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2017

Annual research report

TECHNOLOGIES FOR BIOLOGY AND HEALTH

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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*.

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

DE LA RECHERCHE À L'INDUSTRIE
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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.

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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)

350
industrial partners

1,900
researchers

2,760
patents in portfolio

91,500
sq. ft. cleanroom space, 8" & 12" wafers

64
startups created

2017

Annual research report

TECHNOLOGIES FOR BIOLOGY AND HEALTH

Core R&D competencies of technologies for biology and health unit 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.

CONTENTS

ÉDITO

Patrick Chaton,
Head of Microtechnologies for Biology and Healthcare division

Pr Alim Louis Benabid
Chairman of the Clinatec Board

Pr Stéphan Chabardes
Clinatec Clinical Sector Director

Leti-Health covers a field of investigation ranging from the maturation of new technologies (Department of Technologies for Biology and Health - DTBS) to preclinical animal testing and even to clinical trials for implantable device evaluation (Clinatec, technology and biomedical research Center). Leti-Health is involved in health R&D in the broad sense as defined by the World Health Organization including medical aspects and medical diagnosis but also environmental control, food quality, wellness or safety.

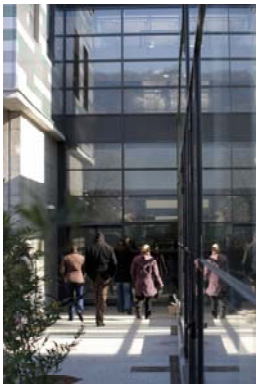
Leti-Health's strategy resides in the maturation of early-stage technologies to bring them towards the realization of operational demonstrators. In this context, our actions in 2017 bring together developments from upstream research to industry. From an upstream perspective, one of our flagship research projects addresses a major societal challenges of the future: personalized medicine. Thus, the year 2017 has seen the emergence of long-term projects around Organ on Chip -OoC- (one scanning the implementation of 3D cell cultures and associated microfluidics, the other on sensor networks to control the metabolism of microorganisms).

Also, personalized medicine represents one of the axes of reflection on artificial intelligence intelligence (AI). Our research is at the heart of this challenge with developments around the artificial pancreas, in collaboration with the start-up Diabeloop, and the brain-computer interface project BCI, with the development of innovative AI-based algorithms for real-time electrocorticography signal decoding via a unique implantable neuroprosthetics named WIMAGINE®. A BCI clinical trial is ongoing and a tetraplegic participant received a bilateral implantation in order to enable the control of external effectors such as the exoskeleton EMY, developed by CEATech/List. This world premiere performed at Clinatec will position Leti-Health at the forefront of neuroprosthetics development and eventual deployment.

2017 also highlights our involvement in the field of microfluidics. Leti Health is indeed an internationally-recognized player in this field with major advances in terms of innovative device design and participates in the creation of an association for its standardization and beyond to coordinate ISO's international standardization group.

In conclusion, 2017 has enabled us to strengthen our health device development strategy by serving industry while responding to societal challenges in the field through miniaturization and integration of streamlined prototypes for rapid industrial transfer to industry.

Key figures



157 permanent researchers

57 PhDs, Post-docs, and short term contracts

56 book chapters & journal articles

49 conferences & workshops

230 m² clean room for biochip packaging and surface chemistry

100 m² biological laboratory

430 m² chemical laboratory

300 m² microfluidics laboratory

6 patient rooms and a room for monitoring technologies

A fully equipped operating room with intraoperative MRI

Multimodal investigation capabilities (MEG, SPECT-CT, gait analysis)

35 patents filed

408 patents in portfolio

9 startups created

Scientific activity

Publications

56 books chapters and journals
49 conferences and workshops

Main papers:

- A. Eliseyev, V. Auboiroux, T. Costecalde, L. Langar, G. Charvet, C. Mestais, T. Aksenova, A-L. Benabid : "Recursive Exponentially Weighted N-way Partial Least Squares Regression with Recursive-Validation of Hyper-Parameters in Brain Computer Interface Applications"
  Scientific Reports 7, 16281 (2017)
  Doi: 10.038/s41598-017-16579-9

- R. Delacroix, S. N. A. Morel, L. Hervé, T. Bordy, J-M Dinten, M. Drancourt, C. Allier: "Cerebrospinal fluid lens-free microscopy: a new tool for the laboratory diagnosis of meningitis"
  Nature Scientific Reports 7, 39893 (2017)
  Doi: 10.1038/srep39893

Prize and awards

L'Oréal-UNESCO "For Women In Science"
Award: Eloïse Pariset.

Experts

4 Research Directors,
14 Senior Experts
22 Experts
10 owning the HDR

Participation in normalization groups

- International Medical Device Regulators Forum (IMDRF), "Software as a Medical Device (SaMD): Clinical Evaluation"

- 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, LIMMS, Politecnico di Milano, University of Pisa, Helmotz Association, University of Twente, UMC Utrecht, SINTEF, Tyndall, VTT, CSEM, EMPA, Fraunhofer, Charité Berlin, University of Liverpool, Helmoltz Association, Nanomedecine European technology platform, School of Medical Sciences, Sydney University.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O1 RADIATION DETECTION AND IMAGING OF LIVING ORGANISMS

- **Lens-free video microscopy**
- **3D lens-free microscopy**
- **X-ray spectroscopic imaging**
- **X-ray diffraction imaging**
- **Multimode gamma camera**
- **X-ray photon counting detector**
- **Spatially resolved DRS**

IMAGING OF DENSE CELL CULTURES BY MULTI-WAVELENGTH LENS-FREE VIDEO MICROSCOPY

RESEARCH TOPIC:

Lens-free microscopy, Video cytometry, Dense cell cultures, Quantitative microscopy

AUTHORS:

C. Allier, S. Morel, R. Vincent, L. Ghenim[1], F. Navarro, M. Menneteau, L. Hervé, X. Gidrol[1], Y. Usso[2] et al.

ABSTRACT:

We present our implementation of lens-free video microscopy for the monitoring of adherent cell cultures. We used a multi-wavelength LED illumination together with a dedicated holographic reconstruction algorithm. It allows to retrieve the phase image of the cells for densities up to those of confluent cell cultures (>500 cells/mm^2). In addition we prove that lens-free microscopy is a quantitative phase imaging technique enabling estimation of several metrics at the single cell level as a function of time.

SCIENTIFIC COLLABORATIONS: [1]CEA BIG, [2]TIMC-IMAG

Context and Challenges

Multi-height acquisitions and multi-angle acquisitions have been used to image dense biological samples such as tissue slides. These methods cannot be applied to the live monitoring of cell culture because the CMOS sensor warming-up, which is generated by the required large number of acquisitions, could kill the cells. A recent approach using four pigtailed lasers in the range of 640 nm to 660 nm has demonstrated the possibility of using only four acquisitions to image and monitor cell cultures up to a density of ~80 cells/mm^2. Inspired by these results, we have developed a novel lens-free video microscopy setup based on the use of three multicolored LEDs delivering red, green, blue illuminations, together with the implementation of a novel holographic reconstruction algorithm [1][2] (Fig. 1). The latter relies on the minimization of the total variation norm of the reconstructed cell phase image with a perfect constraint of data fitting. These results in reconstructed phase images of adherent cells are free of 'twin-image' artefacts up to the densities of confluent cell cultures (>500 cells/mm^2).

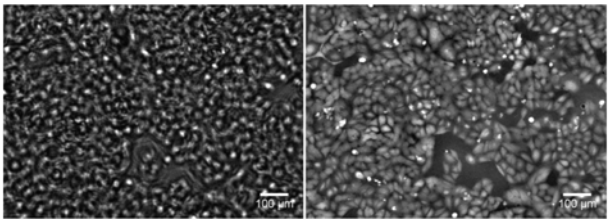


Figure 1: Left: raw acquisition of a culture of A549 cells (detail of the full field of view of 29.4 mm2). Right: multi-wavelength holographic reconstruction.

Main Results

We have demonstrated that a multi-wavelength reconstruction algorithm using three well separated wavelengths allows for an efficient and faithful reconstruction of the phase image of densely packed cells. We could image with high contrast cell colonies at densities up to approximately 700 cells/mm^2 over a field of view of 29.4 mm^2, with local densities as high as 1,650 cells/mm^2. The phase image quality is sufficient to provide robust automatic cell segmentation and tracking up to densities of 350 cells/mm^2. Hence large datasets can be gathered to study e.g. the motility and morphology of individual cells in a cell culture. In addition, our results demonstrate that multi-wavelength lens-free microscopy is a quantitative phase imaging technique. It allows an accurate determination of the cell dry mass, and the monitoring of the intra-cycle cell growth. Overall, multi-wavelength video lens-free microscopy provides a simple performant platform to monitor large cell colonies over several days, and perform downstream label-free automated cell segmentation and cell tracking (Fig.2) [2].

