgery is particularly important because flap failure is a dramatic event for the patient and for the medical team. Noninvasive deep tissue oxygenation monitoring is a challenge. The aim of this experimental study was to assess the performance of time-resolved near-infrared spectroscopy compared with continuous-wave near-infrared spectroscopy and with invasive oxygen partial pressure measurement in pigs.

Main Results

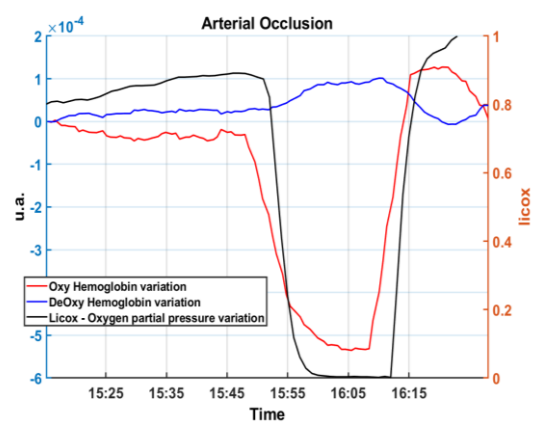
Thirty fasciocutaneous flaps based on the superficial epigastric inferior pedicle were harvested and buried under the transcutaneous dorsal muscle (~ 1 cm thick). An optical probe was placed on the skin above each buried flap. For each pig, two buried flaps were performed, one submitted to arterial occlusion and one to venous occlusion. Time-resolved near-infrared spectroscopy provided specific signals to detect and identify arterial or venous occlusion, even for deep structures. The TR-NIRS specificity and sensitivity was 100% while Oxygen partial pressure failed to detect vascular events in three cases and Continuous-wave near-infrared spectroscopy could not clearly identify vascular occlusions.

Perspectives

There are two major steps in the use of this promising technology: the first is to validate this approach on patients by making the preliminary improvements necessary for clinical use of this system. the second is to make the TR technology more accessible to bring it out of the laboratory.



Monitoring of buried flaps on pig



Monitoring of arterial occlusion

RELATED PUBLICATIONS:

- [1] Planat-Chrétien A., et al., "Non-Invasive Assessment of Deep Buried Flap Viability with Time-Resolved Optical Monitoring: Results on Pigs.", Biomedical Optics Congress 2018, Clinical and Translational, OSA Technical Digest (Optical Society of America, 2018), paper JW3A.27 (2018).
- [2] Planat-Chrétien A., et al., Time-Resolved Optical Monitoring to detect and identify deep flaps, FNIRS2018: The society for functional near-infrared spectroscopy, 2018 biennial meeting, Session 21 Clinical Application II, Sunday Oct 7th, 2018.
- [3] Dot A., et al., "Chromophore reconstruction at depth in bilayered media: a method for quantification.", Biomedical Optics Express 12(3) 1279-1294, 2021

Space-time-frequency multi-sensor analysis for motor cortex localization using magnetoencephalography¹

RESEARCH TOPIC:

Magnetoencephalography, cortical mapping, brain-computer interface, neural data analysis

AUTHORS:

V. Auboiron, C Larzabal, L. Langar¹, V. Rohu, A. Mishchenko, N. Arizumi, E. Labyt, A.-L. Benabid, T. Aksenova

Brain activity cortical mapping is a valuable tool, before surgery, to optimize the placement of implanted brain-computer interfaces and more generally of neural interfaces. Brain source imaging and time frequency mapping are commonly used in magneto/electro encephalography (M/EEG) imaging but suffer from important limitations. We developed a regression-based multi-sensor space-time-frequency analysis (MSA) approach, which integrates co-localized sensors and/or multi-frequency information, to overcome these limitations. In a clinical trial including 14 participants, MSA performance was compared to the weighted minimum norm estimate method and showed statistically improved robustness against ill-defined trigger, typical of motor-deficient patients.

SCIENTIFIC COLLABORATIONS: ¹CHU Grenoble Alpes, France.

Context and Challenges

Magneto/electro encephalography (M/EEG) is routinely used in non-invasive functional brain imaging. In particular, its ability to provide cortical mapping of mental tasks makes it a valuable tool, as it allows the presurgical localization of areas of interest in the brain. The "Brain-Computer Interface (BCI) and tetraplegia" clinical trial (NCT02550522), held at Clinatex, aims at restoring partial motor autonomy to severely motor-impaired patients by the simultaneous use of an implanted brain-computer interface and selected external effectors. Therefore, the capability to localize brain motor activity would allow the optimal positioning of implanted sensors.

Classically, brain activity sources are computed by averaging signals from many trials to increase the signal-to-noise ratio. However, trial averaging is generally sensitive to ill-defined event triggers, which occur for induced response study due to variable subject response time. It is typically the case for BCI studies involving mental tasks and/or patients with motor disabilities, for which precise motor triggers cannot be measured. Another limitation of source imaging approaches concerns high-frequency brain oscillation localization, for which the averaging results in brain signal low-pass filtering. The main goal of this work was to develop a robust brain-mapping method, adapted to tetraplegic patients, which covers the full spectrum of brain activity.

