Committed to Innovation, Leti Creates Differentiating Solutions for its Industrial Partners.

Leti is a research institute of CEA Tech and a recognized global leader in miniaturization technologies. Leti’s teams are focused on developing solutions that will enable future information and communication technologies, health and wellness approaches, clean and safe energy production and recovery, sustainable transport, space exploration and cybersecurity.

For 50 years, the institute has built long-term relationships with its industrial partners, tailoring innovative and differentiating solutions to their needs. Its entrepreneurship programs have sparked the creation of 64 start-ups. Leti and its industrial partners work together through bilateral projects, joint laboratories and collaborative research programs. Leti maintains an excellent scientific level by working with the best research teams worldwide, establishing partnerships with major research technology organizations and academic institutions. Leti is also a member of the Carnot Institutes network*.

*Caron Institutes network: French network of 34 institutes serving innovation in industry.

CEA Tech is the technology research branch of the French Alternative Energies and Atomic Energy Commission (CEA), a key player in research, development and innovation in defense & security, nuclear energy, technological research for industry and fundamental physical and life sciences.

**www.cea.fr/english**

*Leti at a glance*

- **€315 million budget**
- **800 publications per year**
- **ISO 9001 certified since 2000**
- Founded in **1967**
- Based in **France** (Grenoble) with offices in the **USA** (Silicon Valley) and **Japan** (Tokyo)
- **1,900 researchers**
- **2,760 patents in portfolio**
- **91,500 sq. ft. cleanroom space, 8" & 12" wafers**
- **350 industrial partners**
- **64 startups created**
Core R&D competencies of technologies for **Biology and Health Division** are the development, design, integration and qualification of micro- and nanotechnologies in many fields. These include detectors 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 include cleanrooms dedicated to biochip packaging (230 m²) and surface functionalization/bio probes grafting (100 m²), biological laboratories with L2 rooms for bacteria, cells and human samples and biological characterization equipment such as PCR, cell microscopy and FACS (100 m²). We also have a laboratory for synthetic chemistry, electrochemistry and characterization (430 m²) and a microfluidic laboratory dedicated to technologies and system validation (300 m²).

With **Clinatec**, 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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Health is defined by the World Health Organization in a broad sense that includes medicine (from diagnosis to treatment and surgery) but that also covers all the factors that may alter human life including environment, food, wellness and security. Leti-Health is positioned in this global context and is able, through its two constitutive divisions, DTBS (Microtechnologies for Biology and Health Division) and Clinatec (Technology and Medical Research Center), to address the overall field of health from the design and the maturation of new technologies to animal testing and even to clinical trials dedicated to the evaluation of implantable devices.

The LETI Health's DNA fits into a global process ranging from the design and maturation of new technologies to the realization of operational demonstrators. In such a context, 2018 was a key period for the deployment of our technologies toward higher maturity levels owing to strategic partnerships with industrial partners and / or their territorialization in partner French areas. Indeed, France is entering a period of recession in its territorial health coverage with the emergence of medical deserts. Thus, Leti-Health has carried out a full-scale action aimed at implementing a strategy for the deployment of connected health in the Meuse department through the deployment of our e-health technologies and those of our industrial partners. On the industrial level, we have also pushed our nanomedicine technologies towards industrialization with the creation of the V-Nano spin-off in the Toulouse region in collaboration with the Oncopôle de Toulouse. Finally, we have also created the Hub4Aim consortium, in collaboration with our industrial partners and Grenoble-Alpes University Hospital, to develop Point-of-Care systems from design to pre-series production.

From a research perspective, 2018 was also a successful year with, first of all, the ramp-up of our work on organs on chips for their long-term application in personalized medicine. Otherwise, monitoring physiological and metabolic parameters on and in the body is key to connected health and home hospitalization that requires the development of complex interfaces for long-term use of sensors. Thus, the design of biomaterials to improve the body/sensor interface in order to ensure the quality and stability of recorded signals has become a new strategic component of our roadmap. Finally, a new therapeutic device dedicated to the treatment of pharmaco-resistant epilepsy based on local brain cooling has been developed and has produced promising preclinical results.

In summary, 2018 was for us a successful year that saw the deployment of our technologies and the genesis of promising new research themes.
KEY FIGURES

152 Researchers
32 Post-docs and short-term contracts
26 PhDs and apprentices

66 Book chapters and journal articles
36 Conferences and workshops

36 Patents filled
374 Patents in portfolio
9 Start-ups 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
Publications


**36 Conferences and workshops**

Main papers are:


Experts

2 Research Directors
2 International Expert
15 Senior Experts
15 Experts
11 owning the HDR

**Participation in normalization groups**

Convenor (Nicolas Verplanck) of the European (CEN/TC332/WG7 and International ISO/TC48/WG3 regulation groups for the normalization of microfluidic systems.

**International Collaborations**

UCLA, MIT (USA)
Politecnico di Milano, University of Pisa (Italy)
Helmoltz Association, Fraunhauer, Charité Berlin (Germany)
University of Twente, UMC Utrecht (Netherland)
Tyndall (Ireland)
University of Liverpool (United Kingdom)
SINTEF (Norway)
CIDETEC, CSIC (Spain)
University of Sidney (Australia)
CSEM, EMPA (Switzerland)
Nanomedicine European Platform (Europe)
01

CELL CULTURE AND ORGAN-ON-CHIP

- Lens-free microscopy
- Human pancreatic islets encapsulation
- Conducting polymer scaffold for 3D cell culture
- Organoids encapsulation and transfection
LENS-FREE VIDEO MICROSCOPY FOR THE DYNAMIC AND QUANTITATIVE ANALYSIS OF ADHERENT CELL CULTURE

RESEARCH TOPIC:
Quantitative phase microscopy, Live cell imaging, Cell culture

AUTHORS:

ABSTRACT:
We demonstrate that lens-free video microscopy enables us to capture simultaneously the kinetics of thousands of cells directly inside the incubator and that it is possible to monitor and quantify single cells along several cell cycles. We describe the full protocol used to monitor and quantify a HeLa cell culture during 2.7 days. First cell culture acquisition is performed with a lens-free video microscope, then the data is analyzed following a four-step process, namely multi-wavelength holographic reconstruction, cell-tracking, cell segmentation and cell division detection algorithms. As a result, we show that it is possible to gather a dataset featuring more than 10,000 cell cycle tracks and more than 2.10^6 cell morphological measurements.

SCIENTIFIC COLLABORATIONS: CEA-IRIG

Context and Challenges
Monitoring cultured mammalian cells throughout several cell cycles and measuring accurately cell size and cell dry mass is a challenging task. Several label-free optical techniques are able to perform this task as reviewed in [1], e.g., digital holographic microscopy (DHM) and quadriwave lateral shearing interferometry. However, these methods are seldom coupled with automatic cell tracking algorithms and their throughput remains limited when measuring cell mass trajectories (N=20). Hence a novel optical method is needed to measure cell mass trajectories with a large statistics (N>1000). In this paper, we demonstrate the capability of lens-free video microscopy to simultaneously image thousands of cells directly inside the incubator, then quantify single cell metrics along thousands of individual cell cycle tracks.

Fig. 1: Lens-free microscopy. (a) Schematic of the lens-free microscopy acquisition setup. (b) Picture of the lens-free video microscope.

Main Results
We showed that lens-free video microscopy can be used inside an incubator to capture the kinetics of thousands of cells [1,2]. In order to describe the overall methodology we explained how a 2.7 days time-lapse acquisition of HeLa cells in culture can be analyzed with standard cell-tracking algorithms. The result is a dataset featuring 2.2x10^6 cell measurements and 10584 cell cycle tracks. The acquisitions were performed on a culture of HeLa cells with a relatively large cell-to-cell distance (cell density <500 cells/mm^2) and cell morphology that could be approximated with a disc. These two features facilitate the application of an automatic cell-tracking analysis, which makes it possible to gather such a large dataset. Other cell lines with smaller cell-to-cell distance or with more complex morphologies would be more difficult to track and subsequent datasets would be smaller.

Fig. 2: Data analysis of a 2.7-day cell trajectory. (a-d) Plot of the cell area, dry mass, thickness and motility as a function of time. The cell thickness exhibits sharp peaks corresponding to cell divisions. (e) Segmentation results. The image is 200x200 µm^2 centred on the cell of interest. (f) 800x800 µm^2 cropped reconstructed phase image. The cell of interest is surrounded by a yellow circle.

Perspectives
Lens-free video microscopy allows obtaining large datasets of cell measurements. However, the analysis remains supervised, manual inputs are still necessary to adjust the different algorithms parameters. Recently the use of deep learning is changing the field of image processing. In addition, we expect that this technology, with the use of convolution neural networks (CNN), will allow us to provide in a short term a general and unsupervised cell analysis algorithm for lens-free video microscopy.

RELATED PUBLICATIONS:
MULTISPECTRAL TOTAL-VARIATION RECONSTRUCTION APPLIED TO LENS-FREE MICROSCOPY

RESEARCH TOPIC:
Optical microscopy, Lens-free, Reconstruction, Sparsity, Unstained samples, Diffraction

ABSTRACT:
Lens-free microscope is a device only composed by a LED source and a CMOS detector. It collects the intensity of the light diffracted by samples located at a small distance from the detector. Without lens, the image is obtained through computation and quantitative 2D optical property maps can be obtained [1]. Here, we show the numerical approach we followed to compute cell culture images from RGB acquisitions: a novel multispectral total variation criterion is defined and minimized. Reconstruction results show that the method is efficient to recover the phase image of densely packed unstained (transparent) cells.

Context and Challenges
Lens-free microscopy is a minimalistic microscopy technique, which consists in acquiring diffraction patterns of a thin unstained biological sample at a distance around 1 mm and computing (reconstructing) an image of optical properties of the sample. By its simple design, the system is cheap, robust and incubator-proof. It can easily provide videos with a large field of view (here around 30 mm²).

Careful analysis of the problem shows monochromatic measurement information is not sufficient to recover the diffracting object optical characteristics thus basic reconstructions exhibit artifacts known as twin images. Here [2], two strategies are employed to improve image reconstruction: sparsity related priors and multispectral (RGB) acquisitions to enrich the measurements set. Associated reconstruction involves the minimization of a multispectral total variation (MS-TV) criterion.

Main Results
A time-lapse acquisition (3.7 days) of A549 mammalian cell culture was performed with a Cytonote® lens-free microscope (RGB, object-detector distance=1340 µm, field of view of 29.4mm², pixel pitch 1.67 µm).

An example of a frame is shown on the figure below. (a) presents the whole image of optical length difference of the sample. (b) shows a zoom of (a) and (e) a bigger zoom - both obtained with a RGB reconstruction algorithm. (c) and (f) are results obtained from a monochromatic approach. (d) and (g) show zooms of raw diffraction acquisitions. From these results, we see that Lens-free microscopy is able to provide sharp images of biological unstained (transparent) samples and we see that multi-chromatic acquisitions allow better recovery (Fig. e). We also evaluated that the half-pitch resolution of the system (limited by temporal coherence of the LED source) is around 2.5 µm.