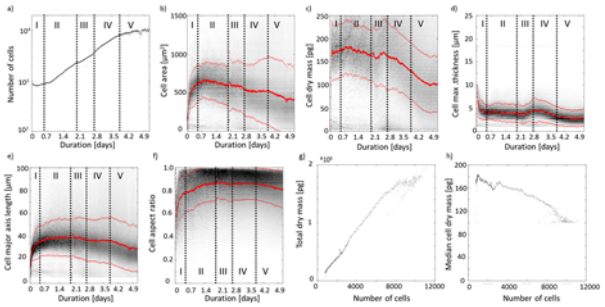


Figure 2: Data analysis of a 5 days lens-free time-lapse of 3T3 fibroblast cells. (a) Total number of cells counted in the full field of view of 29.4 mm2. (b) Scatterplot as a function of time of cell segmented area, (c) cell dry mass, (d) cell maximum thickness, (e) cell major axis length, and (f) cell aspect ratio. Every scatter plot compiles about 3.106 measurements. The median curve is plotted in red and standard deviation curves are plotted with red dashed lines. The phases I–V separated by black dash-lines correspond to different kinetic phases of the cell cultures (g) Plot of the total dry mass. (h) Plot of the mean cell dry mass as a function of the total number of cells.

Perspectives

We have introduced the first results of quantitative phase imaging obtained by means of lens-free microscopy. This is an important milestone and further works will be conducted to improve this ability of lens-free microscopy to perform quantitative measurements of cell cultures.

RELATED PUBLICATIONS:

[1] C. Allier et al., "Imaging of dense cell cultures by multi-wavelength lens-free video microscopy", Cytometry Part A, 2017 May;91(5):433-442. doi: 10.1002/cyto.a.23079.

[2] C. Allier et al., "Dynamic quantitative analysis of adherent cell culture by means of lens-free video microscopy", Proc. SPIE 10503, Quantitative Phase Imaging IV, 105031R (23 February 2018); doi: 10.1117/12.2289525; https://doi.org/10.1117/12.2289525.

COMPARATIVE STUDY OF FULLY 3D RECONSTRUCTION ALGORITHMS FOR LENS-FREE MICROSCOPY

RESEARCH TOPIC:

Diffractive optics, Digital holography, Image reconstruction techniques, Imaging systems, Three-dimensional microscopy

AUTHORS:

A. Berdeu, F. Momey, B. Laperrousaz[1], T. Bordy, X. Gidrol[1], J.-M. Dinten, N. Picollet D'hahan[1], C. Allier

ABSTRACT:

We propose a 3D imaging platform based on lens-free microscopy to perform multi-angle acquisitions on 3D cell cultures embedded in extracellular matrices. Lens-free microscopy acquisitions present some inherent issues such as the lack of phase information on the sensor plane and a limited angular coverage. We developed and compared three different algorithms based on the Fourier diffraction theorem to obtain fully 3D reconstructions of volumes as large as 5 mm^3. These algorithms present an increasing complexity associated with a better reconstruction quality.

SCIENTIFIC COLLABORATIONS: [1]CEA BIG

Context and Challenges

A previous work [1] introduced a method for 3D lens-free tomography for large 3D biological samples but presented various artefacts. To improve both the acquisitions and reconstruction methods, we present here a new design enhancing the angular coverage of the object. Based on this new imaging device, we tested and compared new algorithms to overcome the two pitfalls of lens-free tomographic acquisitions: the lack of phase information in the in-line holographic configuration and the limited angular coverage.

Main Results

We presented a novel tool to perform acquisitions on large 3D cell cultures (Fig 1). Based on the in-line holographic principle, it can image unlabeled and unstained living samples.

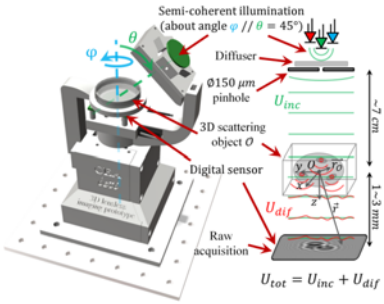


Figure 1: Setup dedicated to lens-free diffractive tomography

To overcome the limitations raised by such a microscope (lack of phase information on the data and limited angular coverage), we developed three dedicated fully 3D reconstruction algorithms all based on the Fourier diffraction theorem as used in standard diffractive tomography [2]. We showed that these algorithms are able to reconstruct the 3D object but with different qualities in terms of contrast to noise ratio and computational time (Fig. 2). The algorithm based on a phase ramp (Fig. 2c) is fast but leads to a signal which can be hard to distinguish from the artefacts and the noise. Providing the best contrast, the algorithm based on the 3D inverse problem approach (Fig. 2e) can nevertheless be extremely time consuming. It appears then that the choice of the algorithm depends on the targeted application. To identify isolated single cells, which provide a strong signal, in a 3D volume, the first algorithm can be sufficient. If one aims at reconstructing complex overlapping structures, 3D regularized iterative reconstruction provides a more pertinent result.

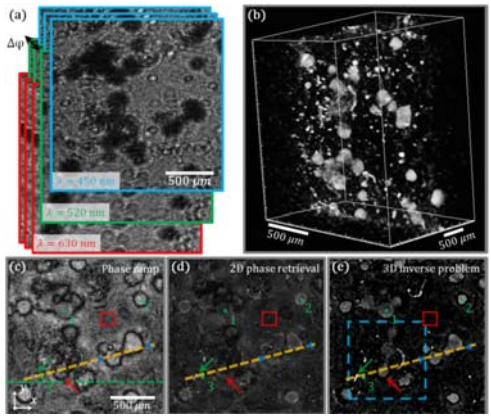


Figure 2: Comparison of the reconstruction methods on a RWPE1 cell culture. The cells tend to form organoids when embedded in Matrigel®. (a) Raw data at the three wavelengths and at different angles. (b) 3D visualization of the reconstructed volume (4.7mm3) with the 3D inverse problem method. (c-e) Comparison of the three methods for a volume slice at z = 0 µm.

Comparison with standard microscope views showed that the fully 3D reconstructions are accurate in terms of morphology and positioning. The proposed lens-free device provides thus a cheap and easy to use tool with a good sectioning in the z-direction on large volumes. To our knowledge, our technique is the only one able to reconstruct very large 3D cell culture volumes (~5 mm^3) by phase contrast imaging.

Perspectives

Following this work, we have conducted for the first time 3D+time acquisitions of 3D cell culture [3]. 3D lens-free video-microscopy becomes hence a very interesting platform to monitor e.g. organ on chips.

RELATED PUBLICATIONS:

[1] F. Momey et al., "Lensfree diffractive tomography for the imaging of 3D cell cultures", Biomedical Optics Express 7, 949–962, 2016.
[2] A. Berdeu et al., "Comparative study of fully 3D reconstruction algorithms for lens-free microscopy", Appl. Opt., 2017 May 1;56(13):3939-3951. doi: 10.1364/AO.56.003939.
[3] A. Berdeu et al., "3D+time acquisitions of 3D cell culture by means of lens-free tomographic microscopy", Proc. SPIE 10499, Three-Dimensional and Multidimensional Microscopy (23 February 2018); doi: 10.1117/12.2289474;https://doi.org/10.1117/12.2289474.

AN FPGA-BASED ALGORITHM FOR THE CORRECTION OF THE INSTABILITY OF HIGH-RESOLUTION AND HIGH-FLUX X-RAY SPECTROSCOPIC IMAGING DETECTORS

RESEARCH TOPIC:
X-ray imaging, Radiation detection, Signal processing

AUTHORS:
C. de Cesare, A. Brambilla, P. Ouvrier-Buffet, S. Stanchina, O. Rossetto[1]

ABSTRACT:
Advances in pixilated Cadmium Telluride detectors and fast readout electronics have resulted in the development of Photon Counting X-ray imagers able to provide energy dependent information which can be exploited for material characterisation and identification (for medical and security applications). However, instabilities affect the response of these detectors and degrade their performances. The aim of this work is to develop real-time digital algorithms implementable in an FPGA (Field-Programmable Gate Array) to correct these effects and to give a stable response of the detector even at very high fluxes.

SCIENTIFIC COLLABORATIONS: [1]LPSC -UGA

Context and Challenges
We have developed a pixelated CdTe detector coupled to a novel fast readout circuit that provides hyperspectral images of the transmitted X-rays on 256 energy bins [1]. The spectral information provides compositional information on the imaged objects [2] which can be used for the detection of illicit materials in luggage control. In the medical field, it is used to quantify tissue composition or contrast agent concentration [3].

Main Results
To be able to work at very high count rates required by these X-ray imaging applications, the signal from the detector is processed by a fast shaping amplifier. As a consequence, the detector is operated under ballistic deficit regime: the output signal from the first amplification stage is incompletely collected during the short integration window of the shaping amplifier. This allows to limit pile-up rate while preserving acceptable spectrometric performance. However due to the small ballistic deficit the gain of the conversion of the detector is sensitive to small temporal variations of the electric field profile due to charge trapping in the semi-conductor. These variations result in instabilities of the spectrometric response of the detector during the first hours of operation and degrade the detector performance.