Main Results

To estimate task-specific brain activations, the multi-sensor space-time-frequency analysis (MSA) uses cross-validated, shifted, multiple Pearson correlation, calculated from the time-frequency transformed brain signal and the binary signal of stimuli. The results are then projected from the sensor space onto the cortical surface.

To assess MSA performance, the proposed method was compared to the weighted minimum norm estimate (wMNE) source imaging method, in terms of spatial selectivity and robustness against ill-defined trigger. This first study was performed over a cohort of 14 healthy volunteers, with an

experimental setup usable with tetraplegic patients later on. Our results show that the MSA approach provides favourable localization performances when compared to wMNE (Figure 1), as well as a statistically-significant improvement of robustness against ill-defined triggers. These developments are being used to help conduct the "BCI and tetraplegia" clinical trial at Clinatex.

Perspectives

This study, based on healthy volunteers, will be completed using MEG and electrocorticography data gathered with tetraplegic patients enrolled in the 'BCI and tetraplegia' clinical trial. On-going developments will also allow us mitigating the need for a Magnetic Resonance Imaging (MRI) acquisition as the anatomical support for brain-mapping projection, thus making the method accessible to patients with MR-unsafe implanted devices.

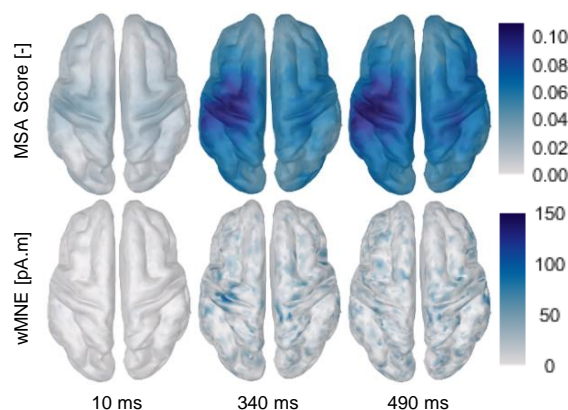


Fig. 1: Group-level brain mapping obtained using MSA and wMNE methods for a motor task (right elbow flexion), based on a visual cue.

RELATED PUBLICATIONS:

- [1] V. Auboiron, C. Larzabal et al., "Space-time-frequency multi-sensor analysis for motor cortex localization using magnetoencephalography", *Sensors*, 2020. doi: 10.3390/s20092706
 [2] A.-L. Benabid et al., "An exoskeleton controlled by an epidural wireless brain-machine interface in a tetraplegic patient: a proof-of-concept demonstration", *Lancet Neurol.*, 2019. doi: 10.1016/S1474-4422(19)30321-7

Adjuvant therapeutic potential of moderate hypothermia for glioblastoma¹

RESEARCH TOPIC:

Glioblastoma, therapeutic hypothermia, chemotherapy

AUTHORS:

C. Fulbert, S. Chabardès and D. Ratel

Therapeutic hypothermia is a promising approach in various medical applications. We evaluated its adjuvant therapeutic potential in the treatment of glioblastoma, an aggressive glial tumor with poor survival. To achieve this, we performed *in vitro* experiments on human glioblastoma cell lines and we explored the adjuvant potential of moderate hypothermia (28 °C) by studying the reversibility of its inhibitory effects on cell proliferation and comparing them to currently used Temozolomide. Results demonstrated an inhibition of glioblastoma cell proliferation by moderate hypothermia even after rewarming and showed that hypothermia had more uniform effects than temozolomide, which ranged from 15% to 95%, and also potentiated these effects. These results support a therapeutic role for hypothermia as an adjuvant therapy for treating glioblastoma.

SCIENTIFIC COLLABORATIONS: Neurosurgery Department, CHU Grenoble Alpes, France.

Context and Challenges

Glioblastoma is the most common malignant brain tumor, currently treated by surgery followed by concomitant radiotherapy and temozolomide-based chemotherapy [2]. Despite these treatments, median survival is only 15 months as a result of tumor recurrence in the resection margins in over 90% of patients, due to the activation of residual glioblastoma cells. We previously demonstrated that continuous moderate hypothermia at 28 °C is able to significantly inhibit both cell proliferation and migration [3]. Here, we assessed the impact of hypothermic preconditioning on cell proliferation following rewarming to 37 °C, and we investigated several intermittent hypothermia sequences. We also compared and combined its inhibitory effects with current temozolomide (TMZ)-based chemotherapy protocols.

Main Results

We performed *in vitro* experiments on four glioblastoma cell lines. First, we studied the impact of different sequences in which cells were successively placed in hypothermic conditions then rewarmed to 37 °C. We demonstrated that moderate hypothermia inhibited glioblastoma cell proliferation even after rewarming. Indeed, hypothermic preconditioning duration strongly enhanced inhibitory effects from 35% after 3 days to 100% after 30 days (Figure 1).

Then, we compared the effects of moderate hypothermia to those of standard chemotherapy on glioblastoma cell proliferation, a key process involved in tumor growth. We demonstrated that moderate hypothermia reduced cell proliferation far more than chemotherapy alone, with inhibitory effects up to eight times higher for chemoresistant cell lines such as U251 (Figure 2).

Finally, combining hypothermia with chemotherapy further reduced proliferation, with more than 95% inhibition even after returning glioblastoma cells to 37 °C.