Perspectives
Lens-less microscopes are robust and easy-to-use systems, which enable cell cultures observation without cumbersome preparation - directly inside the incubator. It will be used to get time-lapse acquisitions of numerous cell lines in order to check their natural behavior or responses to drugs or mechanical stresses. It will also be used to monitor bio-productions by enabling easy counting of cells. It is envisioned that deep-learning techniques will accelerate and improve image reconstruction, and will also help to compute efficiently the data analysis (cell detection, cell segmentation, cell tracking, etc.).

RELATED PUBLICATIONS:
NEW TOOLS FOR CELL THERAPY AND REGENERATIVE MEDICINE

RESEARCH TOPIC: Regenerative medicine, Cell Therapy, Cell imaging

AUTHORS: F. Rivera, F. Bottausci, M. Pierron, D. Rabaud, R. Crouigneau, N. Sarrut and J-L Pesce

ABSTRACT: Immune reaction is the main challenge faced by allogeneic or xenogeneic cell therapy. To counteract such immune reaction, one option is to increase the furtiveness of cells towards the host immune system. To do so, one solution is to encapsulate the cells into biocompatible and polymeric shell. These polymeric capsules allow the immune protection of the cells while preserving the diffusion of nutrients, oxygen and secreted molecules (i.e. hormones).

CEA-LETI coordinates the BIOCAPAN project funded within the EU-H2020 program. This project is dedicated to the development of a new bioactive implantable capsule for pancreatic islets to treat Type 1 diabetes. New biomaterials have been developed to enhance cell survival and modulate immune system, new tools to automate the process of encapsulation and to characterize the viability of cells once encapsulated. Preclinical tests are ongoing to assess the ability of this new cell therapy to reverse diabetes.

Context and Challenges
Type 1 diabetes mellitus patients need to take insulin several times a day. Among Type II diabetes mellitus patients, one in six patients eventually needs to take insulin. For these patients (about 80 million worldwide) the disease is ever-present in daily life requiring glycemic measurements, calculations of carbohydrate intake, and insulin injections. Maladjusted levels of insulin often lead to serious co-morbidities such as hypoglycemia, stroke, heart diseases, nephropathy, retinopathy, or diabetic foot (amputation). Reducing insulin level controls, insulin injections, and side effects are key elements for a therapy that improves the patients’ quality of life. The best option for such a therapy is the transplantation of allogeneic islet cells, but the current state of the art limits the applicability of this approach. Implanting unmodified islet grafts requires lifelong administration of immunosuppressant, frequently associated with adverse effects such as higher blood pressure, higher susceptibility to infections, and higher risks for cancers. A new therapeutic approach that promotes islet graft function and survival without the need for insulin injection and immunosuppressive drugs would have a tremendous benefit.

CEA-LETI coordinates the EU H2020 BIOCAPAN project aiming at developing an innovative treatment, based on the implantation of smartly microencapsulated allogeneic islet cells in a bioactive microcapsule, to allow an effective long-lasting blood glucose normalization and stabilization, without the need for immunosuppressant. CEA-LETI mainly focus on the development of the bioactive microcapsule and tools to establish a method to encapsulate and characterize freshly harvested islets quickly.

Main Results
CEA-LETI developed a new GMP grade compatible platform to automate the encapsulation process of pancreatic islets and to provide standardized and reproducible bioactive microcapsules. This platform is composed of an instrument and microfluidic sterile and disposable modules in which the process of cell encapsulation is performed. The process is fully automated and only few steps of the process has to be done under a safety cabinet, mainly cells preparation steps. Thanks to this platform, it is possible to process unique composition of microcapsule.

Pancreatic islets are a cluster of different type of cells acting all together to release insulin in response to glucose level. The shape and size of islets are highly variable (Size going from 50µm to 400µm). These islets can be encapsulated alone or with other types of cells [1]. Characterization of viability after encapsulation is then a challenge as it is necessary to characterize a 3D structure and to discriminate different type of cells. To get relevant and reliable results, it has been necessary to develop new images analysis algorithm [2] (Work in collaboration with UGA).

Fig. 1: GMP compatible cell encapsulation platform

Fig. 2: Encapsulated human pancreatic Islets and confocal images analysis to characterize the viability

Perspectives
Within BIOCAPAN, a new bioactive microcapsule composition has been patented [3], new tools developed. Preclinical tests are ongoing to assess the ability of this new cell therapy to reverse diabetes.

RELATED PUBLICATIONS:
[3] Europe n° 19305415.2, 29 of March 2019
CONDUCTING POLYMER SCAFFOLDS FOR HOSTING AND MONITORING 3D CELL CULTURE

RESEARCH TOPIC:S
Cell culture, PEDOT-PSS, 3D scaffold, In situ monitoring

AUTHORS:

ABSTRACT:
This work reports the design of a live-cell monitoring platform based on a macroporous scaffold of a conducting polymer, poly(3,4-ethylene dioxythiophene): poly(styrenesulfonate) (PEDOT-PSS). The conducting polymer scaffolds support 3D cell cultures due to their biocompatibility and tissue-like elasticity, which can be manipulated by inclusion of biopolymers such as collagen. Integration of amedia perfusion tube inside the scaffold enables homogenous cell spreading and fluid transport throughout the scaffold, ensuring long-term cell viability. This also allows for co-culture of multiple cell types inside the scaffold. The inclusion of cells within the porous architecture affects the impedance of the electrically conducting polymer network and, thus, it utilized as an in situ tool to monitor cell growth. Therefore, while being an integral part of the 3D tissue, the conducting polymer is an active component, enhancing the tissue function, and forming the basis for a bioelectronic device with integrated sensing capability.

SCIENTIFIC COLLABORATIONS: Department of Bioelectronics, Ecole Nationale Supérieure des Mines Gardanne

Context and Challenges
Advances in tissue engineering have demonstrated that physical architecture of tissues is extremely important for correct differentiation and it is now well accepted that the in vivo environment, comprising both the composition as well as the 3D structure, can have a tremendous influence on the function and differentiation of the cells [1]. Considerable attention has thus focused on the development of biocompatible scaffold materials for hosting cells in 3D. A variety of synthetic and bio-derived polymers has been used to mimic the extracellular matrix or connective tissue. In terms of technology integration the major advances so far for 3D cell biology have been related to materials and methods used for scaffold preparation and integration of microfabrication techniques, for example for fluidics. What appears to be missing, however, are techniques for evaluation of tissues. A significant challenge relates then to integration of their monitoring tools with 3D models, such as impedance spectroscopy which is frequently used to monitor barrier properties of epithelial and endothelial tissues [2]. We propose here to implement 3D structured PEDOT-PSS, an electronically conductive polymer network and, thus, is utilized as an in situ tool for tissue engineering (Fig. 1a and 1b) owing to its recognized biocompatible and electrochemical properties [3].

Main Results
3D PEDOT-PSS architecture were fabricated according to an original protocol, based on freeze drying process, developed at CEA using PEDOT-PSS commercial ink. PEDOT-PSS foam (Fig. 1c) is formed and then used concomitantly as scaffold for the (co-)culture of cells and as internal electrode for the in situ monitoring of afferent tissue evolution. To enable this dual use, the pristine PEDOT-PSS ink was modified through the addition of GOPS (a silane-based cross-linker that ensure the mechanical stability of the scaffold without compromising its stiffness), collagen (a biopolymer that improves cell adhesion and growth) and DBSA (a sulfonated surfactant that improves PEDOT conductivity). The obtained 3D conductive material was proved to exhibit excellent biocompatibility using (co-)culture of different cell lines (Fig. 1d for fibroblast epithelial cells co-culture) and their viability characterization and colonization evolution using fluorescent dye staining.

Fig 1: (a) Schematic of the cell culture system embedded PEDOT foam and (b) image of the system. SEM picture of the PEDOT foam before (c) and after (d) fibroblast and epithelial cells co-culture.

PEDOT-PSS conserves its electrochemical activity and is then used to sense the cell culture evolution in real time. To exemplify such capabilities, electrochemical impedance spectroscopy measurement were made before and after cell growth and further after proteolytic treatment using trypsin.

Fig 2: Impedance spectroscopy of the PEDOT foam in absence, after growing and after digestion of MDCK cells.

Perspectives
The 3D scaffold infiltrated by cells herein can now be envisaged as a bioelectronic device that enables electronic, label-free sensing of cells. Here, the conducting polymer performs two distinct functions: (1) housing for cell culture with 3D physical contacts with cells, (2) “soft” electrode function allowing for monitoring of the presence of cells. Such scaffold-engineered living electrodes can be an alternative platform measuring cell viability against toxic components in cell environments.

RELATED PUBLICATIONS:
DIRECT TRANSFECTION OF CLONAL ORGANOIDS IN MATRIGEL MICROBEADS: A PROMISING APPROACH TOWARD ORGANOID-BASED GENETIC SCREENS

RESEARCH TOPIC:
3D organoids, 3D transfection, Microfluidics, Microencapsulation

AUTHORS:
B. Laperrousaz, S. Porte, S. Gerbaud, V. Harma, F. Kermarec, V. Hourtane, F. Bottausci, X. Gidrol and N. Picollet-D'hahan

ABSTRACT:
Organoid cultures in 3D matrices are relevant models to mimic the complex in vivo environment that supports cell physiological and pathological behaviors. Effective genetic engineering in organoids would bring new insights in organogenesis and carcinogenesis. However, direct 3D transfection on already formed organoids remains challenging. One limitation is that organoids are embedded in extracellular matrix and grow into compact structures that hinder transfection using traditional techniques. To address this issue, in collaboration with the BIOMICS laboratory (CEA-DRF), we developed a microfluidic device for single-cell encapsulation in Matrigel microbeads. It enables an innovative approach for transgene expression in 3D organoids by combining it to electroporation. We demonstrate that direct electroporation of encapsulated organoids reaches up to 80% of transfection efficiency.

Context and Challenges
While traditional 2D cultures on rigid surfaces fail to reproduce in vivo cell behavior, 3D matrices are becoming increasingly popular supports for cell cultures because they mimic the complex environment that supports cell physiological functions [1] to better predict in vivo responses and thus to limit the need for animal models [2]. Focus is made on the 3D transfection ability and therefore, deciphering the key genetic networks underlying epithelial differentiation and polarity in organoids to bring new insights in organogenesis and allow us to better understand how they may be disrupted in disease states such as cancer. Formation of 3D monodispersed microspheres of matrix as microenvironments for the organoids is key since it affects the transfection capability [3]. Even if microspheres provide thinner ECM, direct 3D transfection on already formed organoids remains challenging.

Main Results
For automatic generation of microencapsulated organoids, a new microfluidic, low temperature (4°C), instrument has been developed to process Matrigel and form monodispersed microspheres embedding organoids of controlled size and composition (Figure 1). Because the process of droplet formation is highly repeatable under laminar flow conditions, it is possible to produce thousands of identical bioreactors. Furthermore, this setup would be compatible with high-throughput screening applications.