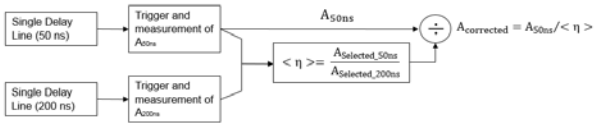


Figure 1: Diagram of the real-time digital algorithm implemented on FPGA for the ballistic deficit compensation

We introduced [4] a processing algorithm implemented in an FPGA and working in real time that effectively corrects these instabilities (Fig. 1). A fast Single Delay Line (SDL) shaping circuit is used to record the X-ray spectra at high count rate. A second slow SDL circuit is used to measure the complete signal in order to calculate the ballistic deficit and to compensate its effect in real time.

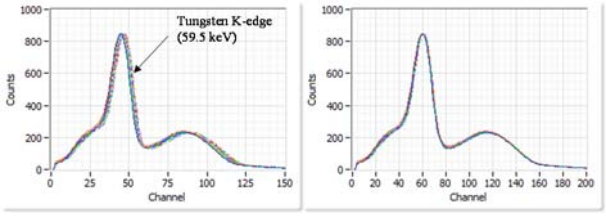


Figure 2: 160kV X-ray spectra transmitted by 0.5mm of tungsten without (left) and with ballistic deficit compensation (right) measured at different times.

Thanks to this correction method, the detector stability is reached in less than 20 minutes of operation, compared with 1 hour or more without ballistic deficit compensation. The major challenge is to correctly measure the ballistic deficit at high count rate. With the use of an effective pile-up rejection algorithm, the correction has been successfully used at count rates up to 1.6×10^6 c/s.

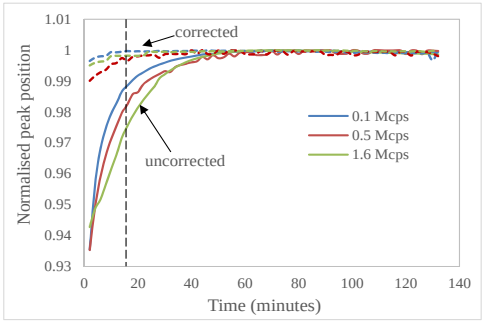


Figure 3: Normalized position of the tungsten K-edge peak at different count rates, before (full lines) and after ballistic deficit real-time compensation (dotted lines) of the ballistic deficit.

Perspectives
The developed algorithm preserves the spectrometric performance of the detector in terms of energy resolution and count rate, while ensuring stable operation. Future work will consist in implementing the correction method at the pixel level in 2D matrix detectors.

RELATED PUBLICATIONS:
[1] A. Brambilla et al., “Fast CdTe and CdZnTe semiconductor detector arrays for spectroscopic X-ray imaging”, IEEE Transactions on Nuclear Science, 60 (1), art. no. 6395225, pp. 408-415, 2013.
[2] A. Brambilla et al., "Basis material decomposition method for material discrimination with a new spectrometric X-ray imaging detector”, Journal of Instrumentation, 12 (8), art. no. P08014, 2017.
[3] Y. Pavia et al., “Breast density and iodine quantification in spectral mammography”, Biomed. Phys. Eng. Express 4, 015008, 2018.
[4] C. de Cesare et al., "An FPGA-Based Algorithm for the correction of the Instability of high-resolution and high-flux X-Ray Spectroscopic Imaging Detector". RTSD, 2017.

PERFORMANCE IMPROVEMENT OF AN X-RAY DIFFRACTION IMAGING SYSTEM USING SUB-PIXEL POSITIONING WITHIN CZT-DETECTORS

RESEARCH TOPIC:

X-Ray diffraction, Baggage control, CdZnTe detectors

AUTHORS:

J. Tabary, D. Kosciesza[1], O. Monnet, G. Montémont, J-P. Schlomka[1], S. Stanchina, J-M. Casagrande, L. Verger

ABSTRACT:

X-Ray diffraction (XRD) is a prevailing technique to detect illicit materials under polycrystalline, liquid or amorphous state. However, XRD systems designed for baggage screening generally suffer from poor photon count statistics, because of the tight collimations and the small scattering angle. To improve these factors, techniques of sub-pixelation can be implemented in CdZnTe detectors to enable the collimation opening without angular resolution degradation. In this paper, we illustrate this sensitivity gain on the XDi system by using an upgraded version of the IMADIF module, which was developed in Leti.

SCIENTIFIC COLLABORATIONS: [1]Smiths Detection)

Context and Challenges

X-ray diffraction (XRD) is proven to be an effective technique for baggage screening at airports, as it can reveal inter- and intramolecular structural information of any solid substances, but also of liquids, aerosols and gels. The XDi screener has been designed by Smiths Detection with an innovative architecture to enable XRD imaging with reasonable throughput of baggage (up to 600 bags per hour) for cabin baggage screening (CBS).

Leti has developed an autonomous detector module, called IMADIF, as crucial component of the XDi screener (Fig.1). The IMADIF module is a pixelated CZT detector, equipped with specific ASICs and an FPGA for signal read-out and control. Charge-sharing and depth of interaction corrections are performed on the fly in order to meet the performances required for diffraction: high energy-resolution, efficiency, and stability [1].

Figure 1: XDi system: CBS system with XRD imaging for automatic detection of dangerous liquids

We present in this paper how those detectors have been enhanced with sub-pixel positioning, and how it can be exploited to improve the whole system performance.

Main Results

A new version of the IMADIF module firmware (including FPGA bitcode and control software) has been developed to exploit the signals induced on neighboring anodes within CZT detectors and then estimate the 3D positioning of each interaction inside the detector. This technique is called sub-pixelation [2] and it enables subdivision of each line of the IMADIF module in 8 virtual sub-lines, without degrading the energy resolution.

Peak resolution of an XRD spectrum is a combination of angular resolution (defined by the collimation) and energy resolution of the detector used to detect scattered beam. Thus, one can adjust the collimation aperture to improve either angular resolution (or so of peak resolution) or sensitivity. For that, the sub-pixelation along the angular direction is interesting as it can improve the tradeoff between angular resolution and sensitivity [3].

Experimental validation has been performed using the Leti diffraction test bench to illustrate the improvement brought by sub-pixelation of IMADIF modules to the XDi system. The idea was to keep the current XDi system geometry (parallel architecture) and to open the secondary collimation through a new positioning of collimation stacks.

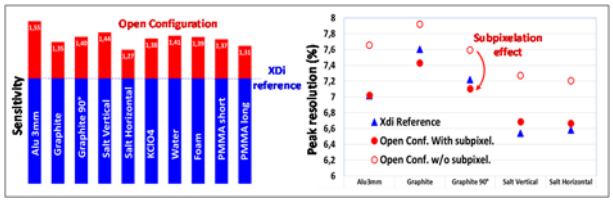


Figure 2: Performance improvement (sensitivity on left, peak resolution on right) with sub-pixelation.

For that, two configurations have been tested and compared (Fig 2): the XDi reference (in blue), and a more open configuration (in red). Several acquisitions done on amorphous and crystalline materials (such as PMMA, water, graphite, salt) have confirmed that sub-pixelation in combination with a more open collimation could improve sensitivity (up to around 40%) without degrading angular resolution.

Perspectives

Next studies will be to consider how sub-pixelation can be exploited in even more open collimations where each pixel sees the object through different slots at different angles.

The use of such multiplexed geometries will require the development of sophisticated reconstruction algorithms, but it may provide even greater sensitivity (to achieve throughput requirements) and potentially better spatial resolution.

RELATED PUBLICATIONS:

[1] G. Montémont et al., “An Autonomous CZT Module for X-ray Diffraction Imaging”, in Room Temperature Semiconductor Detector Conference Record, 2013, RTSD IEEE, 2013.
[2] G. Montémont et al., “Studying Spatial Resolution of CZT Detectors Using Sub-Pixel Positioning for SPECT”, IEEE Transactions on Nuclear Science, vol. 61, no. 5, pp. 2559–2566, Oct. 2014.
[3] J. Tabary et al., "Performance Improvement of an X-ray Diffraction Imaging System Using Sub-pixel Positioning Within CZT-Detectors", in Room Temperature Semiconductor Detector Conference Record, 2016, RTSD IEEE, 2016.

NUVISION: A PORTABLE MULTIMODE GAMMA CAMERA BASED ON HISPECT IMAGING MODULE

RESEARCH TOPIC:
Gamma-ray imaging, CdZnTe semiconductor detectors

AUTHORS:
G. Montémont, P. Bohuslav[1], J. Dubosq[1], B. Feret[1], O. Monnet, O. Oehling[1], L. Skala[1], S. Stanchina, L. Verger, G. Werthmann[1].