Our results demonstrate that moderate hypothermia has the potential to reduce inter-patient variability of treatment efficiency, and thus appears to be a relevant adjuvant therapy for use when treating glioblastoma patients.

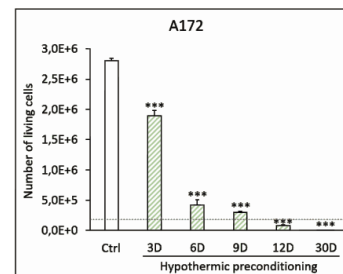


Fig. 1: Influence of the duration of hypothermic preconditioning (28°C) on glioblastoma cell proliferation after rewarming in A172 cell line. Bar graphs represent adherent living cells, *p < 0.001 versus normothermic Control.**

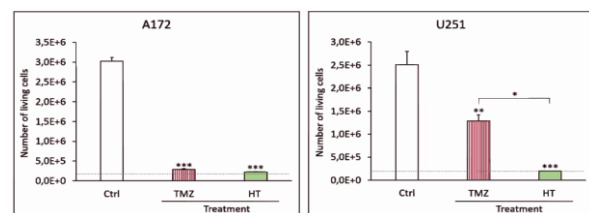


Fig. 2: Temozolomide chemotherapy (TMZ) and hypothermia (HT) effects on glioblastoma cell proliferation. These treatments were applied for 6 days and glioblastoma cells were counted. Bar graphs represent numbers of adherent living cells, **p < 0.01, *p < 0.001.**

Perspectives

Further *in vivo* experiments will be required to confirm these *in vitro* results, with intermittent sequences based on 30-day hypothermia in combination with chemotherapy.

If the latter corroborate our findings, moderate hypothermia could be a suitable adjuvant treatment for glioblastoma when applied locally at the resection margins.

RELATED PUBLICATIONS:

- [1] C. Fulbert et al., "Adjuvant therapeutic potential of moderate hypothermia for glioblastoma", J. Neurooncol., 2021.
- [2] R. Stupp et al., "Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival...", Lancet Oncol., 2009.
- [3] C. Fulbert et al., "Moderate hypothermia inhibits both proliferation and migration of human glioblastoma cells", J. Neurooncol., 2019.

Contact, high-resolution spatial diffuse reflectance imaging system for skin condition diagnosis: a first-in-human clinical trial

RESEARCH TOPIC:

skin characterization, optical properties, diffuse reflectance, multi-pixel sensor, tissue oxygen saturation, wearable device

AUTHORS:

Nils. Petitdidier¹, Henri Grateau, Samamar Characoun², Abdallah Ghaith², Samuel Verges², Stéphane Doutreleau², Sadok Gharbi, Rémi Gerbelot, Sylvain Gioux^{1,3}, Jean-Marc Dinten, **Anne Koenig**

Long-term monitoring of oxygenation or such parameters is needed for patient care management, at the patient bed as well as outside the hospital. The aim of this work was to propose a low-cost, in contact system for the long-term measurement of skin physiological parameters. We have developed a low-cost, wearable, Complementary Metal Oxide Semiconductor based device. We propose an original method for processing diffuse reflectance data in order to calculate the tissue oxygen saturation. We tested the device for the assessment of tissue oxygenation during a first-in-human clinical trial that took place at the Grenoble University Hospital France. The results of this clinical trial show a good accordance between our sensor and commercial devices used as a reference.

SCIENTIFIC COLLABORATIONS: ¹ Université de Strasbourg, 4 rue Blaise Pascal, F-67081 Strasbourg, (FR), ² Laboratoire HP2 (U 1042), Université Grenoble Alpes, CHU Grenoble Alpes, INSERM, Hôpital Sud, Avenue Kimberley, 38 434 Echirolles, (FR), ³ Laboratoire ICube, Télécom Physique Strasbourg, 300 bd Sébastien Brant, F-67412 Illkirch, (FR)

Context and Challenges

Knowledge of tissue properties such as oxygen saturation (StO_2) is important in patient care management for many diseases. Monitoring systems of physiological parameters at the patient bed as well as outside the hospital should be of great interest for the remote medicine of tomorrow. Diffuse reflectance spectroscopy (DRS) has been widely used for biological tissue characterization. To miniaturize the technology, we have developed a wearable device using a commercially available Complementary Metal Oxide Semiconductor (CMOS) sensor coupled to LEDs placed in contact with the skin [1-2] Fig 1. We conduct a clinical trial using this CMOS-DRS device to monitor in vivo the StO_2 where desaturation followed by an ischemia was controlled.

Main Results

This clinical trial showed that the CMOS-DRS allows qualitative continuous monitoring of StO_2 . In addition, the use of a third wavelength makes our system autonomous and independent of a reference system. The experiments demonstrate that our approach is suitable for qualitative detection of ischemia, showing potential for the detection of free flaps failure [3]. However, we observed discrepancies for quantitative StO_2 measurements between the CMOS-DRS and the reference system Fig 2.

Perspectives

Future work will address further validation for quantitative results, the integration aspect of the system, through software embedment and implementation of wireless data transfer, making eventually a low cost, easy-of-use system for continuous StO_2 monitoring.