Direct transfection of organoids in Matrigel microbeads with siRNA was possible by using electroporation, with a GFP silencing efficiencies of almost 80% (P = 0.029). These results highlight the innocuousness of electroporation to transfect RWPE-1 organoids in microbeads compared with other techniques. siRNA transfection was achieved at the center of the organoids in microbeads. These results demonstrate the general applicability of microbeads electroporation to transfect siRNA and to a lower extent plasmids, even into cells that are located at the center of organoids. Moreover, the transfection efficiency was improved by modulating microbeads size and Matrigel concentration.

Gene silencing in Matrigel microbeads validates p63 and PTEN as key genes in mammary and prostatic acinar development (Figure 2)
Altogether, these results demonstrate that electroporation of microencapsulated organoids that are grown in Matrigel microbeads is efficient in decreasing p63 and PTEN expression and leads to the failure of acini development accompanied by the transition toward a tumor-like phenotype.

Perspectives
This approach will also be useful in identifying potential RNAi therapeutics and in probing their effectiveness in a 3D culture environment that will more accurately predict in vivo cell response.

RELATED PUBLICATIONS:
02 SAMPLE PREPARATION AND µFLUIDIC

- µfluidic standardization
- Digital µfluidic using collapsible chambers
- Deterministic Lateral Displacement
- Miniaturized breath sampling
MICROFLUIDIC STANDARDIZATION: STATUS AND ROADMAP

RESEARCH TOPIC:
Microfluidic, Standardization, Manufacturing

AUTHORS:
N. Verplanck

ABSTRACT:
Since the Lab on a Chip concept was introduced in the 1990s, many scientific advancements have occurred. However, large-scale commercial realization of microfluidic technology is being prevented by the lack of standardization. There seems to be a gap between Lab on a Chip systems developed in the lab and those that can be manufactured on a large scale. We propose a modular platform, which makes use of standardized parts. Using this platform, a function-based method of designing microfluidic systems is envisioned based on a library of ready for use microfluidic building blocks that perform specific functions. Such a library of reusable and interoperable microfluidic building blocks is important to fill the gap between lab and fab, as it reduces the time-to-market by lowering prototype time cycles.

SCIENTIFIC COLLABORATIONS: Steering board of the MicroFluidic Association

Context and Challenges
With the development of new applications and the emergence of a few established companies capable of mass production [1], microfluidics has acquired the status of enabling technology. However, PhD students, researchers, start-ups often reinvent the wheel when they have to develop complex microfluidic systems. They spend too much time and money developing necessary but minor components, leak-proof fluidic connections, for example, and they do not focus enough on key functions. Thanks to design guidelines, we propose a modular approach (Figure 1a) and the opportunity for off-the-shelf components such as valves and connectors to develop quickly complex but reliable microfluidic chips.

Fig.1: (a) Complex lab-on-chip with a modular approach (courtesy EVEON), (b) Top view 15x15 Microfluidic Building Block with multiple ports and (c) reference point, nomenclature and pitch spacing for top fluidic connections issued from [2].

Main Results
The starting point has been the MFManufacturing European project (2014-2017). Through this project, we have demonstrated the efficiency of common design guidelines [2-3] to develop within a few months complex and reliable microfluidic products [4]. Then, the project consortium organized an ISO International Workshop Agreement (IWA23) [5], the first step to a standard. The project concluded in December 2017 with the creation of an international microfluidic association and the approval of the International Standard Organization (ISO) to write the first standard covering pitch spacing dimensions and initial device classification.

Technically, we have defined several chip formats and proposed footprints (Figure 1b) [2]. According to the proposed nomenclature, the hole A1 is at (3mm, 3mm) from a reference point (Figure 1c). Then, the pitch between two holes is a multiple of 1.5mm, preferably 3mm. It covers most of the commercial fluidic connectors and is compatible with miniLucer connectors (4.5mm).

With a standardized chip (Microfluidic Building Block or MFBB), you can easily use a standardized test bench and characterize properly your component following standardized testing protocols (Figure 4).

Fig2: Characterization of a CEA Leti valve (MFBB) to the Fluigent test bench (developed within MFManufacturing project) [4].

Perspectives
With more than 100 industrial and academic members, we have structured the association around working groups. The current working groups are modularity, interfacing, flow control and testing protocols. The role of each group is to identify the lack of standards and to propose guidelines for future standards. They are strongly interconnected: modularity with interfacing and flow control with testing protocols. To ensure a link between the association and ISO, some members are involved as experts in International (ISO TC48/WG3) and European (CEN TC332/WG7) working groups, coordinated by Nicolas Verplanck. The ISO group will write the standards based on IWA23 document by 2020. In parallel, this working group will manage new subjects based on the association feedbacks.

RELATED PUBLICATIONS:
QUANTITATIVE BIOLOGICAL ASSAYS WITH ON-CHIP CALIBRATION USING VERSATILE ARCHITECTURE AND COLLAPSIBLE CHAMBERS

RESEARCH TOPIC:
Microfluidics, Lab on Chip, Dilutions, Quantitative assays, Collapsible chambers

ABSTRACT:
A new microfluidic device composed of pneumatically actuated multiple collapsible chambers arranged in an X-Y architecture has been developed. Elementary fluidic functions such as fluid transfer, volumes calibration, mixing, aliquoting and linear dilutions can be parallelized, achieving automatically and rapidly complex operations. A hyper elastic membrane with a high elongation rate and switching between two polymer solid layers has been used to control precisely the fluid volumes. The ultimate aim is to perform fully integrated quantitative assays such as complete enzymatic assays and ELISA assays by using existing commercial kits and including on-chip calibration. This objective require to manipulate a high range of volumes (from 1 µL to 100 µL) while keeping an excellent accuracy.

Context and Challenges
Because of the increase of chronic diseases, there has been a growing interest in integrated, portable, disposable microfluidic systems [1]. To achieve a complete biological assay on a microfluidic chip, major fluidic operations such as dispensing, transport and mixing have to be implemented. Moreover, for a quantitative assay, our strategy is to integrate range of dilutions to generate an internal calibration, as recommended by the laboratory reference protocols. Standards solutions at different concentrations are prepared in the chip to perform a calibration curve and to compare the sample measurement to this curve. The challenge is to control this multi-steps long process as precisely as done in laboratory in an autonomous and portable device. To reach this objective, an original technology combining an X-Y architecture and collapsible chambers has been developed and validated through several protocols.

Main Results
Linear dilutions for a 7 level calibration curve with ratios ranging from 1 to 1/64 have been prepared and quantified by combining different volumes of water and dyed water (Figure 2a). The absorbance intensity depending on the predicted dilution factors is highly linear ($R^2=0.9995$). As examples of quantitative and multi-steps tests, two assays have been conducted using these architecture and technology. A glucose enzymatic assay has validated essential fluidic operations such as volumes calibration and aliquoting. A homogeneous glucagon ELISA test demonstrates an accurate linear calibration together with a precise measurement of a known concentration of glucagon in a sample (Figure 2b). A compact portable instrument [4] actuates the cards.

Fig. 2: (a) Fluidic validation of a homogenous Elisa assay for glucagon by using dyed water. (b) Validation of a homogenous Elisa assay. Plot of HRTF signal depending on the glucagon concentration.[3]

Perspectives
This architecture offers promising possibilities to integrate complex biological protocols with internal calibrations, parallelized analyses and to reduce the external equipment. Other protocol based on a heterogeneous Elisa test was also validated [4]; it integrates additional functions such as washing steps, surface functionalization, samples multiplexing. The next steps will integrate multiplex ELISA and add a detection module to measure the signal directly on the chip.

RELATED PUBLICATIONS:
SEPARATION OF BIOLOGICAL PARTICLES IN A MODULAR PLATFORM OF CASCADED DETERMINISTIC LATERAL DISPLACEMENT MODULES

RESEARCH TOPIC: Deterministic lateral displacement, Microfluidics, Sample preparation, Cell processing, Separation array, Particle isolation

ABSTRACT: Deterministic Lateral Displacement (DLD) has been extensively implemented in the last 15 years as a size-based sample preparation technique, applied to the isolation of blood cells, bacteria at the micrometer scale but also nanoparticles and exosomes at the nanometer scale. Size-based fractionation of complex biological samples into several sub-populations however remains challenging, as a DLD array generates two particles populations. A new modular approach, based on cascaded DLD modules, is proposed in order to process a biological sample through several purification steps. DLD modules are serially connected on an automated platform, which enables to operate independently and successively the sorting modules thanks to their temporary isolation with valves and deformable chambers. This approach is validated on a model sample before being applied to the sorting of bacteria from a blood sample. This work could open clinical perspectives for sepsis diagnosis at the micrometer scale, but also for the isolation of viruses and exosomes at the nanometer scale.

Context and Challenges
Isolation of a targeted sub-population from a complex biological sample is a frequently encountered issue in a diagnosis perspective or as an intermediate step for further analysis. Deterministic Lateral Displacement (DLD) is a passive microfluidic technique largely developed since 2004 for the purification of biological entities, such as blood cells, circulating tumor cells, or parasites, and is currently developed for the isolation of viruses and exosomes. In this size-based sorting device, particles larger than a critical diameter are deviated along a slanted pillar array, while particles smaller than this critical diameter zig-zag between the pillars and show a non-deviated trajectory along the channel length. Two sub-populations can then be isolated around a critical diameter and collected at the DLD outlets, the critical diameter of a DLD device being determined by the geometrical parameters of the pillar array [1] (Fig. 1A). One major challenge however remains the fractionation of a complex sample into several sub-populations: it would require the serial connection of several DLD components. The main issue, when connecting a DLD module to a downstream microfluidic system, is to keep a constant flow velocity across the DLD outlets in order not to disturb particles trajectory. We have developed such a cascaded DLD approach, first with a biphasic microfluidic system [2], and lately with a platform based on independent DLD modules [3].

Main Results
In this new approach, purification steps are performed successively and independently. DLD devices are isolated at each sorting step with valves and storage chambers equipped with a deformable membrane, which are integrated in a microfluidic cartridge and automatically actuated without any intermediate sample manipulation (Fig. 1B). These deformable chambers are intended to both collect the particles at the first DLD outlet (swelling of the membrane), and to inject the sample to the subsequent DLD (collapsing of the membrane under the controlled applied pressure) (Fig.1C). The platform of cascaded DLD modules has been validated with a model sample (polystyrene beads), before being applied to the fractionation of a complex sample, containing three main populations: prostate cancer cells (PC3) (13-19µm), red blood cells (RBC) (6-8µm) and E. Coli bacteria (2-5µm). The first DLD stage enabled the deviation (and removal) of 94% of PC3 cells, while the second DLD stage enabled the deviation of 77% of RBC, leading to the collection of a final sample containing more than 20% of E. Coli bacteria (close to the 25% theoretical recovery yield), with less than 8% of RBC and 0% of PC3 cells.

Perspectives
Working on the device throughput, these results could open perspectives in sepsis diagnosis, while extended at the nanoscale, they could be applied to the isolation of nanoparticles such as viruses or exosomes. Results have been collected in the framework of the on-going H2020 Viruscan project and the FUI-granted BactIDiAG project.