ABSTRACT:
We have recently developed NuVision, a portable gamma camera using CdZnTe (CZT) semiconductor detectors. It is based on the HiSPECT imaging module architecture using IDeFX-HD ASICs [1] with 0.3 mm spatial resolution and an overall energy resolution of 2.5 % at 122 keV and 1.5 % at 662 keV. The system uses coded aperture mask imaging to provide high sensitivity and high angular resolution in a limited frontal field of view (45°). Compton imaging is used to coarsely localize out of field radiation. We then take profit of the complementarity of these imaging methods in a single multimode camera.

SCIENTIFIC COLLABORATIONS: [1]NUVIAtech instruments

Context and Challenges
Far field gamma ray imaging is used in several applications: homeland security, dismantling, safeguards and dose monitoring. They have conflicting requirements: sensitivity, high dose rate capability, energy resolution, field of view, angular resolution.

The CZT gamma imaging module has a 40x40 mm footprint and is four side buttable. The key feature of our system is sub-pixel positioning capability that allows localizing events with high precision inside the crystal. As a result, with only 256 readout channels (2.5mm pitch), it is possible to acquire 128 x 128 pixel images without compromising energy resolution [2]. This design allows Compton and coded aperture imaging at the same time [3].

Main Results
Coded aperture is known to be a sensitive and accurate imaging technique for low energy gamma rays. However, its field of view is limited and performance drops for high energies. Conversely, Compton imaging allows all-directional imaging (on 4π steradians). It works well for high energies (above 300 keV) but has a limited angular resolution. Compton information can be useful to spot out of field sources, allowing the user to reorient the camera afterwards if a more precise image is required.

We combine both techniques to build multi-isotope images (Fig. 1) and cover wide angle (Fig. 2).

Figure 1: Example multi-isotope image from the camera obtained at real time in a 1 s frame – 3.7 MBq Am-241 source in red and 1.7 MBq Co-57 in green.

Gamma images are obtained by using a statistical iterative reconstruction algorithm based on Maximum Likelihood Expectation-Maximization method. The list-mode implementation we use is fast enough to enable fast reconstruction and real time imaging. For example, image from Fig. 1 is obtained by the real time processing of a one-second data frame (containing only 300 photons).

Data from Compton event is integrated to extend the field of view of coded-aperture image and to get rid of artefacts caused by out of field sources. Compton imaging extend the 45° wide central field of view covered by the coded aperture imaging with the full 4π field of view, but with a degraded angular resolution (typically 15° at 662 keV instead of 3.5° with coded aperture).

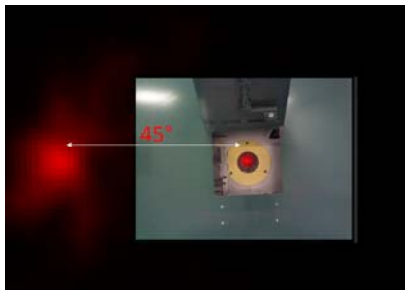


Figure 2: Use of Compton imaging to complement coded aperture imaging with two Cs-137 sources (10 µSv/h at the center and 18 µSv/h located 45° on the left).

Perspectives
We are currently working on new features like absolute dose rate quantification of hot spots by using a rangefinder. We are also refining physical models used for reconstruction to enhance image quality and sensitivity.

At the same time, camera ergonomics and design is being improved to ease camera operation for the end user. Software user interface is also evolving to provide a good trade-off between needs of the expert while keeping enough simplicity for the average user.

RELATED PUBLICATIONS:
[1] G. Montémont et al., "Development and evaluation of a portable CZT coded aperture gamma camera", ANIMMA conference, Lisbon, 2015.
[2] G. Montémont et al., "Studying Spatial Resolution of CZT Detectors Using Sub-Pixel Positioning for SPECT", IEEE Transactions on Nuclear Science, vol. 61, no. 5, pp. 2559–2566, Oct. 2014.
[3] G. Montémont et al., "Nuvision: A Portable Multimode Gamma Camera Based on Hispect Imaging Module", IEEE NSS-MIC-RTSD 2017 conference record, Atlanta, 2017.

HIGH ACCURACY AND LOW POWER X-RAY DETECTOR FOR MEDICAL IMAGING USING PHOTON COUNTING AND CHARGE INTEGRATION

RESEARCH TOPIC:

X-rays, Medical imaging, Spectrometry, Charge integration

AUTHORS:

A. Habib, M. Arques, J.-L. Moro, M. Accensi, S. Stanchina, B. Dupont, P. Rohr[1], G. Sicard, M. Tchagaspanian, L. Verger

ABSTRACT:

Most X-ray digital flat-panels available on the market today are based on indirect detection using a scintillator and basic amorphous silicon pixels. On the other hand, photon-counting detectors provide spectrometric (or multi-energy) information but suffer by their small size and high price. A small integrated circuit - that can provide spectrometric information and quantification at pixel level and which could be compatible with the production of large radiographic flat-panels - has been designed as a proof of concept. First performance measurements for this test chip are presented. Noise was found to be ~80 e-rms in photon counting mode with a power consumption of only 0.9 µW/pixel for the static analog part and 0.3 µW/pixel for the static digital part.

SCIENTIFIC COLLABORATIONS: [1]Trixell

Context and Challenges

Radiography X-ray medical imaging requires 2D large surface detectors (from 20 cm to 40 cm width) equipped with rather large pixels (from 50 µm to 200 µm width). These detectors use indirect detection and charge integration imaging. However, the current trend is to develop photon-counting detectors as they present numerous advantages. They may have several energy threshold levels, and therefore are capable of capturing different images corresponding to different photon energies. This imaging technique is very advantageous for medical applications and allows distinguishing different components in a patient, such as bones, soft tissues, or injected products such as iodide or gadolinium (K-edge imaging). The major problem with large-area photon counting detectors is the development of large-area radiation detectors, which are currently not available. In addition, photon-counting detectors tend to be limited by the incident flux when photon pile–up occurs (i.e. count rate is affected when two photons arrive simultaneously or follow each other very closely).

The goal of this proof of concept circuit [1,2] is to design a system that can provide spectrometric information and which is compatible with the production of large radiographic flat-panels.

Main Results

The pixel architecture is based on a charge balancing concept. In this concept, the electrons created by the detection of an X-ray cause the decrease of the voltage of a capacitor. When this voltage crosses the threshold of a comparator, a positive calibrated counter charge is injected back on the capacitor. This counter charge corresponds to the quantification step. The energy of each detected X-ray is then measured. The counter-charge concept makes in-pixel digitization of the data possible, and eliminates the need for external analog-to-digital converters. This innovative design allows detection of radiation either by photon counting (low flux) or integration mode (high flux).

Counter charge homogeneity is a key parameter to get good yields when fabricating large-area CMOS circuits with thousands of pixels. Pixel mismatch is the ratio of the standard deviation for pixels responses over the average response, and was found to be better than 15% for all areas. The pixel mismatch was measured even better (<10%) for low input capacitance pixel areas such as the two darker area of the matrix response shown in Fig. 1-left. The histogram of normalized pixels response is also plotted (Fig. 1-right). This plot shows the number of pixels which have the same response to a uniform current applied.

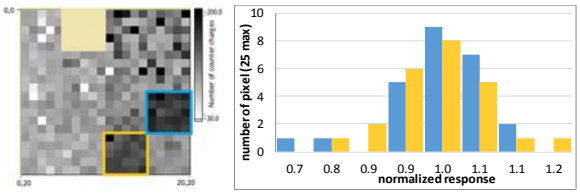


Figure 1: Pixel mismatch – whole matrix (left) and histogram of two areas (right) (normalized pixel response to a current stimulus).

Noise was measured in photon counting mode. The Equivalent Noise Charge (ENC) value can be calculated from pixel response to electrical stimulus emulating photons with increasing energy. It was measured for several areas with different detector capacitances (Fig. 2).

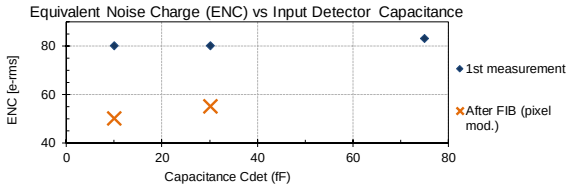


Figure 2: Equivalent Noise Charge versus detector capacitance

Noise was measured >80 e-rms. A Focused Ion Beam (FIB) edit was used to remove a parasitic capacitor. Noise with this circuit modification and new setup was found to be 50 e-rms.
Integral non-linearity measurement is 8% at full dynamic. We are pursuing simulations to improve this key parameter.

Perspectives

This proof of concept demonstrates the possibility to design a new X-ray spectrometric detector using photon counting and charge integration, scalable to large 2D panels. Associated with a specific spectrometric data processing, it is the first step to demonstrate the potential interest of a large field of view spectrometric detector to improve diagnosis in medical applications.