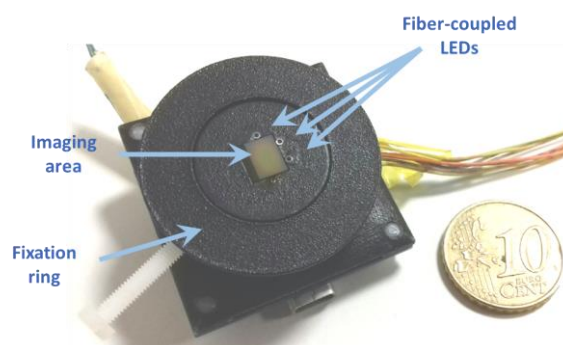


Fig. 1 CMOS-DRS system photograph.

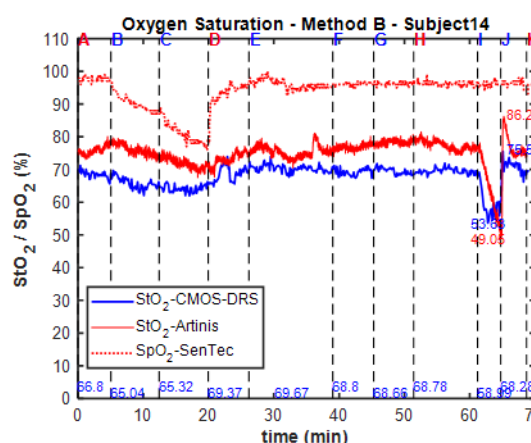
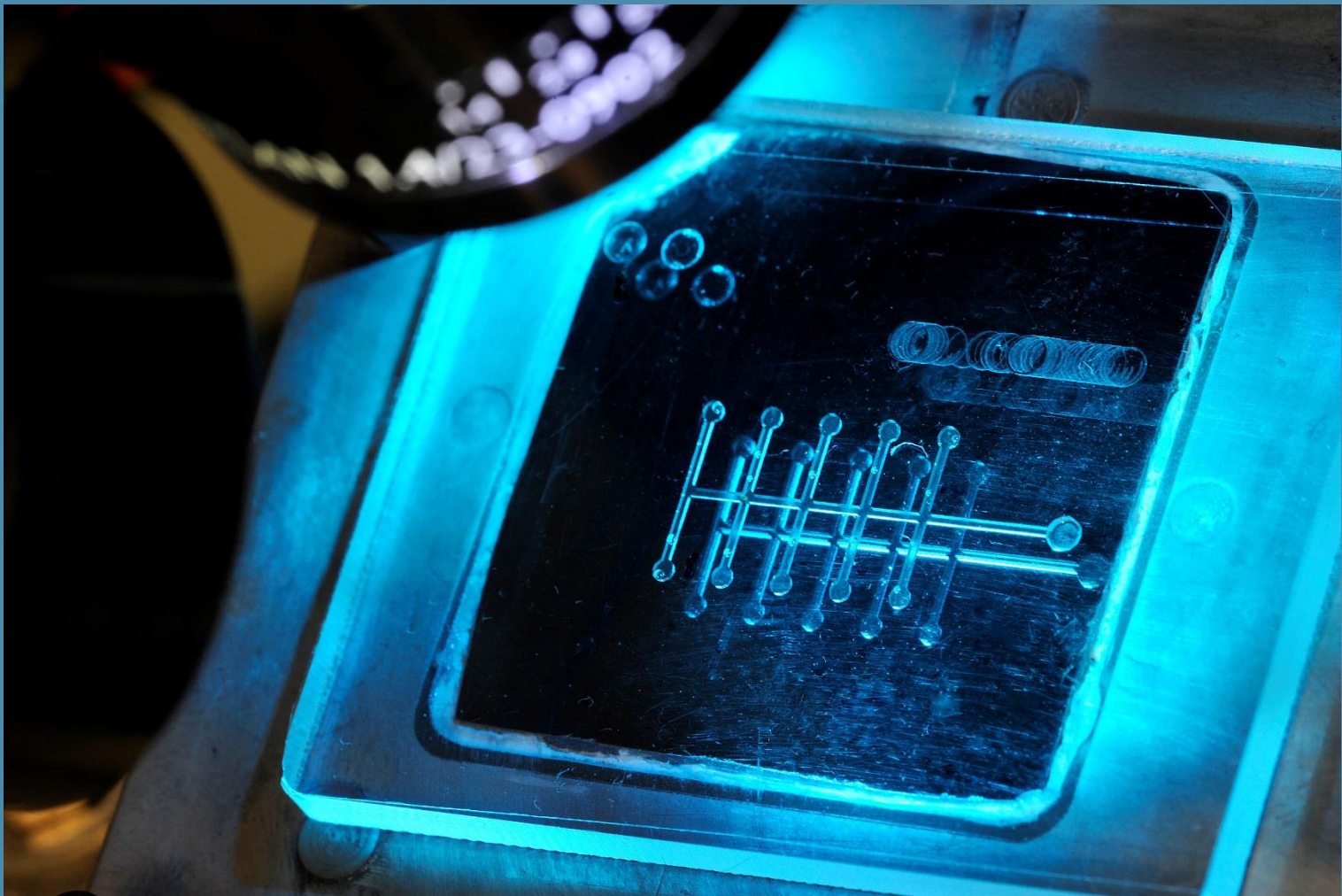


Fig. 2 StO_2 readings systems comparison.