RELATED PUBLICATIONS:

AUTHORS: E. Partiset, C. Parent, Y. Fouillet, F. Boizot, N. Verplanck, F. Revol-Cavalier, A. Thuaire and V. Agache

Fig. 1: DLD channels (A). Experimental set-up with cascaded modules (B) and Illustration of the actuation steps of the deformable membrane (C).

Fig. 2: Validation with a complex biological sample: DLD outlets before and after centrifugation.


ABSTRACT:
This work presents the performances of silicon micro-preconcentrators (µPC) chips for breath sampling [1]. The silicon chips were coupled to a handheld battery powered system for breath sampling and direct injection in a laboratory GC-MS system through thermal desorption (TD). Similar performances of µPC were obtained compared to commercial TD while the volume of sample needed was reduced by a factor of 5. The performances of µPC for breath sampling on a single expiration were illustrated with the example of volatile tobacco markers tracking. Concentrations of benzene and toluene were found to be 10 to 100 higher in the breath of smokers. 2,5-dimethylfuran was only found in the breath of smokers. The efficiency of silicon chips for breath sampling could be in the future a key technology towards portable breath sampling and analysis.

Context and Challenges
The fine analysis of breath biomarkers is proposed in research laboratories as a non-invasive medical approach for disease screening, diagnostic and follow-up, in rupture to classical medical tests (blood/urine analysis, lumbar puncture and biopsy). This is possible because breath contains hundreds of volatile organic compounds (VOCs) related to the human metabolism and its environment. The concentrations of analytes present in breath vary from % to ppt. This explains that thermal desorption (TD) coupled with gas phase chromatography (GC) and mass spectrometry (MS) is the method of choice to resolve the complexity of breath. But the size of these analytical instruments confines breath analysis to the laboratory. Miniaturization is a general trend in analytical chemistry. It is mainly based on microfabrication techniques transferred from the semiconductor world. Despite the promising field of applications allowed by the combination of breath analysis and miniaturized systems, very few examples are described in the literature. In this work the use of this µPC is reported for breath sampling and injection in a laboratory gas chromatograph coupled with mass spectrometry.

Main Results
Performances of µPC were first compared to commercial TD for benzene trapping: similar chromatographic peaks after GC separation were observed while the volume of sample needed was reduced by a factor of 5. In addition, µPC were tested for breath samples collected in Tedlar® bags. Three analyses of the same breath sample displayed RSD values below 16 % for eight of the ten most intense peaks. Finally, the performances of µPC for breath sampling on a single expiration were illustrated with the example of volatile tobacco markers tracking. The signals of three smoking markers in breath, benzene, 2,5-dimethylfuran, and toluene were studied. Concentrations of benzene and toluene were found to be 10 to 100 higher in the breath of smokers. 2,5-dimethylfuran was only found in the breath of smokers.

The elimination kinetics of the markers were followed as well during 4 h: a fast decrease of the signal of the three markers in breath was observed 20 min after smoking in good agreement with what is described in the literature.

Perspectives
The smaller amount of breath necessary for analysis with µPC opens the possibility of single breath analyses. Finally, the ultimate goal is the development of a system integrating direct preconcentration separation and detection of the whole breath in a portable system for medical applications. This work has been sponsored by the French joint-ministerial CBRN-E R&D Program

Fig. 1: Handheld battery powered system used for breath sampling and direct injection in a laboratory GC-MS system

Fig. 2: Comparison of chromatograms after sampling the breath of 3 smokers and 3 nonsmokers. Smoking markers studied here are: benzene, toluene and 2,5-dimethylfuran.

Fig. 2: Comparison of chromatograms after sampling the breath of 3 smokers and 3 nonsmokers. Smoking markers studied here are: benzene, toluene and 2,5-dimethylfuran.

Related Publications:
Bacteria gram type differentiation

Elastic Light Scattering for pathogens identification

Quantification of gas mixtures

Biological tissues optical analysis

Encephalopsin immunoreactivity
ABSTRACT:
Fast and label-free techniques to analyze viruses and bacteria are of crucial interest in biological and bio-medical applications. For this purpose, optofluidic systems based on the integration of photonic structures with microfluidic layers were shown to be promising tools for biological analysis, thanks to their small footprint and to their ability to manipulate objects using low powers. In this collaboration the optical trapping of living bacteria in a 2D silicon hollow photonic crystal cavity has been proven. This structure allows for the Gram-type differentiation of bacteria at the single cell scale, in a fast, label-free, and non-destructive way.

SCIENTIFIC COLLABORATIONS: EPFL, CNRS-LTM, CEA-INAC
ELASTIC LIGHT SCATTERING FOR CLINICAL PATHOGENS
IDENTIFICATION: APPLICATION TO EARLY SCREENING OF
STAPHYLOCOCCUS AUREUS ON SPECIFIC MEDIUM

RESEARCH TOPIC:
Optics, Diagnosis, Artificial Intelligence, Elastic light scattering,
Bacteria identification

ABSTRACT:
Elastic Light Scattering (ELS) is an innovative technique to identify bacterial pathogens directly on culture plates. Here, we have developed ELS for clinical diagnosis, starting with S. aureus (SA) early screening. Our goal is to bring a result (positive/negative) after only 6 h of growth to fight surgical-site infections. The method, which combines optical instrument and statistical learning, was first applied on a reference dataset of 38 strains of SA and other Staphylococcus species (5459 images) collected on ChromID-SAID/MDRSA bi-plates, and then on a validation set including 20 patients. The best correct-identification rate between SA and non-SA (94.7%) has been obtained using a support vector machine (SVM) classifier trained on a combination of Fourier-Bessel moments and Local Binary Pattern operators. This statistical model applied to the validation set provided a sensitivity and a specificity of 90.0% and 56.9%, or alternatively, a positive predictive value of 47% and a negative predictive value of 93%. These results pave the way toward the WHO’s requirements for rapid, low-cost, and automated diagnosis tools.

Context and Challenges
This study addresses SA infections considered as the first cause of Surgical Sites Infections (SSIs) and the third cause of hospital acquired infections (HAIs). These infections drastically increase morbidity, mortality and healthcare costs, especially because of the rapid emergence and dissemination of resistant, or multi-resistant, SA strains. The best means to fight this clinical issue is to screen and decolonize all patients pre-operatively [1]. To successfully implement this protocol, there is a need for appropriate diagnosis tools able to communicate rapidly a positive (SA) or negative (non-SA) result to patients, caregivers and medical departments. The proposed ELS and AI based approach has unique advantages to improve the standards of care (Fig. 1) in terms of rapidity, affordability and automation [2,3].

Main Results
A SVM classifier was trained on two different types of descriptors (Fourier-Bessel moments and Local Binary Pattern operators) extracted from images of the whole dataset. The best Correct Identification Rates (CIRs), estimated using a 5-fold cross validation procedure, was obtained by directly learning at the species level and by merging the descriptors. The results are presented in the form of a confusion matrix (Fig 2). Starting with the reference dataset, the best CIR is 94.7%, which is in accordance with performance generally reported for chromogenic tests on 24-h cultures [4]. Once the model trained on the reference database, it was applied to classify images collected from clinical samples. Optimizing appropriately the classifier led to a specificity of 56.9% and to positive and negative predictive values of 47.2% and 93.0%, respectively. From a clinician point of view, such a high NPV value, means that a high degree of confidence can be assigned to the test.

Perspectives
The result of this study positions Elastic Light Scattering favorably on the major public health application represented by S. aureus screening. The technique will gain from producing and analyzing larger datasets, so opening up to the investigation of new classifiers such as neural networks and deep-learning algorithms.

Fig. 1: Description of the ELS identification technique, and comparison of its advantages with current standards of care.

Fig. 2: Confusion matrices for the best classification configuration. (A) reference dataset, (B) clinical trial

RELATED PUBLICATIONS:

A LINEAR-QUADRATIC MODEL FOR THE QUANTIFICATION OF A MIXTURE OF TWO DILUTED GASES WITH A SINGLE METAL OXIDE SENSOR

RESEARCH TOPIC:
Exhaled breath analysis, Acetone, Ethanol, MOX sensor, Source separation, Linear-quadratic model, Inverse problem

AUTHORS:
S. Madrolle, P. Grangeat and Ch. Jutten

ABSTRACT:
The aim of our work is to quantify two gases (acetone and ethanol) diluted in an air buffer using only a single metal oxide (MOX) sensor. We took advantage of the low selectivity of the MOX sensor, exploiting a dual-temperature mode to obtain diversity in the measures. Two virtual sensors were created to characterize our gas mixture. We presented a linear-quadratic mixture sensing model which was closer to the experimental data. To validate this model and the experimental protocol, we inverted the system of quadratic equations to quantify a mixture of the two gases. We presented an experimental evaluation on mixtures made of a few ppm of acetone and ethanol, and we obtained a precision close to the ppm. This is an important step towards medical applications, particularly in terms of diabetes, to deliver a non-invasive measure with a low-cost device.

SCIENTIFIC COLLABORATIONS: 
GIPSA-lab, Univ. Grenoble Alpes, UMR CNRS 5216, Saint Martin d’Hères

Context and Challenges
Gas sensors, especially metal oxide (MOX) gas sensors, are increasingly used in electronic noses to detect volatile organic compounds (VOCs). This is of particular relevance to medical applications because VOCs are abundant in breath and several VOCs are disease biomarkers. For example, acetone is linked to both diabetes and weight loss. The MOX sensors are low cost, small, and reversible. They present the all the necessary features for a portable device. However, because of their low selectivity, they are sensitive to several VOCs. Therefore, we have studied a dual temperature acquisition mode to allow to separate the targeted and the interfering gases. This digital separation is based on a signal model [1].

Main Results
We proposed to use two temperatures of the MOX sensitive layer, which corresponds respectively to 131 °C (T1) and 462 °C (T2). For a given sample of acetone and ethanol gas mixtures diluted in the air, we acquired data in the air buffer alone (V0) and in mixtures diluted in the air buffer (V1) and calculate the resistance using the equation given by the constructor. We present on the following figure the temporal curve of the sensor voltage during a measurement cycle.

![Sensor temporal responses presented for four mixtures of the two gases. On the right, enlarged portions near the signal peaks are presented for T1 and T2.](image)

We choose as measurement points the peak height values after the temperature transitions, to optimize the discrimination of the two gases. The peak height includes the dynamic behavior of the sensor, which is known to contain more information. The linear-quadratic model consists of linear, bilinear (interaction), and quadratic terms of non-integer power of the gas concentrations.

To evaluate the performance, we analyze a set of 39 mixtures with different concentrations of acetone and ethanol. Acetone concentration varied between 0 ppm and 20 ppm, and ethanol one between 0 ppm and 40 ppm (corresponding to the typical breath content after the absorption of a small glass of wine, approximately 8 cl). The correlation coefficient between the model and the experimental data was 0.97 and 0.96, respectively, for the first and the second temperature; and the relative error was low, 6.7% and 3.6%, respectively. To validate the linear-quadratic model proposed, we selected a K-fold cross-validation methodology. We achieved a precision on concentrations less than 1.5 ppm for both gases (acetone and ethanol). This precision of 1.5 ppm for acetone could allow detection for all types of diabetes (Type 1 and Type 2).