RELATED PUBLICATIONS:

[1] A. Habib et al., "Sphinx1 : Spectrometric Photon Counting and Integration Pixel for X-Ray Imaging with a 100 electron LSB", IEEE Trans. Nucl. Sci., vol. 62, Issue: 3, June 2015.
[2] A. Habib et al., "Characterization of Sphinx1 ASIC X-ray detector using photon counting and charge integration", Journal of Instrumentation, Volume 13, January 2018 (P01024).

QUANTITATIVE 2D MAPS OF OPTICAL PROPERTIES RECONSTRUCTION: PRE-CLINICAL RESULTS ON RATS

RESEARCH TOPIC:
Imaging systems, Tissue characterization

AUTHORS:
V. Sorgato, M. Berger, C. Emain, C. Vever-Bizet[1], G. Bourg-Heckly[1], J.-M. Dinten, A. Planat-Chrétien

ABSTRACT:
We present the Dual-Step system and method that we developed to achieve 2D quantitative maps of optical properties. It is non-contact, quantitative for both absorption and scattering, large field, and spectrally resolved. The present study shows the results obtained on rats and figures the interest of the approach to address complex in-vivo samples.

SCIENTIFIC COLLABORATIONS: [1] Laboratoire Jean Perrin UMR8237 CNRS- UPMC Univ. Paris 6

Context and Challenges
Spectral imaging techniques have to address two main challenges of the clinical requirements: the consideration of a wide spatial field, which considerably reduces the measurement time of the region of interest, and the non-invasiveness of the measurement when used in non-contact set-ups. Yet, to cover a wide spatial field, the use of non-punctual illumination leads to mixed effects of absorption and scattering properties that cannot be separated from the detected diffuse reflectance. Thus, the quantification of individual optical properties, necessary for an accurate diagnosis, is not achieved. We propose to address this quantification problem by combining the quantification capacity of Diffuse Reflectance Spectroscopy (DRS) [1] with the spatial wide field of view offered by the staring Multispectral Imaging (MSI) system. The resulting Dual-Step combination technique achieves wide field maps of absolute quantitative scattering and absorption optical properties.

Main Results
The Dual-Step Multispectral Imaging technique we have developed couples a system of Non-Contact DRS with a system Large Field of View MSI (LFOV MSI) (Fig. 1).

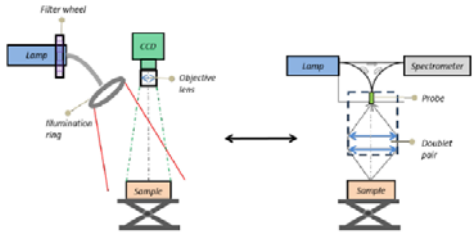


Figure 1: Combination of Non-Contact srDRS (right) with LFOV MSI system (left) is achieved via a common focal object plan. The sample object is moved between them (in x-y directions) with a translational stage.

The technique achieves wide-field quantification of optical properties through a two-step process: an initial estimation of common scattering properties is performed in specific zones of the imaged sample with the Non-Contact srDRS (spatially resolved DRS) modality; these srDRS-estimated scattering coefficients are used to quantify absorption over the whole image with LFOV MSI. As a proof of concept, the experimental set-up connects the two modalities through a translation stage on which the sample is positioned at a common focal object plane. The punctual srDRS systems estimate the local optical properties based on a preliminary Monte Carlo computed Look Up Table (LUT) and calibration measurements on reference phantoms. The scattering estimation is used further in the dual-step approach, to estimate absorption properties with LFOV MSI through an adapted ACA-PRO algorithm [2] (Fig. 2).

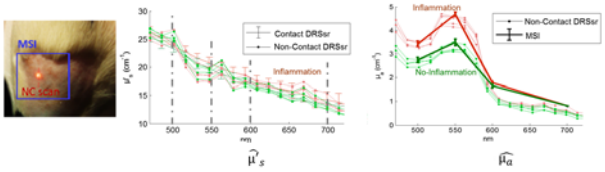


Figure 2: In-vivo rat skin inflammation model. (Middle) Local estimation of the skin scattering according to contact and non-contact DRS for validation of the non - contact instrument. (Right): Absorption estimation by MSI and non-contact DRS for validation of the LFOV MSI instrument.

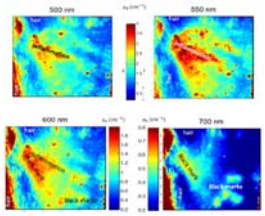
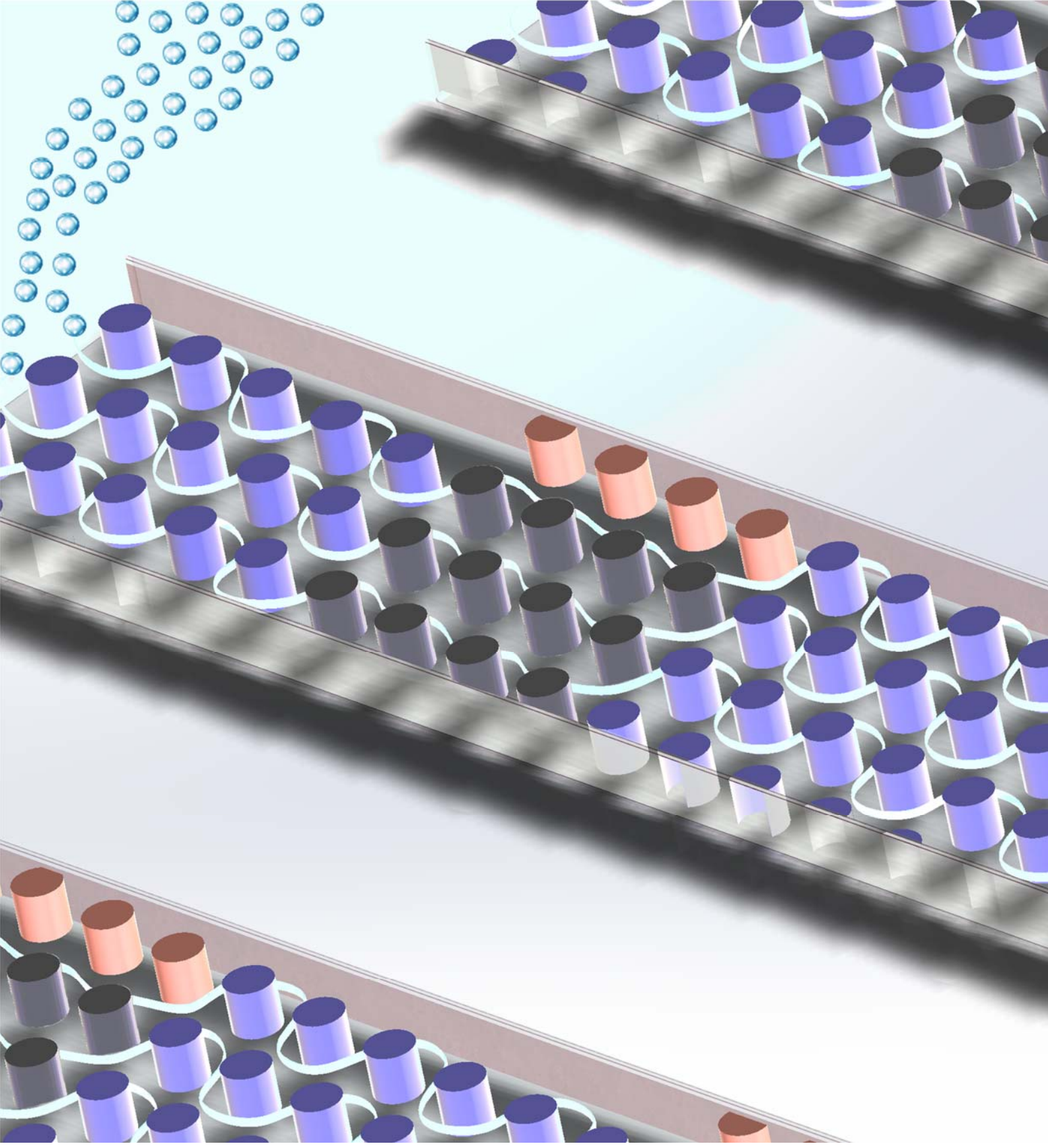


Figure 3: In-vivo rat skin inflammation model. Estimated 2D quantified absorption maps obtained by the Dual-Step set-up at each wavelength.

The Dual-Step set-up offers wide-field quantitative maps of both absorption and scattering coefficients on real complex samples such as inflamed in-vivo rat skin model in Fig. 3.

Perspectives
The next step is to couple the DRS and MSI on a single matrix sensor to move towards integration of the two modes [1]. Solutions to take into account the sample curvature should be considered.

RELATED PUBLICATIONS:
[1] V. Sorgato et al., "Aca-pro: calibration protocol for quantitative diffuse reflectance spectroscopy. Validation on contact and noncontact probe-and ccd-based systems", Journal of Biomedical Optics, 21(6) (2016).
[2] V. Sorgato et al., "Wide-Field Absolute Quantification of Absorption in Turbid Media", Clinical and Translational Biophotonics. Optical Society of America, pp. JM3A.32. (2016).