RELATED PUBLICATIONS:

- [1] N. Petitdidier, "Imaging system for the characterization of skin layers using diffuse reflectance (Système d'imagerie pour la caractérisation en couches de la peau par réflectance diffuse)," PhD Thesis, Université de Strasbourg, <http://www.theses.fr/s158721> (2018)
- [2] N. Petitdidier et al., "Contact, high-resolution spatial diffuse reflectance imaging system for skin condition diagnosis," J. Biomed. Opt. 23(11), 115003 (2018) [doi:10.1117/1.JBO.23.11.115003]
- [3] A. Koenig et al., "Contact, high-resolution spatial diffuse reflectance imaging system for skin condition diagnosis: a first-in-human clinical trial," J. Biomed. Opt. 26(1) 012706 (2021) [doi/10.1117/1.JBO.26.1.012706.]



05

MICROFLUIDICS FOR DIAGNOSTICS

- Digital microfluidic for gas manipulation
- Microfluidic preparation for bedside proteomics
- Sorting of extracellular vesicles

A microfluidic device for digital manipulation of gaseous samples

RESEARCH TOPIC:

Digital microfluidics, gas sampling and analysis microfluidic device, micropreconcentrators

AUTHORS:

J.Vial², D.Thiébaud², A. Enel^{1,2}, A. Bourrelier¹, **B. Bourlon¹**

Digital microfluidics is known for diverse applications from biological samples preparation to diagnostic testing, all limited to liquid phase manipulations. We present a new system based on a digital microfluidic platform (DMFP), which is able to digitally manipulate gaseous samples, such as alkanes from n-hexane to n-nonane. The DMFP relies mostly on interconnected micropreconcentrators to trap and release the samples depending on their controlled temperature. We show that the DMFP is capable of performing all basic operations of digital microfluidics: trapping/releasing, moving samples, adding samples and separating samples. This DMFP promises great possibilities for the development of new digital gas sample preparation and analysis methods.

SCIENTIFIC COLLABORATIONS: ¹ Univ. Grenoble Alpes, CEA, LETI, MINATEC Campus, F-38000 Grenoble, France, ² UMR8231 CBI, LSABM, ESPCI Paris-CNRS, PSL Institute, Paris, France

Context and Challenges

Microfluidics has been progressing steadfastly in the last few decades, in particular with the emergence of digital microfluidics. This type of microfluidics allows step by step, synchronized, computer-like control of liquid droplets. This approach makes possible the realization of digital complex operations. No such works have been reported on digital manipulations of gas samples within a carrier gas. Here, using interconnected temperature controlled silicon chips filled with adsorbent, we show that it is possible to perform all the key digital elementary operations. This new method differs from classical techniques used for gas samples manipulations and analysis based on "analog" operations and limited by gas diffusion.

Main Results

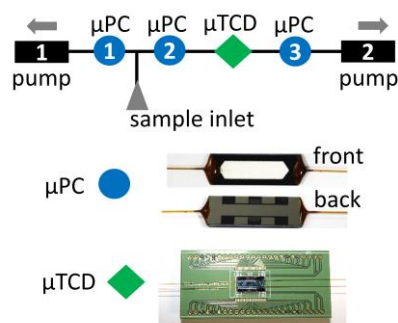
In this work, a digital microfluidic platform (DMFP) using pumps, silicon CEA-LETI micropreconcentrators (μ PC) and a silicon CEA-LETI thermal conductivity detector was assembled. We showed that the digital manipulation of gas samples was possible with the DMFP, and demonstrated all elementary operations: trapping/releasing and moving a compound, merging two compounds and separating two compounds. Several alkanes, ranging from n-hexane to n-nonane were used to demonstrate these operations. These elementary operations were carried-out by controlling precisely, step by step, the state of the pumps and the temperature of the μ PCs. These elementary operations were also the first step towards a more complex device, which could be used to perform high-level functions, such as separation of mixed samples.

As a first proof of concept of a more complex programmable application, the DMFP was used to measure the breakthrough volume of these alkanes on a classical adsorbent (Tenax TA). Breakthrough volume measurements with standard laboratory instruments, such as a gas

chromatograph, are labor intensive, as they need several injections, one for each temperature studied. As an illustration of potential applications, it was possible to measure automatically with the DMFP the breakthrough volume on a wide range of temperatures using only one injection, by manipulating without losses the same initial gas sample.

Perspectives

Such digital system could open in the future a new field of technologies and applications regarding gas or volatile compounds samples preparation and analysis. For example, one could imagine separation of a gas mixture, by using the temperature control of the μ PCs network and the retention differences between the chemical compounds, similarly to a "digital" distillation or gas chromatographic analysis.



Schematics of the DMFP

PepS: An Innovative Microfluidic Device for Bedside Whole Blood Processing before Plasma Proteomics Analyses

RESEARCH TOPIC:

Proteomic analysis, mass spectrometry, innovative and automated microfluidic.