Based on this model, we have constructed source separation approaches associated with supervised [2] or unsupervised calibration [3]. The unsupervised approach requires a reduced number of calibration samples, coupled to a database of clinical samples. Those results are described in detail in the Ph. D. thesis of S. Madrolle [4].

Perspectives
The final goal is to apply the proposed method directly to human breath. This implies to increase the complexity of the gas mixture to be closer to the human breath, taking into account humidity, naturally present in human breath, or reducing the sample complexity to a smaller sub-space of unknown concentrations. Such a fingerprint will help to discriminate between samples for diagnostics, monitoring or treatment follow-up.
VALIDATION OF OPTICAL PROPERTIES QUANTIFICATION WITH A DUAL-STEP TECHNIQUE FOR BIOLOGICAL TISSUE ANALYSIS

RESEARCH TOPIC:
Quantification, Optical properties, Multispectral imaging, Diffuse reflectance, Spectroscopy, Calibration algorithm

ABSTRACT:
To approach wide-field optical properties quantification in real heterogeneous biological tissue, we developed a Dual-Step method that couples a punctual diffuse reflectance spectroscopy (DRS) technique with multispectral imaging (MSI). The setup achieves wide-field optical properties assessment through an initial estimation of scattering with DRS, which is used to estimate absorption with MSI. The absolute quantification of optical properties is based on the ACA-Pro algorithm that has been adopted both for DRS and for MSI. We validate the Dual-Step system on homogeneous Intralipid phantoms and heterogeneous gelatin phantoms with different scattering and absorbing properties.

Context and Challenges
The absolute quantification of both absorption and scattering optical properties of a heterogeneous tissue by using wide field MSI remains a scientific challenge; however it is essential to improve the accuracy of the clinical diagnosis. To address this issue, we propose a new method, namely the Dual-Step technique [1], which combines the quantification capacity of DRS with the spatial wide field of view (FOV) offered by MSI. Similar instrumental combinations have been developed for endoscopy offering wide field imaging and punctual Diffuse Raman, and fluorescence spectroscopies, to improve the sensitivity and specificity of cancer diagnosis [2-3] ; however, no optical property quantification maps are considered.

Main Results
The Dual-Step imaging technique combines Non-Contact DRS and MSI to quantify wide-field absolute scattering and absorption properties through noncontact measurements. The optimal scattering properties $\mu_s^*$ are estimated with local Non-Contact DRS measurements performed on the homogeneous zones of interest. Based on these $\mu_s^*$ estimations, 2-D absorption maps $\mu_a$ are obtained with MSI. The Dual-Step technique relies on a thorough instrumental calibration performed by the developed ACA-Pro algorithm [4], which has been extended for wide-field absorption quantification in this work. This includes the use of a correction factor CF reference base, CF interpolation, and an additional factor scaling the illumination intensity fluctuations between images [1].

Fig. 1: Scheme of the Dual-Step technique instrument, DRS and MSI are coupled via a translation table
As a proof of concept, we implemented the Dual-Step approach

Fig. 2: Flowchart describing the absorption quantification ($\mu_a$) method of MSI with the ACA-Pro approach (in blue), based on the Non-Contact DRS scattering estimation $\mu_s^*$

The wide-field quantification of the Dual-Step technique is validated with a range of well-characterized homogeneous Intralipid phantoms. Using Non-Contact DRS to estimate $\mu_s^*$ with an error inferior to 4.1%, the depth of field (DOF) of the technique lies within 1.2 mm. This leads to a maximum average $\mu_a$ relative error of 5.5% ± 4.03% for the studied range in this work with the MSI ACA-Pro calibration. Following the tests on gelatin heterogeneous phantoms, promising results have been obtained when using the Dual-Step quantitative approach to estimate optical wide-field properties on different abdominal ex-vivo human skin samples and in-vivo specific rat models (bicolored and inflammatory zones).

Perspectives
The DOF may be a limitation for biological samples where the sample curvature is higher than 1.2 mm leading to less accurate $\mu_s^*$ and $\mu_a$ estimation. Prospective work should focus in controlling the curvature of the sample to improve quantification accuracy on biological samples.

RELATED PUBLICATIONS:

AUTHORS:
V. Sorgato, M. Berger, C. Emain, C. Vever-Bizet1, J.-M. Dinten, G. Bourg-Heckly1 and A. Planat-Chrétien

SCIENTIFIC COLLABORATIONS: 1 Sorbonne Universités, UPMC University Paris 06, France
EVIDENCE FOR ENCEPHALOPSIN IMMUNOREACTIVITY IN INTERNEURONES AND STRIOSOMES OF THE MONKEY STRIATUM

RESEARCH TOPIC:
Neurosciences, Photobiomodulation

AUTHORS:
N. El Massri, K. M. Cullen, S. Stefani, C. Moro, N. Torres, A.-L. Benabid, J. Mitrofanis

ABSTRACT:
We examined the cellular distribution of encephalopsin expression in the striatum of nonhuman primates. In addition, because of our long standing interest in Parkinson’s disease and neuroprotection, we examined whether parkinsonian insult and/or photobiomodulation (670 nm) had any impact on encephalopsin expression in this key area of the basal ganglia. Striatal sections of monkeys were processed for immunohistochemistry. Our results revealed two populations of striatal interneurones that expressed encephalopsin, one of which was the giant, choline acetyltransferase-containing, cholinergic interneurones. The other population had smaller somata and was not cholinergic. Neither cell group expressed the calcium-binding protein, parvalbumin. There was also rich encephalopsin expression in a set of terminals forming striosome-like patches across the striatum. Finally, we found that neither parkinsonian insult nor photobiomodulation had any effect on encephalopsin expression in the striatum.

Context and Challenges
Recent studies have shown that photobiomodulation (600–1070 nm) improves motor behaviour and offers neuroprotection in a number of animal models of Parkinson’s disease. The precise cellular mechanism and neural pathways used by photobiomodulation are not entirely clear. Previous studies have shown that red to infrared light is absorbed by cytochrome c oxidase that acts as a chromophore. Recently, a population of neurones has been found within the brain that are activated by light using a distinct pathway to the one described above. These neurones have been shown to contain encephalopsin, or OPN3, a light-sensitive membrane proteins. Encephalopsin has been suggested to be involved in non-visual phototransduction, encoding circadian rhythms. Indeed, transcranially applied light has been shown to penetrate the cranium and, moreover, influence levels of encephalopsin expression within the brain.

In this study [1], we had two main aims: (1) to explore whether there was an extensive encephalopsin neuronal network system in the striatum, the main synaptic hub of the basal ganglia and a major region of lesion in Parkinson’s disease (2) to examine whether parkinsonian insult and/or photobiomodulation (670 nm) had any impact on encephalopsin expression.

Main Results
Encephalopsin immunoreactivity was found in two types of striatal cells. The total number of larger Eno+ cells in the striatum was estimated at 200,000±5000. The overall morphology of both types of Eno+ cells indicated that that they were interneurones. Cholinergic interneurones of the striatum also expressed encephalopsin. Both types of Eno+ cells were found uniformly across all areas of the striatum, not being localised to a particular striatal zone. However, patches of Eno+ terminals were very striosome-like in organisation.

Parkinsonian insult and/or photobiomodulation had no impact on the number and somal sizes of encephalopsin+ cells in the striatum.

RELATED PUBLICATIONS:
• HIV vaccination primate clinical trial
• Si-RNA delivery
• Standard methods for nanomedicine evaluation
• Closed-loop insulin delivery clinical trial
OVERCOMING IMMUNOGENICITY ISSUES OF HIV P24 ANTIGEN BY THE USE OF INNOVATIVE NANOstructured LIPID CARRIERS AS DELIVERY SYSTEMS: EVIDENCES IN MICE AND NON-HUMAN PRIMATES

RESEARCH TOPIC:
Nanomedicine, HIV vaccine, Adjuvant delivery system, HIV Gag p24, Lipid nanoparticles

AUTHORS:

ABSTRACT:
HIV is one of the deadliest pandemics of modern times, having already caused 35 million deaths around the world. Spread of the virus remains uncontrolled, thus exposing the worldwide population to HIV danger, due to the lack of efficient vaccines. Here, we describe the use of nanostructured lipid carriers (NLC), called Lipidot®, for the delivery of p24 protein as a model HIV antigen, with the aim of increasing its immunogenicity. We have designed vaccine formulations comprising NLC grafted with p24 antigen, together with cationic NLC optimized for the delivery of immunostimulant CpG. This tailored system significantly enhanced immune responses against p24, in terms of specific antibody production and T-cell activation in mice. More importantly, the capacity of NLC to induce specific immune responses against this troublesome HIV antigen was further supported by a 7-month study on non-human primates (NHP). This work paves the way toward the development of a future HIV vaccine, which will also require the use of envelope antigens.

Context and Challenges
Fighting the HIV pandemic is one of the major priorities for healthcare worldwide. Until now, only three prophylactic vaccine candidates have completed the efficacy trials of phases II-b and III. The results of these trials were unexpectedly disappointing. Particulate systems, including viral vectors and synthetic carriers, have proven to be excellent tools for the delivery of antigens to antigen-presenting cells (APC), promoting immune-specific responses with the production of antibodies and activation of cytotoxic T lymphocytes. Indeed, for their efficient capture and processing by APC, antigens must be in a particulate state. We recently described the delivery of protein antigens to DC for efficient antigen presentation to T cells using nanostructured lipid carriers (NLC) [1]. Here, we selected p24 as a relevant HIV antigen and to evaluate the efficacy of NLC for targeting it to DC. p24 represents a true challenge considering its poor immunogenicity, especially at inducing a humoral response. Therefore, we also analyzed p24-specific antibodies to assess the efficacy of our carrier at enhancing p24 humoral immunogenicity. Taking advantage of NLC’s versatility, we specifically designed particles bound with oligodeoxynucleotides (ODN) containing unmethylated cytosine-guanine repetitions (CpG) as an immunostimulant to be administered alongside p24 antigen. CpG is an adjuvant of great interest, known to induce an antiviral immunity with a pronounced Th1 profile. In this study, we examined the immunogenicity of p24-bearing NLC in mice and non-human primates (NHP), as the most relevant animal model to assess anti-HIV immunity.

Main Results
Despite significantly increased antibody responses, the cellular immune responses obtained, induced through the delivery of Gag p24, were very weak, suggesting weak immunostimulation by CpG. Here, we show that CpG vectorization by NLC+ not only enhanced antibody production, but also induced significant T-cell activation. More importantly, NLC+ strikingly promoted IFN-γ secretion by p24-specific T cells. The use of NLC is therefore highly valuable considering the impact on the resulting cellular activation. Altogether, these results demonstrated the advantages of using NLC to deliver both antigen and immunostimulant in order to combine the benefits from each of them to induce complex and potent immune responses.