O2 MICROSYSTEMS FOR DIAGNOSIS AND BIOLOGY

- **Deterministic lateral displacement**
- **Bacteria grinding lysis**
- **Electrochemical isothermal PCR**
- **Pollutant lab-on-chip extraction**
- **Gas analysis**
- **Method for biomarker selection**

ANTICIPATING CUTOFF DIAMETERS IN DETERMINISTIC LATERAL DISPLACEMENT MICROFLUIDIC DEVICES FOR AN OPTIMIZED PARTICLE SEPARATION

RESEARCH TOPIC:

Deterministic lateral displacement, Nanometer and micrometer-sized particle separation, Multiple critical diameters

AUTHORS:

E. Pariset, C. Pudda, F. Boizot, N. Verplanck, J. Berthier, A. Thuaire, V. Agache

ABSTRACT:

Determinist Lateral Displacement (DLD) devices enable to separate nanometer to micrometer-sized particles around a cutoff - or critical - diameter, thanks to slanted rows of microfluidic pillars. In order to design appropriate DLD geometries, robust models are required to anticipate the value of the critical diameter. We show that the critical diameter varies along the DLD channel, especially in narrow pillar arrays. Experimental and numerical results reveal that the variation of the critical diameter is induced by boundary effects at the channel side walls, called the wall effect. The wall effect generates unexpected particle trajectories that may compromise the separation efficiency.

SCIENTIFIC COLLABORATIONS: Bio-Rad, MIT (Scott Manalis Laboratory)

Context and Challenges

When particles are injected in a DLD channel, their trajectory is determined by their position relatively to the different streamlines [1]. Particles larger than the critical diameter (D_c) are deviated along the pillars (displacement trajectory), while smaller particles remain in the channel axis and follow a zigzag trajectory. D_c is determined by the geometrical characteristics of the pillar array, such as the inter-pillar gap (G), the slant angle (θ), the pillar shape and orientation [2]. For a given geometry of the pillar array, a single value of D_c can be found in the literature.

In our DLD devices, an intermediary trajectory was observed in addition to the classical zigzag and displacement modes. This new mode of trajectory strongly influences the separation efficiency of DLD devices. Here we present a general model to anticipate this effect based on both experimental and numerical results.

Main Results

A schematic representation of the intermediary trajectory is given in Fig. 1. While flowing in a DLD channel, a monodisperse population of particles display a zigzag mode in some areas of the channel and a displacement mode in the others.

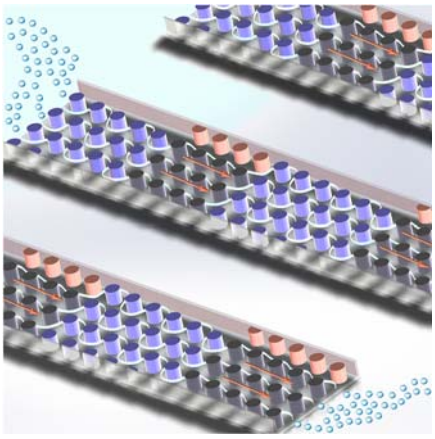


Figure 1: Representation of the intermediary trajectory

Our COMSOL Multiphysics finite element model demonstrated that the critical diameter actually varies significantly along the DLD channel. This variation is represented on the graph of Fig. 2. Therefore, a given particle size can be above D_c in some channel areas (displacement mode) and below D_c in the other areas (zigzag mode). A very good agreement was found between experimental and numerical evidence of this intermediary mode.

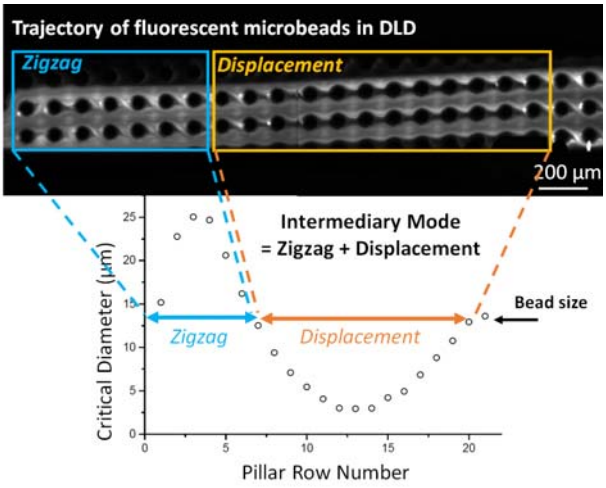


Figure 2: Evolution of the critical diameter along the DLD channel, and experimental observation of the trajectory of 13µm fluorescent beads.

The intermediary mode results from the boundary effect of the channel walls that force the flow to follow the direction of the pillars where the side pillars are adjacent to the walls. This effect increases in narrow DLD channels. A general model was proposed in order to anticipate the value of D_c by taking into account the channel width, in addition to the classical array parameters (G and θ).

Perspectives

The intermediary mode could be implemented for multi-separations, with several D_c values in a unique DLD channel [3].

RELATED PUBLICATIONS:

[1] E. Pariset et al., "Extracellular vesicles: isolation methods", Advanced Biosystems, vol. 1, no. 5, May 2017.
[2] E. Pariset et al., "Anticipating cutoff diameters in deterministic lateral displacement (DLD) microfluidic devices for an optimized particle separation", Small, vol. 13, no. 37, October 2017.
[3] E. Pariset et al., "Purification of complex samples: implementation of a modular and reconfigurable droplet-based microfluidic platform with cascaded deterministic lateral displacement separation modules", under review.

GRINDING LYSIS: A MICROFLUIDIC DEVICE FOR SAMPLE PREPARATION ALLOWING RAPID AND SENSITIVE BACTERIA DETECTION

RESEARCH TOPIC:
Sample preparation, Spores, Bacteria, DNA, PCR

AUTHORS:
M. Flaender, M. Baqué, R. den Dulk, J. Ventosa, D. Gosselin, J. Berthier, A.-G. Bourdat

ABSTRACT:
Rapid identification of health threatening bacteria and/or spores present in low concentration is of utmost importance. Efficient sample preparation and molecular detection aim to achieve this goal.
We present an original approach combining an efficient concentration together with purification of bacteria or spores from various samples. This is coupled with a rapid and efficient Grinding Lysis (GL) step to provide accessible DNA templates. Conventional or Isothermal PCR is then used for bacteria identification.
The method is very efficient and rapid: it can concentrate and detect bellow 10 targets in 1 mL of sample in less than 1h and is compatible with food and environmental samples.

Context and Challenges
The need for rapid and sensitive methods for pathogenic bacteria detection keeps growing. Molecular detection is the method of choice for rapid pathogens recognition due to its high sensitivity and specificity. In food samples, pathogens are present at very low concentrations. Thus, a long culture enrichment (10-48h) is performed prior to molecular detection in standard procedures. Shortening time-to-result is challenging especially for perishable food.

To reduce time to result, the challenge concern the limitation of the culture enrichment duration. To achieve this goal, our strategy was to search for bacteria from a larger sample volume compared to the usual commercial system. This was possible using a new efficient concentration and purification system. Our device integrates enrichment of the sample by filtration, purification and mechanical lysis on the same platform.

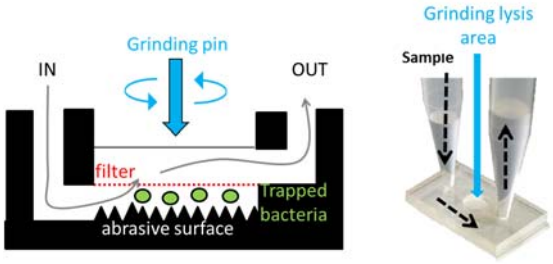
Main Results


Figure 1: Schematic representation and view of the device. Sample is introduced in the inlet port, the bacteria are retained on the filter and the excess liquid is removed. The microorganisms are then lysed by mechanical grinding on the rough surface. A recovery buffer (qPCR reagent) is injected and the bacterial DNA is directly eluted in qPCR reagent through the filter membrane via the outlet port. The eluted solution is ready to be transferred in a tube and analyzed with a standard qPCR machine.

We have developed a simple, portable, inexpensive and manual method to concentrate, lyse the spores and bacteria and purify their DNA, contained in 1mL of sample (Fig. 1) [1]. Molecular detection (qPCR, LAMP or RPA) could then be performed using standard equipment.

The GL method, in combination with molecular detection, delivers quantitative results over a very wide range of concentrations from 5 to 10^6 pathogens/mL in less than 1h (Fig. 2). The sensitivity of the system is better than the state-of-the-art sample preparation system, as concentrations down to 5 spores or bacteria per mL are detected in less than 37 PCR cycles. In addition, the method exhibits high robustness toward interfering species which are removed during injection and purification steps.