AUTHORS:

B. Gilquin, M. Cubizolles, R. Den Dulk, F. Revol-Cavalier, M. Alessio, C.-E. Goujon, C. Echampard, G. Arrizabalaga, A. Adrait, M. Louwagie, P. Laurent, F. P. Navarro, Y. Couté, V. Brun, **M.-L. Cosnier**

Immunoassays used in laboratories to quantify proteins are inappropriate in some cases. Mass spectrometry has emerged as an alternative method to assess panels of biomarkers to monitor health status. Translation of MS-based proteomics to the clinic has been hampered by its complexity and the necessary time and human resources for sample preparation. We designed a microfluidic device automating and accelerating this preparation. The microfluidic cartridge is operated through an instrument providing automated fluid processing and thermal control. In less than 2h, the device allows bedside plasma separation to stabilization of peptides. The performance was assessed using discovery proteomics and targeted proteomics.

Context and Challenges

The plasma proteome includes over 200 biomarkers that are generally measured using immunoassays. They are adapted to clinical chemistry platforms but have limitations. Advances in instrumentation have improved the performance of MS-based proteomics but has limitations too like need of skilled operators and complex procedures. To automate, simplify and standardize the processing of samples, we develop a microfluidic device.

Main Results

The system consists of a microfluidic cartridge and a transportable instrument that drives the functionalities. Samples are collected at the patient's bedside by venipuncture and prepared, while proteomics analysis can be realized in an appropriate environment.

We assessed the performance and repeatability and compared it to manual preparation for plasma proteome profiling. The peptide digests obtained using the automated or manual protocols were analyzed. The number of proteins identified was slightly increased using the PepS protocol.

We evaluated the approach for multiplexed assay of biomarkers. Three selected proteins were reproducibly quantifiable after automated sample processing. The physiological and pathological ranges for these biomarkers should be detected.

Perspectives

Future developments will aim to assess analytical performance for PepS applied to the quantification of selected clinical biomarkers in line with guidelines issued by health authorities.

RELATED PUBLICATIONS:

[1] A. Enel, A. Bourrelier, J. Vial, D. Thiébaud, and B. Bourlon, A microfluidic device for digital manipulation of gaseous samples, *Lab. Chip*, vol. 20, no. 7, 2020, 1290–1297

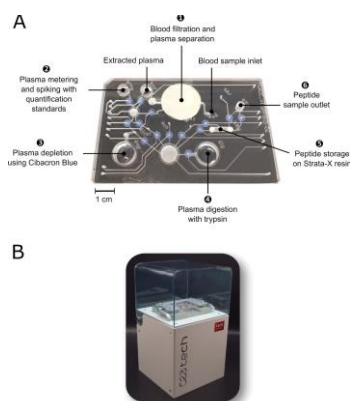


Fig. 1: Microfluidic cartridge and instrumentation

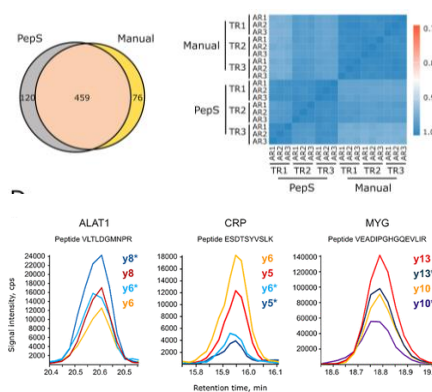


Fig. 2: Identified proteins and targeted biomarkers

Deterministic Lateral Displacement for the sorting of extracellular vesicles from complex biological samples

RESEARCH TOPIC:

Extracellular Vesicles, Deterministic Lateral Displacement, Size Sorting, Microfluidics, Sample Preparation, Liquid Biopsy

AUTHORS:

M. Gaillard, N. Sarrut-Rio, L. Viro, F. Boizot, N. Verplanck, C. Raillon, V. Agache, Y. Roupioz, **A. Thuair**

Extracellular Vesicles (EVs) are secreted by cells and found in many biofluids, with concentration correlated with oncogenic signals, making them attractive biomarkers for liquid biopsy. The current gold-standard method to isolate EVs requires an ultracentrifugation (UC) step among others. The cost and complexity of this technique are forbiddingly high for many researchers, as for clinical implementation. Here is presented an alternative microfluidic technique for EVs isolation based on particles size sorting, namely Deterministic Lateral Displacement (DLD). We improved the design from our initial device and demonstrated the capacity to isolate EVs from THP1 cell culture media, with size distribution results consistent with UC technique.

Context and Challenges

EVs are lipid nanoparticles secreted by cells for communication purpose that circulate in biological fluids as blood. They convey information from a parental cell to another cell through surface proteins and their DNA/RNA content [1]. Thus, they are considered as promising biomarkers in the field of liquid biopsy. EVs isolation is a technological challenge currently addressed using UC. Even though this method is widely used, it has a significant number of limitations, so the International Society of Extracellular Vesicles (ISEV) has encouraged the development of more reproducible methods for EVs isolation in 2018. Previous works have demonstrated the ability to sort EVs using nanoscale DLD arrays but required restrictive preparation steps. Our approach aims at sorting nanometric particles using micrometer-sized pillar array thus decreasing the fluidic resistance and significantly increasing the flow rate. Improving our initial DLD device [2], in terms of DLD design, fabrication and fluidic packaging, has allowed the sorting of submicronic biological particles, as EVs, as we report here.