After a 7-month study comprising four intradermal immunizations and bi-monthly sampling to analyze immune markers, our results confirmed the huge potential of NLC for promoting both p24-specific humoral and cell-mediated immune responses [2].

Perspectives
In terms of perspectives, NLC can be associated with other rational HIV antigens, such as envelope glycoproteins gp120 or gp41. With such a multi-antigen vaccine formulation, we aim at inducing multi-targeted immune responses, namely Env-specific broadly neutralizing antibodies and p24 specific cytotoxic responses, for protection against HIV.

RELATED PUBLICATIONS:
ARE EXISTING STANDARD METHODS SUITABLE FOR THE EVALUATION OF NANOMEDICINES: SOME CASE STUDIES

RESEARCH TOPIC: Preclinical characterization of nanoparticle-enabled medicinal products


ABSTRACT:
The use of nanotechnology in medical products has been demonstrated at laboratory scale, and many resulting nanomedicines are in the translational phase toward clinical applications, with global market trends indicating strong growth of the sector in the coming years. The translation of nanomedicines toward the clinic and subsequent commercialization requires the development of new standards for the physical characterization of nanoparticles-enabled medicinal products (NEMPs). The particle size distribution (PSD) and stability of NEMPs in complex biological environments are key attributes to assess their quality, safety and efficacy. Despite its low resolution, dynamic light scattering (DLS) is the most common sizing technique since the onset of NEP in pharmaceutical technologies. The work developed by the European Nanomedicine Characterization Lab (EUNCL) addresses the shortcomings of batch mode DLS and propose alternative physicochemical methods with the potential to qualify as future standards, including asymmetric flow field flow fractionation (AF4) and analytical ultracentrifugation (AUC).

SCIENTIFIC COLLABORATION: On the behalf of EUNCL consortium

Context and Challenges
The application of nanotechnology in healthcare has a tremendous potential to address a variety of medical conditions by providing better diagnostics and therapy. Unfortunately, when compared to the extensive research activities in research laboratories and industries, the clinically approved nanoparticle-enabled medicinal products (NEMPs) are still very limited. Very often NEMPs fail to reach late clinical phases due to the lack of pre-clinical characterization protocols, which are needed to correlate their physico-chemical (PC) properties with their biological effects. In this context, CEA is involved in multiple collaborative actions among global regulatory bodies, standardization authorities, and research agencies as well as stakeholders. Since 2015, CEA is an active part of the European Nanomedicine Characterization Laboratory (EUNCL) infrastructure, which was H2020 funded to unbiasedly support developers of NEMPs, by providing access to their multidisciplinary characterization facilities which are built on a comprehensive set of integrated preclinical methodologies and techniques (e.g., physical, chemical, in vitro and in vivo biological testing), and also by promoting knowledge and educational exchange with the NEMPs community.

Main Results
CEA, in collaboration with the US National Cancer Institute Nanotechnology Characterization Laboratory (NCI-NCL) and the EUNCL consortium has developed multiple robust standard operating procedures (SOPs), with suitable quality controls to measure particle size distribution and aggregation propensities in simple and complex biological media that are relevant for industrial and regulatory purposes [1]. NCI-NCL and EUNCL have jointly developed a multi-step approach of incremental complexity to measure particle size distribution and size stability of NEPs, consists of a quick preliminary step to assess sample integrity and stability by low resolution techniques such as batch mode DLS (pre-screening), followed by the combination of complementary high resolution sizing measurements performed both in simple buffers and in complex biological media [2-4]. Thus, the use of asymmetric flow field fractionation (AF4) with on-line size light scattering measurement (DLS and/or MALS), and analytical ultracentrifugation (AUC) were demonstrated to be very powerful approaches to measure particle size distribution (PSD) with high resolution and to assess size stability in simple and complex biological media. AF4-MALS-DLS was successfully applied to characterize pristine Med-NPs to (i) resolve the PSD of complex mixtures, (ii) discriminate between populations of particles with different shapes, (iii) discriminate between larger particles and small aggregates, and (iv) to monitor small changes of PSD due to instances like batch to batch variability or instability during long-term storage, all cases where batch mode DLS fails. AUC has been shown to be a fast and simple method for determining both the PSD and the free/encapsulated drug ratio [2]. In fact, AUC combines separation, concentration and detection steps into one single measurement improving total analysis times and reducing experiment complexity. Importantly both AF4-DLA-MALS and AUC allows to study the NEMPs behavior in presence of serum proteins, which is crucial to understand the biological effects of NEMPs in vitro and in vivo [2-4].

Fig. 1: Importance of particle size and particle size distribution for the quality and safety of Med-NPs [1].

Perspectives
The available PC standards applicable to NEMPs, and the current gaps in the characterization were recently discussed leading to the creation of a collaborative group including R&D, regulators, metrology and industrial experts, which will aim at proposing new standard methods addressing these gaps. Collaborative initiatives are starting to develop documentary standards to assess PC properties of liposomal formulations. CEA, is involved in a collaborative action to push AF4-DLS-MALS and AUC through the International standardization process.

RELATED PUBLICATIONS:
**CHITOSAN-LIPID NANOPARTICLES (CS-LNP): APPLICATION TO siRNA DELIVERY**

**RESEARCH TOPIC:**
Gene therapy, siRNA vectorization, Nanomedicine

**AUTHORS:**
Ö. Tezgel, A. Szarpak-Jankowska¹, A. Arnould, R. Auzély-Velty¹, I. Texier

**ABSTRACT:**
Small interfering RNA (siRNA) has received increased attention as gene therapeutic agent in a variety of diseases. However, siRNA has high negative charge and stiffness, is highly instable in serum, and should overcome numerous biological barriers before reaching its site of action, namely RISC (RNA induced silencing complex) in cell cytoplasm. Small chitosan/lipid nanoparticles (CS-LNPs) dedicated to siRNA delivery were formulated to benefit from the biocompatibility of lipid nanoparticles associated with the transfection ability of chitosan. Formulations of CS-LNPs were optimized for their physicochemical properties (size, zeta potential, colloidal stability) according to their shell composition, and their ability to complex and transfect siRNA into fibroblast cells and down-regulate ERK1 protein was evaluated.

**SCIENTIFIC COLLABORATIONS:** Ö. Tezgel, A. Szarpak-Jankowska, A. Arnould, R. Auzély-Velty, I. Texier

Context and Challenges
Small interfering RNA (siRNA)-based therapies hold great promises for a variety of diseases; however, their delivery to their site of action is highly challenging [1]. There is a variety of carriers available for siRNA delivery in the literature, such as cationic polymers, cationic lipids and nanoparticles. Among investigated cationic polymers, chitosan is well known as a biocompatible and low immunogenic material, with additional benefits such as muco-adhesive potential, cell permeation enhancement properties, and favoring siRNA endosomal escape. In parallel, lipid nanoparticles have gained great importance as therapeutic delivery systems as well as for siRNA delivery in the last two decades. Hybrid lipid core/chitosan shell nanoparticles have therefore been designed as innovative efficient siRNA nanocarriers for cell transfection, combining the advantageous characteristics of lipid nanoparticles and biological features of chitosan (Fig. 1).

**Main Results**
Chitosan-lipid nanoparticles (CS-LNPs) were synthetized in one step with particle diameter below 120 nm and zeta potential above +40 mV. Particle lipid core was composed of an amorphous mixture of soybean oil and wax, solid at ambient temperature but melting at 37°C. This lipid core can encapsulate a fluorescent payload for particle tracking, and is well tolerated [2]. The particle shell includes soybean lecithin, cationic (DOTAP), fusogenic (DOPE) phospholipids, and amphiphilic chitosan. To ensure their good anchoring at the nanoparticle surface, amphiphilic polymers were designed by hydrophobically modifying chitosan with dodecyl (C12) chains at different degrees of substitution. It was previously reported that 12 is the optimal number of carbons for chitosan to act as a polymeric surfactant. Optimized formulations were tested to determine their ability to protect siRNA in cell culture media and transfact cells (Fig. 2) [3]. Extracellular signal-regulated kinase1 (ERK1) was chosen as a target protein for the delivery of functional siRNA in NIH3T3 mouse fibroblasts. ERK1 is a member of mitogen activated protein (MAP) kinase family. It is an important player in various cellular processes involved in wound healing. Lipid nanoparticles formulated with 15,000 g/mol 2% C12 substituted chitosan, DOTAP and DOPE, mediated 40% ERK1 downregulation efficiency, comparable to lipofectamine™ RNAiMax, while displaying no cytotoxicity up to 500 µg/mL (Fig. 2).

![Fig. 1: Scheme of lipid/chitosan nanoparticles for siRNA complexation and cell delivery and transfection.](image1)

![Fig. 2: ERK1 down-regulation by CS-LNP/SiRNA.](image2)

**RELATED PUBLICATIONS:**
CUSTOMIZATION OF HOME CLOSED-LOOP INSULIN DELIVERY IN ADULT PATIENTS WITH TYPE 1 DIABETES: THE PILOT WP7 DIABELOOP STUDY

RESEARCH TOPIC:
Type 1 diabetes mellitus, Artificial pancreas, Closed-loop insulin therapy, Telemedicine, Remote monitoring

AUTHORS:
P.-Y. Benhamou, E. Huneker, S. Franc, · M. Doron, G. Charpentier on behalf of the Diabeloop Consortium

ABSTRACT:
Improvement in closed-loop insulin delivery systems could result from customization of settings to individual needs and remote monitoring. This pilot home study evaluated the efficacy and relevance of this approach.

SCIENTIFIC COLLABORATIONS: CERITD EVRY, CHU GA, Diabeloop SA, CHSF, CHU de Besançon, Caen, Lyon, Marseille, Montpellier, Nancy, Nantes, Reims, Strasbourg and Toulouse

Context and Challenges
Closed-loop insulin delivery [1] holds great promise for patients with type 1 diabetes. Several studies conducted at home in various experimental settings (population, duration of studies, type of algorithms, single or dual hormone systems) have established the relevance of this approach in improving metabolic outcomes. The pilot WP7 Diabeloop study was requested by ANSM in order to address the following objectives: a) to experiment the various manual settings made available to healthcare providers and patients that impact the algorithm reactivity and b) to check the reliability of the remote monitoring system using a web-based platform.

Main Results
The Diabeloop (Regulation v2017.04.20) algorithm is an MPC (model predictive control) system based upon [2]:
• personalization of the Hovorka et al. model using a window of past CGM (Continuous Glucose Monitoring) measurements, insulin delivery and meals.
• prediction of glucose variations over a 3-h window.
• prescription of change in insulin delivery from current time, to maximize the time spent in normoglycemia based on changes in the basal rate. This prescription is computed over a period of a few hours, but only the first rate is applied.

The algorithm also features a decisional matrix. This decisional matrix is based on diabetologists expertise to adapt insulin basal rate or insulin bolus based on current glycaemia and extrapolated glycaemia. A safety module aiming at preventing hypoglycemic events is implemented. It also deals with specific cases such as calibration events or management of pronounced physical activity. Iteratively, a recommendation is performed every 5 min.