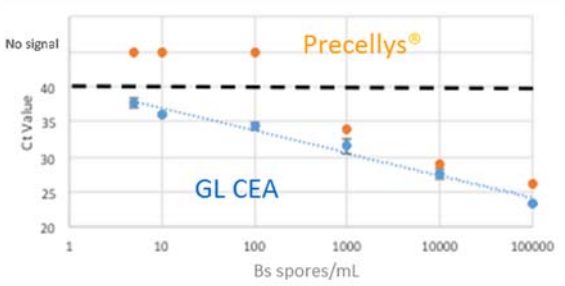


Figure 2: Comparison between manual GL device (■) and commercially available Precellys® (●) for Bacillus subtilis (Bs) spores sample preparation. At equal starting concentration of Bs spores, a lower Ct value indicates a more efficient concentration and lysis. Note that all points above the dotted line (Ct>40) have not shown any positive signal.

The system could be actuated manually or integrated in an automated microfluidic system, which consists of a single-use microfluidic cartridge and an associated instrument.

Perspectives
In the future, it is planned to render the device totally portable by including a low energy system to perform isothermal DNA amplification on the same platform.

RELATED PUBLICATIONS:
[1] M. Flaender et al., "Grinding Lysis (GL): A microfluidic device for sample enrichment and mechanical lysis in one", Sensors and Actuators B: Chemical 258, 148-155, 2018.
[2] D. Gosselin et al., "Low-cost screen printed and embossed LAMP micro-reactors", Biotech, Biomaterials and Biomedical TechConnect Briefs 2017, Micro & Bio Fluidics, Lab-on-Chip Chapter 6:158 61, 2017.
[3] D. Gosselin et al., "Screen-Printed Polyaniline-Based Electrodes for the Real-Time Monitoring of Loop-Mediated Isothermal Amplification Reactions", Analytical Chemistry 89, no 19, 10124 28, 2017.

SCREEN-PRINTED POLYANILINE-BASED ELECTRODES FOR A REAL-TIME MONITORING OF LAMP REACTIONS

RESEARCH TOPIC:
PolyAniline, Potentiometry, LAMP, Point-of-care diagnostics, NAAT, Screen-printing

AUTHORS:
D. Gosselin, M. Gougis, M. Baque, F. Navarro, M. N. Belgacem[1], D. Chaussy[1], A.-G. Bourdat, P. Mailley, J. Berthier

ABSTRACT:
Nucleic Acid Amplification Testing (NAAT) is a very powerful method to perform efficient and early diagnostics. However, the integration of a DNA amplification reaction with its associated detection in a low-cost, portable and autonomous device remains challenging. The detection of the DNA amplification reaction is performed by a real-time monitoring of the H^+ ions concentration, a by-product of this reaction. Such measurements are done by potentiometry using PolyAniline (PAni)-based working electrodes and Ag/AgCl reference electrodes. The developed screen-printed potentiometric sensor allows real-time monitoring of a LAMP reaction with an initial number of DNA strands as low as 10 copies and with similar results compared to fluorescence measurements.

SCIENTIFIC COLLABORATIONS: [1]LGP2, INPG, Université Grenoble Alpes

Context and Challenges

To lead an efficient fight against infectious diseases and epidemics, reliable and sensitive diagnostics are of utmost importance. Diagnostic is the first step toward treatment, and without it, broad-spectrum antibiotics are generally used, which may result in an increase in microbial resistance. Nucleic acid amplification tests (NAAT) are powerful tools to perform such diagnostic. In fact, due to the amplification step, a very small amount of DNA copies can be detected, allowing for early diagnostics. Presently, such diagnostics are routinely performed in medical laboratories but their availability at the point-of-care is still limited. This raises an issue when medical environments are not easily accessible, in particular in developing countries. In fact, the World Health Organization has established criteria for diagnostic systems which are aimed to be used at the point-of-care: they must fulfill the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and delivered) requirements. In the case of NAAT diagnostics, the detection remains challenging to integrate in a portable device. Numerous detection techniques have been used for NAAT. The development of an electrochemical detection based on printed electrodes can ensure a low-cost and high throughput production of the detection system using screen-printing technologies

Main Results

PAni-based electrodes are used for the detection and the monitoring of a LAMP reaction as presented in Fig. 1.

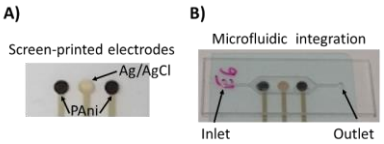


Figure 1: Pictures of the screen-printed electrodes (A) and of its integration in a microfluidic chamber (B).

Such a detection relies on the fact that each time a DNA polymerase adds a nucleotide during DNA elongation, a hydronium ion is released. This lead to a large increase of the concentration of hydronium ions, along with the number of DNA strands, during the amplification process. Thus, continuously measuring the pH with the PAni-based electrodes allows the monitoring of the DNA amplification.
The potentiometric responses of the screen-printed PAni-based electrodes were studied for different initial DNA quantities. The DNA amplification was successfully detected for an initial number of DNA strands ranging from 10^5 to 10 (Fig. 2, this figure was obtained with a baseline correction using the reactive blank).

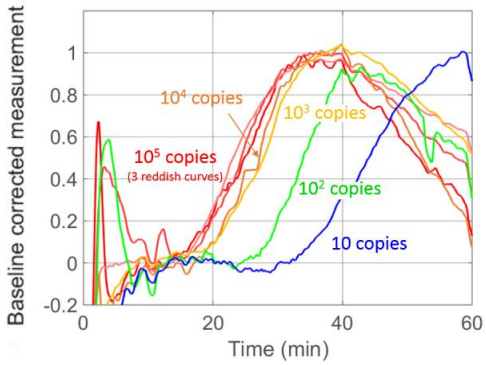


Figure 2: Comparison of the baseline corrected potential measurements for different initial DNA amounts (10^5, 10^4, 10^3, 10^2, and 10 copies).

The performances of the presented detection method were assessed in comparison with fluorescence measurements and exhibited same temporal evolution. The lower the number of DNA strands in the sample, the longer it is before obtaining a signal.

Perspectives

The presented detection method is able to detect a quantity as low as 10 copies of DNA and exhibits a very similar response to fluorescence measurements. In addition, the fabrication of these electrodes by screen-printing processes allows for low-cost production with high throughput. Moreover, such screen-printed electrodes can be easily embedded in microfluidic devices and the potentiometric measurement allows for a compact and portable acquisition system. Therefore, PAni-based electrodes appear to be a convenient way for a simple, integrated, reliable and cheap detection method for nucleic acid amplification diagnostics at the point-of-care.

RELATED PUBLICATIONS:
[1] D. Gosselin et al., "Screen-Printed Polyaniline-Based Electrodes for the Real-Time Monitoring of Loop-Mediated Isothermal Amplification Reactions", Anal. Chem., vol 89, no.19, pp.10124-10128, October 2017.

DEVELOPMENT OF A NEW PHASE FOR LAB-ON-A-CHIP EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM WATER

RESEARCH TOPIC:
Porous SiOCH, Microfluidic device, PAHs, Extraction

AUTHORS:
L. Foan, J. El Sabahy, F. Ricoul, B. Bourlon, S. Vignoud

ABSTRACT:
Nanoporous organosilicate (SiOCH) were implemented as solid phase for lab-on-a-chip extraction of organic pollutants from natural waters and have exhibited improved performances. Validations were carried out for polycyclic aromatic hydrocarbons (PAHs) extraction, and the results were compared with the commonly used SBSE laboratory technique (Stir-Bar Sorptive Extraction). An optimized microfluidic device in term of channel width and chip total area could be selected and the high specific surface area of the nanoporous phase reduces the matrix effects related to interferences with dissolved organic matter.

Context and Challenges

PAHs detection in environmental waters is a public health issue. Indeed PAHs are known for their toxic, mutagenic and carcinogenic effects and are widely present in the environment, mainly because of human activity. To check water quality, PAHs detection is usually carried out in centralized laboratories. This involves important costs and labor, and requires sampling, transport and storage steps, which can induce biases on the final results due to possible loss of analyte or sample contamination.

In this context, our team has developed a miniaturized pre-concentration device intended to be used in a portable system for monitoring PAHs directly in environmental waters. Our lab-on-a-chip system presents several considerable advantages, compared to other extraction techniques: (i) fast extraction time (20 min against 24h with SBSE); (ii) compact device; (iii) easily automatable process using valves and pump; (iv) reduced energy and solvent consumption.