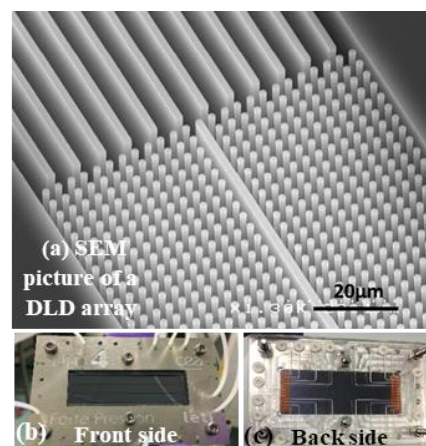
Main Results

The DLD fluidic system integrates a DLD silicon-based chip mounted on a fluidic plastic cartridge and connected to a pressure controller. The DLD device is based on a channel including a tilted pillar array, which geometrical parameters define a cut-off diameter (D_c). Particles smaller than D_c are not deviated by the pillar array as opposed to larger particles. Our design features pillars diameter and gap of 1.5 μm , leading to an estimated D_c of ~ 345 nm. This D_c has been targeted to collect the most abundant EVs population, presumably distributed between 50 and 300 nm in diameter, while the gap size has been chosen to minimize hydraulic resistance. The separation performance of the DLD system was compared to results obtained on similar samples processed by UC. Both samples were characterized in terms of particles size distribution and concentration using a NTA system, which is considered as the standard method for EVs

characterization. Data show multi-modal populations in both purification methods, within the 50nm-300nm range with a main population around 130 nm. These results highlight the potential of our DLD system as an interesting technique for reproducible and standardized EVs isolation from complex biological samples.

Perspectives

Through a specific DLD design and optimization of hydraulic resistance, these encouraging results of EVs isolation from THP-1 cell culture medium pave the way for an original strategy enabling the fast and easy-to-operate isolation of EVs as promising biomarkers.



DLD channel and microfluidic cartridge

RELATED PUBLICATIONS:

[1] Gaillard, M., Thuair, A., Nonglaton, G., Agache, V., Roupioz, Y., Raillon, C., Biosensing extracellular vesicles: Contribution of biomolecules in affinity-based methods for detection and isolation, *Analyst*, 145 (6), 1997-2013, 2020.

[2] Pariset, E., Parent, C., Fouillet, Y., Boizot, F., Verplanck, N., Revol-Cavalier, F., Thuair, A., Agache, A., Separation of Biological Particles in a Modular Platform of Cascade Deterministic Lateral Displacement Modules, *Scientific Reports*, 8, 17762, 2018.



06

**PHD DEGREES
AWARDED IN 2020**

- **Mathilde Champeau**
- **Claire Desfrancois**
- **Prisca Perlemoine**
- **Maude Savonnet**



MATHILDE CHAMPEAU

DISSOLVING MICRONEEDLES FOR AN OPTIMAL TRANSDERMAL DELIVERY OF AN ACTIVE PRINCIPLE USED IN PHOTODYNAMIC THERAPY: DEVELOPMENT AND PROOF OF CONCEPT

Ecole doctorale Biologie Santé, University of Lille

Non-melanoma skin cancers are on the rise with 2 to 3 million people diagnosed each year and are sometimes treated by local ablation therapy. To avoid this surgery, photodynamic therapy (PDT) appears as an advantageous treatment. Currently used in clinics, PDT consists of applying a cream containing a photosensitive precursor to the damaged skin, which, then metabolizes and under light excitation induces cell death. However, this technique is not fully effective if the skin lesion extends into the deep skin layers. To improve the therapeutic treatment of this type of skin cancer, a patch with dissolving microneedles (MNs) was developed to reach the deep layers of lesions that are difficult to treat. Hyaluronic acid, known for its biocompatibility, solubility and biodegradability, was chosen as the constituent material, and mixed with the 5-aminolevulinic acid (photosensitive precursor, 5-ALA). To ensure the best penetration without causing pain by touching the nerve endings, an optimal "pencil-tip" design was defined with MNs length going from 400 to 750 μm . A simple and robust manufacturing process

called solvent casting molding method, has been set up which is an asset for potential industrialization. In absence of realistic skin lesions model, we chose to establish one on rats skin by applying daily UV-B doses. Histology and pharmacokinetic studies validated the presence of precancerous skin lesions and the MN-patch in vivo efficiency was therefore tested. After one hour application on the injured rat skin, the MN-patch dissolved and released the 5-ALA that further metabolized to protoporphyrin IX (PpIX). A significant level of PpIX fluorescence was recorded suggesting that after light excitation, a PDT session could be effective. In parallel, to reduce pain felt during PDT treatment, a light device with suitable optical and thermal properties was conceived and coupled to the MN-patch. The idea would be to start the illumination directly after MN-patch application in order to avoid a painful photochemical reaction. This wearable and easy to use system purpose a all-in-one PDT processing which fulfills the criterion of patient compliance, better efficiency and speed of treatment.