Eight patients with type 1 diabetes (six men and two women) were included. Their characteristics were as follows: age 41.8 ± 11.4 years; BMI (Body Mass Index) 23.3 ± 2.7 kg/m²; HbA1c 7.7 ± 1.0% [interquartile range Q1–Q3: 6.9–8.6%]; duration of diabetes 25.1 ± 12.7 years; total daily insulin dose 43.0 ± 4.7 units. All patients have been on insulin pump therapy for more than 6 months and have been trained to flexible insulin therapy and carbohydrate counting in their history, with no retraining for purpose of the current study. Time spent in the 70–180 mg/dl range was 70.2% [67.5; 76.9]. Time in hypoglycemia < 70 mg/dl was 2.9% [2.1; 3.4]. Eleven SIMs led to phone intervention. Original default settings were modified in all patients by the intervention of the nurses.

<table>
<thead>
<tr>
<th>Metrics</th>
<th>Overall Closed-Loop Period (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% time</td>
<td></td>
</tr>
<tr>
<td>&gt; 360 mg/dl</td>
<td>0.0 [0.0; 0.3]</td>
</tr>
<tr>
<td>&gt; 300 mg/dl</td>
<td>0.0 [0.0; 1.1]</td>
</tr>
<tr>
<td>&gt; 250 mg/dl</td>
<td>4.2 [2.4; 6.3]</td>
</tr>
<tr>
<td>&gt; 180 mg/dl</td>
<td>27.3 [19.6; 29.7]</td>
</tr>
<tr>
<td>&gt; 50 mg/dl</td>
<td>0.2 [0.1; 0.4]</td>
</tr>
<tr>
<td>&lt; 70 mg/dl</td>
<td>2.9 [2.1; 3.4]</td>
</tr>
<tr>
<td>&lt; 50 mg/dl</td>
<td>0.4 [0.1; 0.1]</td>
</tr>
<tr>
<td>Number of episodes</td>
<td></td>
</tr>
<tr>
<td>&lt; 50 mg/dl</td>
<td>3.5 [0.5; 6.0]</td>
</tr>
<tr>
<td>&gt; 360 md/dl</td>
<td>0.0 [0.0; 1.0]</td>
</tr>
<tr>
<td>Average glucose (mg/dl)</td>
<td>146.9 ± 8.4</td>
</tr>
<tr>
<td>Median glucose (mg/dl)</td>
<td>149.0 [138.2; 153.8]</td>
</tr>
</tbody>
</table>

Table 1 Description of interstitial glucose profiles during closed-loop period.

This pilot trial suggests that the Diabeloop closed-loop system could be efficient regarding metabolic outcomes, whereas its telemedical monitoring feature could contribute to enhanced efficacy and safety [3].

Perspectives
This 3-weeks pilot trial is the first report of in-home achievements with the Diabeloop system. Two main information can be drawn from our data. First, we observed metabolic outcomes that were similar to the recently published various home studies (However, this was an uncontrolled pilot study with a small number of patients). Second, we showed that remote monitoring with dedicated nurses were allowed to adjust the settings of the algorithm is not only feasible and reliable but also safe as 10% of the SIMs led to a direct intervention of these dedicated nurses.

RELATED PUBLICATIONS:
• AI treatment of EEG for sleep stage scoring
• Physical activity expenditure prediction
• Standard methods for nanomedicine evaluation
• Review on internally placed motor brain computer interfaces
A CONVOLUTIONAL NEURAL NETWORK FOR SLEEP STAGE SCORING FROM RAW SINGLE-CHANNEL EEG

RESEARCH TOPIC: EEG, Supervised Learning, Convolutional Neural Network

ABSTRACT: A single-channel sleep scoring method will be interesting because it will allow light, wearable and unobtrusive system to study sleep disorders. We have developed a deep convolutional neural network (CNN) applied on raw EEG samples for building a 5-class sleep stage classification model. The system is trained using supervised data from a large multi-center cohort study including expert-rated polysomnographic records. Performance metrics reach the state of the art, with accuracy of 0.87 and Cohen kappa of 0.81.

SCIENTIFIC COLLABORATIONS: CHU Grenoble-Alpes, CHU Dijon

Context and Challenges
Sleep is an essential ingredient for good human health. Polysomnography (PSG) is the main tool for diagnosing, following, or ruling out sleep disorders based on the collection of various signals (EEG, EMG…). Sleep staging consists of dividing a PSG record into short successive epochs of 30 s and classifying each of these epochs into one sleep stage amongst a number of candidate ones, according to standardized classification rules [2]. Sleep staging is a tedious task, which requires considerable work by human experts. We propose to use deep learning tools to automate this task using a CNN model applied on a single-EEG channel.

Main Results
The input to the convolutional neural network (CNN) consists of the unprocessed EEG signal for the epoch to be classified, concatenated with two preceding/following epochs in order to conform to scoring rules [2]. No feature extraction was used and the CNN applies directly on raw EEG data. We use 12 convolutional layers followed by one fully connected layer and one last fully connected layer of size 5 with softmax activations. The activation functions for all others layers are leaky rectified linear units. A multiclass cross-entropy was used as a cost function, and mini-batch training for stochastic optimization of the weights and biases was carried out. A view of the CNN architecture is given in Fig.1.

The dataset is split randomly between a training, a validation and a test set, with respective proportions 50%, 20%, and 30%. Validation cost is monitored during training and a full pass on the validation set is done every 20,000 training batches. The test is performed on unseen participants. Models are implemented in Tensorflow and trained on an Nvidia GTX980Ti GPU for 300,000 mini-batches. The overall multiclass classification accuracy is 87% and the overall kappa is 0.81. Stage N2 is occasionally confused with stage N3 (4%) and rarely with other stages.

To understand deep signal representation, we also generate artificial inputs that maximize the output activation of each of the five output neurons, corresponding each sleep stage. A gradient descent algorithm is again used to reconstruct the input signals.

Perspectives
In this work, no domain-specific feature extraction step, as commonly done in EEG, is required and the network is learned end-to-end. We showed that the method is competitive in terms of performance and we also demonstrated that the network learned sensible pattern detection that can be visualized. Multimodality could be easily incorporated in this framework to obtain better sleep scoring results.

RELATED PUBLICATIONS:

Fig. 1: CNN architecture for EEG sleep scoring

Fig. 2: Visualization of reconstructed 10-s inputs that maximize the activation of each output neuron.

Fig. 2: Visualization of reconstructed 10-s inputs that maximize the activation of each output neuron.

Fig. 2: Visualization of reconstructed 10-s inputs that maximize the activation of each output neuron.
The variance explained by the predictions from one, and the reference protocol improves physical activity scores, and an index of the

In all three studies, the performances of the prediction model which, coupled with an automatic activity-recognition algorithm, improved the variance explained by the predictions from triaxial accelerometer counts by 43% of daily PAEE compared with models relying on a simple relationship between accelerometer counts and EE.

Context and Challenges
Accelerometry is often used to quantify physical activity energy expenditure (PAEE). Linear models are employed to regress EE based on accelerometer counts. In order to improve the accuracy of such models, we propose in [1] to first classify activity types based on accelerometer and machine learning and then use activity-specific linear models to estimate EE in a more robust manner.

Main Results
The present work relies on data collected during three distinct studies carried out at CRNH, Lyon, cf. Fig. 1. An activity-specific model predicting PAEE from accelerometer-counts was developed using data collected from subjects performing a set of standardized activities in the laboratory, with simultaneous PAEE measurement by indirect calorimetry. This activity-specific model was further coupled to a previously validated automatic activity-recognition (AAR) algorithm [2] to build the AAR model that enables activity-specific PAEE to be estimated directly from raw triaxial accelerometer data. The algorithm uses different signal features from the time and frequency domains recorded on the vertical axis, and an index of the signal scattering on all three axes, to classify 6-s consecutive epochs into 11 posture/activity classes.

In all three studies, the performances of the activity-specific models were systematically compared with those of a simple count-based linear model (SLM model) estimated during study 1 and with two models provided by the companion software of the Actigraph (Freedson) and Acticheart devices, cf. Fig. 2.

The study shows that AAR associated with an activity-specific model improves EE prediction from accelerometer counts with a unique hip-worn device, for both activity-by-activity and mean daily EE measures in unrestrained free-living conditions. The performances of the prediction model were similar to those from combined accelerometer and heart rate [3] monitoring but, importantly, without any need to individually calibrate the equations.

Perspectives
High convenience and good performances in both long-term and almost real-time PAEE monitoring favor the implementation of such models in epidemiological studies, as well as tools to promote physical activity in the general population or in patients. We hope this study will pave the way for future AAR algorithm development by others using different signal features, or coupled to more complex modelling approaches to lead to even better estimates, which should definitely be put to similar tests.

RELATED PUBLICATIONS:
DATA-DRIVEN TRANSDUCER DESIGN AND IDENTIFICATION FOR INTERNALLY-PACED MOTOR BRAIN COMPUTER INTERFACES: A REVIEW

RESEARCH TOPIC:
Brain Computer Interface, Data-driven transducer design and strategy, Clinical application requirements

AUTHORS:
M-C Schaeffer and T Aksenova

ABSTRACT:
Motor Brain Computer Interface (BCI) aim to open new opportunities to motor disabled subjects to regain limb mobility by controlling orthoses/prostheses. BCIs are systems that establish a direct communication pathway between the users’ brain and external effectors. The challenges of BCI systems is to adapt the brain signal decoder to the user’s brain to reach a high decoding accuracy. The paper review data-driven and user-specific decoders, their design and identification methods as well as decoding strategies. The article specified the approaches, which correspond / are mandatory for achieving a challenging goal of neuroprosthetics control out of laboratory and without assistance. The conclusions are used to design BCI system for clinical trial “BCI and tetraplegia” (in progress at CLINATEC®).

Context and Challenges
The context is CLINATEC® Brain Computer Interface (BCI) project. BCIs are systems that establish a direct communication pathway between the users’ brain activity and external effectors. The goal of the project is to provide the proof of concept that it is possible to control complex effectors, such as a 4-limb exoskeleton [1], thanks to brain activity monitoring [2] and decoding [3]. Motor BCIs aim to permit severely motor-impaired users to regain limb mobility by controlling orthoses/prostheses. This opens new opportunities to motor disabled subjects.

Main Results
The current progress and challenges related to the design of clinical-compatible motor BCI systems are discussed. Motor BCI systems benefit patients if the decoded actions reflect the users’ intentions with an accuracy that enables them to efficiently interact with their environment. Thus, one of the main challenges of BCI systems is to adapt the brain neuronal signal decoding/translation block to the user brain in order to reach a high decoding accuracy. This paper review the literature of data-driven and user-specific transducer design and identification approaches. Static and dynamic decoding approaches, linear and non-linear, offline and real-time incremental/adaptive identification algorithms are revised. More generally, popular BCI decoding strategies ‘Direct’ biomimetic kinematic decoding vs ‘Indirect’ mental-task decoding are revised in the context of perspectives of out-of-laboratory neuroprosthetics clinical applications. ‘Direct’ biomimetic decoding which exploits the activity of neurons naturally devoted to the control of a specific limb is considered as more natural and easy-to-use compared to ‘Indirect’ decoding which exploit the activity elicited in brain areas not exclusively devoted to the control of the limb of interest.