CLAIRE DESFRANCOIS

DESIGN, SYNTHESIS AND CHARACTERIZATION OF HYDROGELS WITH HIGH COMPRESSIVE STRENGTH FOR THE SUSTAINABLE DELIVERY OF HYDROPHOBIC DRUGS

Université Grenoble Alpes (France)

The local and sustained delivery of a drug of interest requires the use of a conformable support matrix, able to adjust to patient daily movements. This matrix should be biocompatible, resistant, and should show suitable compressive and stretchable properties. New composite materials for the delivery of hydrophobic active pharmaceutical ingredients (API) have been designed from stretchable hydrogels and lipid nanoparticles able to encapsulate high payloads of hydrophobic drugs. The double encapsulation system – API-loaded lipid nanoparticles embedded in a polymer network with stretchable meshes – could achieve finely-tuned control over drug

delivery while improving patient compliance. First, water-soluble polyrotaxanes (PR) were synthesized with a controlled number of threaded cyclodextrins thanks to an original method. These PR were further used as cross-linkers in a photo-crosslinked network of poly(ethylene glycol)-methacrylate. This novel polymeric matrix showed increased compressive properties as demonstrated by mechanical tests. In parallel, new formulations of lipid nanoparticles loaded with two API of interest and their drug release profile were studied, for further inclusion in the stretchable hydrogel matrix.



PRISCA PERLEMOINE

WIDE-FIELD LENSLESS IMAGING FOR PHAGE SUSCEPTIBILITY TESTING

École doctorale électronique, électrotechnique, automatique, traitement du signal, University Grenoble Alpes

Drug resistance is one of the main issue to be tackled in the next few decades. Indeed it is estimated that infectious disease could be the most common cause of death by 2050. One promising solution lies in phage therapy which is based on the use of highly specific bacterial viruses called bacteriophages or phages. These viruses are ubiquitous and harmless for human cells. Through a lytic life cycle, some phages are able to replicate within the cytoplasm of bacterial cells before being released through host lysis. Contrary to antibiotics, which are broad spectrum antimicrobials, phages' activity spectrum is highly specific to strains among the same bacterial species. Therefore, to increase chances of therapeutic success during phage therapy, it is required to test the susceptibility of the pathogenic bacterial strain to several viral strain. That test is called phagogram, or phage susceptibility testing. However, to the best of our knowledge, direct visual evaluation of phage plaques by the experimenter is still the sole technique routinely used in

laboratories to perform the phagogram. This method is time-consuming (at least 12h) and does not comply with the automated environment of hospital laboratories. The aim of this research is to investigate innovative approaches to perform phagogram inspired by lensless imaging technique. Lensless imaging consists of imaging biological sample placed directly on top of an imaging sensor without the use of any optical objectives. The main advantages of this technique is that it allows observation of larger field-of-views than optical microscopy. We designed a custom wide-field lensless imager that assesses phage-bacteria interaction in both liquid broth and solid media, on a field-of-view of 3.3 cm². Using this prototype, we report proof of concept of a phagogram in both in liquid and solid culture media. We are able to detect bacteria susceptibility to a phage in less than 2hrs30min. Moreover, the monitoring of phage-bacteria interaction on agar media can yield the infectious titer of viral suspension in less than four hours.



MAUD SAVONNET

DEVELOPMENT OF AN INNOVATIVE DETECTION METHOD FOR DIAGNOSIS OF CARDIAC PATHOLOGIES

Ecole Doctorale de Physique, University Grenoble Alpes

Today, early diagnosis of cardiac pathologies is a major issue in healthcare world. Indeed, the speed of myocardial infarction diagnosis has an impact not only on the patient's health, but also on the management of emergency hospital services. The use of diagnostic devices at the patient's bedside is a relevant solution to overcome effectively such a challenge. Consequently, the number of Point-Of-Care systems dedicated to the diagnosis of cardiac pathologies is growing. However, these devices have some disadvantages that need to be overcome. This thesis work has been conducted in this context. Research and development of an innovative method for the detection of cardiac biomarkers

has been carried out. The objective of this method is the detection of any type of analyte in a complex medium with a good sensitivity allowed by the biomolecular amplification used. This generic method is based on the LAMP amplification of an oligonucleotide probe. It uses aptamer probes, specific to the target to be detected, which have been validated by surface plasmon resonance imaging. This method has been implemented in a relevant manner on different models and applied to the detection of a cardiac biomarker of interest, troponin I. The integration of this method in a portable microfluidic device was finally addressed for future use in field diagnostics.



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GRAPHIC DESIGN

Eve Issartel, Design by
Eve, Hélène Vatouyas

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Contacts

Patrick CHATON
Head of Microtechnologies
for Biology and
Healthcare division
patrick.chaton@cea.fr

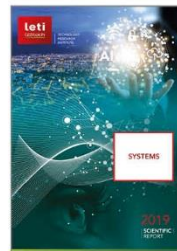
Jean-Marc DINTEN
Deputy head of
Microtechnologies for
Biology and Healthcare
division
Partnerships management
jean-marc.dinten@cea.fr

Pascal MAILLEY
Chief Scientist of
Microtechnologies for
Biology and Healthcare
division
pascal.mailley@cea.fr

Jean-Claude ROYER
Clnatec Director
of Operations
jean-claude.royer@cea.fr

Abdelmadjid HIHI
Clnatec Scientific
program Manager
abdelmadjid.hihi@cea.fr

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