One of the major issue for motor BCI clinical applications is the ability to provide users with asynchronous control over the effector. Most motor clinical trials have been completed using a synchronous protocol, i.e., user intentions were not processed outside predefined, cued windows. Potential BCI users, however, express a strong desire for stand-alone BCI systems. Asynchronous (self-paced) BCI continuously available to users, and corresponding to stand-alone BCI systems, were specially considered.

Article is focused on internally-paced motor BCIs which rely on the brain patterns that are voluntarily elicited by users and are not system dependent.

The invited review article analyzes more than 300 references.

Perspectives
The article specified the approaches and methods which correspond / are mandatory for achieving a challenging goal of neuroprosthetics control out of laboratory and without assistance: internally-paced and self-paced adaptive BCI, using dynamic decoder and providing direct biomimetic effector control. The conclusions are used for BCI system design used for clinical trial “BCI and tetraplegia” (in progress at CLINATEC®).

RELATED PUBLICATIONS:
06 PHD DEGREE AWARDED

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- Pierre-Antoine RODESCH
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The development of vaccines was one of the major health advances of the last century, with the success of smallpox eradication in 1980. Historical vaccines, based on attenuated or killed pathogens thus strongly immunogenic were finally replaced by subunit candidates, much safer but also poorly immunogenic. Therefore, adjuvants such as vectors and immunostimulants were incorporated in vaccine formulations in order to generate immune responses of high magnitude. However, actual adjuvants authorized in human vaccines only trigger humoral immune responses, with the production of antibodies, which neutralize extracellular pathogens. Yet, some pathogens such as HIV require the induction of a cell-mediated immunity, necessary to eliminate viral reservoirs in infected cells. In this context, new adjuvant systems are being developed in order to identify the most efficient and safe candidates. Here we describe the approach followed to prepare a stable, safe and versatile vector consisting in lipid nanoparticles (LNP), named Lipidot® for the delivery of antigens. We first report the proof of concept of antigen delivery based on the model ovalbumin, leading to the significant enhancement of humoral responses in vivo in mice. Thereafter, we focused on the induction of cell-mediated immune responses through the vectorization of both antigens and immunostimulants. Several combinations and vectorization strategies were assessed in the aim to identify the best prototype for a study of protection against tumour challenge. Finally, we applied these systems to HIV and its capsid antigen p24, which allowed us to conduct an immunogenicity study on a non-human primate model. Altogether, these results highlight the versatility of LNP and their ability to induce potent humoral and cell-mediated immune responses.

Breath is of interest for medical applications such as screening, monitoring pathologies or exposures. Indeed, breath contains endogenous or exogenous volatile markers and its sampling is non-invasive. Although the sampling is simple, the complexity and variability of breath explain the few tests authorized by the health authorities. This thesis focused on the development of two analytical bricks dedicated to the analysis of breath: preconcentration chip and 2D-gas chromatography. Works on the former brick have shown that the micropreconcentrators developed in the laboratory show performances close to standard systems with two major advantages, the reduction of breath sampled volumes and their integration into simple, portable and battery-powered systems. Three smoking markers present in exhaled breath were studied using micropreconcentrators in real conditions to track their kinetics in human’s breath (3 smokers and 3 non-smokers). We finally performed preliminary integration work in dedicated samplers to exploit the benefits of micropreconcentrators for the collection of breath within a single expiratory sampling. We then reproduced a simple fluidic modulator architecture based on a Dean’s switch. This modulator was shown to be compatible with injection by thermodesorption and was compared to GC for the analysis of the same breath sample. We show that this architecture is of interest in modulating volatile exhaled compounds that could be co-eluted. Finally, we confronted our tools with breath samples of a patient suffering from a rare disease, phenylketonuria. Samples of urine head space and exhaled air from the patient were analysed.
The emergence of CdTe Photon Counting Detectors (PCD) with energy discrimination capabilities, opens up new perspectives in X-ray imaging. Medical and security applications are characterized by very high X-ray fluxes and consequently require a very fast shaper in order to limit dead time losses due to pile-up. However, if the shaper is faster than the collection of the charges in the semiconductor, there is a loss of charge called ballistic deficit. Moreover, variations of the electric field profile in the detector over time cause a change in the collection time of the charges. As a result, the conversion gain of the detector will be affected by these variations. The instability of the response is visible over time as a channel shift of the spectra, resulting in a false information of the photon energy. The aim of this work is to develop a method to correct this effect. We proposed a correction algorithm based on the use of two Single Delay Line (SDL) shaping amplifiers. A fast SDL is used to measure the X-ray spectra at high-count rates with limited count rate losses. A slow SDL is used to measure the full collected charge to estimate a correction factor for the compensation of the ballistic deficit fluctuations of the fast SDL. An important step is to sort the processed pulses to reject undesirable effects that may degrade the measurement of the correction factor. The proposed method was implemented in an FPGA to correct the ballistic deficit in real-time and to give a stable response of the detector at very high fluxes. The method was tested with a 4x4 pixels CdTe detector (3 mm thick and 800 µm) pitch, enabling to measure transmitted X-ray spectra in the range of 20-160 kV on 256 energy bins. The developed method was initially tested at low count rate with $^{57}$Co and $^{241}$Am sources, then at high count rates with an X-ray source. We prove the ability of our algorithm in providing a stable response of the detector over time without affecting the energy resolution (~7% at 122 keV) and the dead time (~70 ns).

Non-invasively taken, exhaled breath contains many volatile organic compounds (VOCs) whose amount depends on the health of the subject. Quantitative analysis of exhaled air is of great medical interest, whether for diagnosis or for a treatment follow-up. As part of my thesis, we propose to study a device to analyse exhaled breath, including these VOCs. This multidisciplinary thesis addresses various aspects, such as the choice of sensors, materials and acquisition modes, the acquisition of data using a gas bench, and then the processing of the signals obtained to quantify a gas mixture. We study the response of a metal oxide sensor (MOX) to mixtures of two gases (acetone and ethanol) diluted in synthetic air (oxygen and nitrogen). Then, we use source separation methods to distinguish the two gases, and to determine their concentration. To give satisfactory results, these methods require first to use several sensors for which we know the mathematical model describing the interaction of the mixture with the sensor, and which present a sufficient diversity in the calibration measurements to estimate the model coefficients. In this thesis, we show that MOX sensors can be described by a linear-quadratic mixing model, and that a dual temperature acquisition mode can generate two virtual sensors from a single physical sensor. To quantify the components of the mixture from measurements on these (virtual) sensors, we have developed supervised and unsupervised source separation methods, applied to this nonlinear model: independent component analysis, least squares methods (Levenberg Marquardt algorithm), and a Bayesian method were studied. The experimental results show that these methods make it possible to estimate the VOC concentrations of a gas mixture, accurately, while requiring only a few calibration points.
Over the past decades, Extracellular Vesicles (EVs) have demonstrated strong potential as new biomarkers for liquid biopsy. Indeed, since EVs are fingerprints of parent cells, they can be exploited as early diagnostic tools. However, owing to their small size and high heterogeneity, EVs are challenging to extract from biofluids. In particular, reproducible and standardized protocols are required to perform fast, efficient, and cost-effective preparation of undamaged EV subpopulations from limited sample volumes. Deterministic Lateral Displacement (DLD) appears to be a promising microfluidic technology for this preparation by means of passive and label-free separation. DLD performs size-based separation of particles around a critical diameter that can be fine-tuned according to design parameters in an array of micropillars. This thesis aims at making DLD separation more predictable, efficient, and easy-to-integrate. Based on both numerical / experimental developments, predictive models are proposed to anticipate particle behavior and to help in the design of DLD devices. In addition, this thesis also addresses the issue of system integration. An innovative approach of serial connection between DLD modules is proposed to address the sequential sorting of particles from a complex biofluid. Two biological applications illustrate the potential of these systems: the isolation of E. coli bacteria from human blood samples for sepsis diagnostics and the extraction of EVs from cell culture media with the perspective of liquid biopsy applications. And as sample preparation cannot be dissociated from detection or characterization, this thesis moreover highlights the potential integration of DLD in an all-in-one microfluidic device...
This work presents the development of a low-cost, wearable instrument for quantitative monitoring of skin physiological parameters toward non-invasive in vivo diagnostics. The instrument is based on the spatially resolved Diffuse Reflectance Spectroscopy (srDRS) technique, which provides absolute quantification of absorption and scattering endogenous properties of the probed tissue volume with a potential to discriminate between properties of individual skin layers. In the developed instrument, this potential is maximized by the use of a multi-pixel image sensor to perform contact, high resolution imaging of the diffuse reflectance. This study comprises the specification and validation of a novel srDRS system architecture based on the proposed approach, the implementation of this architecture into a low-cost, wearable device and the evaluation of the device performance both on tissue-simulating phantoms and in vivo. Results validate the potential of the instrument for the non-invasive, quantitative monitoring of tissue properties. The described approach is promising for addressing the analysis of layered tissue such as skin and paves the way for the development of low-cost, wearable devices for continuous, passive monitoring of tissue optical properties.

X-ray tomography is a non-destructive but radiating 3D imaging technology based on the transmission of X-rays through the object of study. This visualization technique is used mainly in three areas: medical diagnosis, non-destructive testing and security. Recent technological advances in the field of X-ray spectrometric detectors open up prospects for improving this imaging technique. We have developed a statistical reconstruction method called MLTR-ONE-STEP which allows to reconstruct the energy variability of the linear attenuation coefficient of the studied object. This approach is called "one-step" because it directly reconstructs the final volume from raw measurements issued from spectrometric detectors, which are in practice spread over 64 energy measurement channels. Nevertheless, the physical phenomena within the detector cause an energy distortion of the attenuation spectrum which is taken into account during reconstruction. The method used is part of the Bayesian framework and maximizes the log-likelihood of the model while taking into account the spatial a priori on the reconstructed volume. The objective of the method is to improve the quality of the final image and the quantification of the materials present. The experimental implementation of the MLTR-ONE-STEP reconstruction requires a calibration step before measurement. This one aims to estimate certain parameters of the direct physical model. This depends on the acquisition conditions (geometry, parameter of the source) and the detector. The final result is sensitive to the correct estimation of these parameters.
The goal of this thesis is to explore methods that allow the extraction of indices out of a multivariate EEG signal from comatose patients. These indices should have prognostic value or be useful for ‘event’ detection, the main targeted application being the early prognostication of post-anoxic coma. For this purpose we are using modern tools of statistical learning (neural networks, unsupervised feature learning), first as classifiers for the end-task (classification or detection) but also as medical indices extractors in order to provide instant feedback to the practitioner.
GREETINGS

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ANNUAL RESEARCH REPORT 2018

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