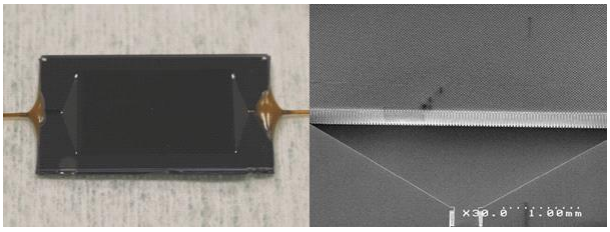


Figure 1: Left: Picture of glass/silicon chip with glued capillaries (outer dimensions: 2.1 × 1.2 cm); Right: SEM view of the microfluidic chamber entrance and the micro pillars array (view from above, before sealing).

Main Results

This work presents the extraction of PAHs at trace levels in natural waters with a microfluidic glass/silicon device embedding porous SiOCH as novel extraction phase (Fig. 1). Compared to PDMS, the most commonly used polymeric extraction phase, SiOCH has the advantage of being deposited by PECVD, (Plasma Enhanced Chemical Vapor Deposition). PECVD process enables collective and repeatable fabrication with improved chip to chip reproducibility and offers the possibility to get lower cost. Moreover, this novel nanoporous coating showed higher affinity than PDMS with the 16 PAHs studied [1].

Following the study of the influence of the chip geometry on the extraction recoveries, an optimized microfluidic device (14cm^2 chamber with micro pillars spaced by 10µm gaps) functionalized with 180 nm porous SiOCH was fabricated. It exhibited equivalent extraction performances than SBSE (Stir bar Sorptive Extraction), the reference laboratory technique used for extraction of organic micropollutants (Fig. 2).

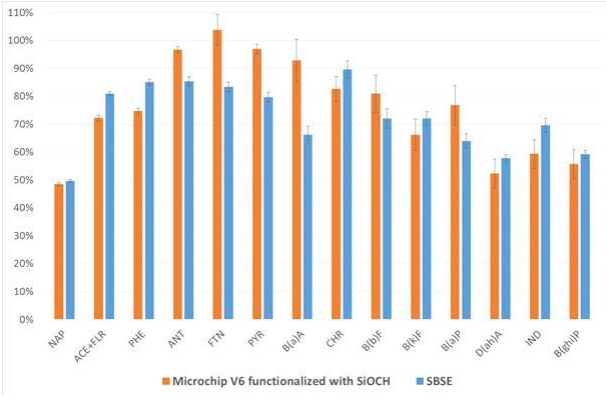


Figure 2: Comparison of the extraction recoveries obtained with the lab-on-a-chip functionalized with porous SiOCH and SBSE. Tests were carried out with milli-Q water spiked with 5 µg L-1 of 16 PAHs.

Moreover tests with spiked natural waters have shown that the high specific surface of porous SiOCH limits the matrix effects related to interference with dissolved organic matter [1].

Perspectives

Porous SiOCH coated lab-on-chips are promising devices for PAHs capture. We are currently integrating this technology brick within a fully portable device (including separation and detection systems) for monitoring of PAHs in natural waters.

RELATED PUBLICATIONS:
[1] L. Foan et al., "Development of a new phase for lab-on-a-chip extraction of polycyclic aromatic hydrocarbons from water," Sensors and Actuators B-Chemical, vol. 255, pp. 1039-1047, Feb 2018.

DUAL-TEMPERATURE MODE FOR QUANTITATIVE ANALYSIS OF GAS MIXTURES WITH MOX SENSOR

RESEARCH TOPIC:
Quantification, Exhaled breath analysis, Acetone, Ethanol, Sensor response model, MOX sensor, Dual temperature mode

AUTHORS:
S. Madrolle, P. Grangeat, Ch. Jutten[1]

ABSTRACT:
Quantitative analysis of gas mixtures for detecting low concentrations of gases like acetone or ethanol in exhaled breath is of high interest in medical diagnostics and patient monitoring. We intend to use source separation methods for separating the gas sources measured with metal oxide (MOX) sensors. These unsupervised methods require a relevant mathematical model of MOX sensor responses to gas mixtures, but only a few calibration measurements for recovering concentrations. We propose an experimental investigation of a linear quadratic response model of MOX sensor in a dual temperature mode for quantitative analysis.

SCIENTIFIC COLLABORATIONS: [1]GIPSA-lab, UMR CNRS 5216, Univ. Grenoble Alpes

Context and Challenges

Metal-oxide gas sensors (MOX) are low cost sensors based on variation of electrical conductivity. They detect a wide range of gases such as hydrogen, carbon monoxide, ethanol, acetone, or other volatile organic compounds (VOCs). In exhaled breath, the amount of some VOCs can vary according to the health status of the patient and thus be useful for diagnosis. Among others, acetone is considered as a breath biomarker for some diseases such as diabetes or for weight loss monitoring. However, acetone might interact with ethanol on MOX sensors, which induces interferences. Thus there is a need to propose a method to separate them.

Main Results

With regard to data processing, we want to investigate a new approach based on blind source separation (BSS) method. Used currently with ion-selective electrodes in liquid, this non-supervised method can deal with nonlinear model and the calibration is simplified since only few points are necessary. However, this method requires a robust sensor response model for mixtures and a diversity in the measures. To get this diversity, the classical operation is to have as many sensors as sources (i.e. as gases). But, here, we propose another method, using a dual-temperature mode instead of one fixed temperature [1]. Thus, a single sensor behaves as 2 virtual sensors, and could estimate the concentration time variations of two gases imbedded in an air buffer. To identify several gases using a single sensor, we introduce a new data processing approach based on model inversion for quantification.

It is known that for one single gas, the resistance R_{gas} of a tin oxide MOX gas sensor follows the power law:

$$\mathrm{R_{gas}} = \mathrm{A}.\mathrm{C_{gas}^{r}}$$

where A and r are coefficients to determine and C_{gas} is the gas concentration. Based on this power law, we propose the following linear quadratic experimental model for a mixture of 2 gases:

$$R_0/R_{gas} = A_1.C_1^{r_1} + A_2.(C_1^{r_1})^2 + B_1.C_2^{r_2} + B_2.(C_2^{r_2})^2 + K_1.C_1^{r_1}.C_2^{r_2}$$

The ratio R_0/R_{gas} is directly linked to the output voltage; R_{gas} is the sensor resistance in presence of gas and R_0 is the baseline.

Considering this model at 2 different temperatures results in a system of two equations. Then, solving the inverse problem allows to recover the gas concentrations. Here a Levenberg-Marquardt algorithm has been used.

The experimental evaluation has been done using an analytic cell of 200 mL made of a commercial SB-30 (FIS) MOX sensor, associated with a temperature and humidity sensor (SHT75, Sensirion) and an oxygen sensor (O2/M-100, Membrapor) to check environmental parameters. We have studied mixtures of acetone and ethanol injected in an air buffer. The data are presented as function of the concentration range of acetone (Fig. 1) considering respectively the 2 heater voltages 0.5 V and 0.9 V, for the linear quadratic model. We have compared with other models (linear, linear bilinear, log). The linear quadratic model seems the most correlated with our measures and the one which have the best balance between higher determination coefficient R^2 and smaller mean square error MSE.

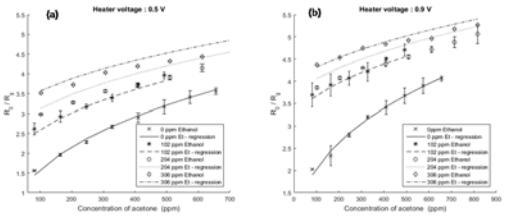


Figure 1: Measured values (points) and associated estimated linear quadratic model (lines) for gas mixtures of acetone and ethanol using a tin-oxide gas sensor. For same mixtures, two heater voltages are presented: 0.5V (a) and 0.9V (b), corresponding to the dual-temperature mode.

We also introduced nonlinear, blind and non-blind source separation methods combining Newton-Raphson minimization and independent component analysis algorithms to estimate gas concentrations and the linear quadratic model coefficients [2].

Perspectives

The first perspective is to increase the complexity of the analyzed gas up to the one of exhaled breath sample and to decrease the gas concentrations to fit with the clinical concentration ranges.

The second perspective is to introduce Bayesian algorithms to regularize this very ill-posed problem, to optimize the calibration protocol and to reduce the number of calibration samples.

RELATED PUBLICATIONS:
[1] S. Madrolle et al., "Dual-temperature mode for quantitative analysis of gas mixtures with MOX sensor", ISOCS/IEEE International Symposium On Olfaction And Electronic Nose (ISOEN 2017), Montréal, Canada, May 28th-31st 2017.
[2] S. Madrolle et al., "Méthodes de séparation de sources non linéaires pour des capteurs gaz à oxyde métallique", XXVIème Colloque GRETSI, 5-8 septembre 2017, Juan-les-Pins, France.

BAYESIAN INFERENCE FOR BIOMARKER DISCOVERY IN PROTEOMICS: AN ANALYTIC SOLUTION

RESEARCH TOPIC:
Variable selection, Model selection, Optimal decision, Bayesian approach, Evidence, Hierarchical model, Proteomes, Biomarker

AUTHORS:
N. Dridi[1], A. Giremus[1], J.-F. Giovannelli[1], C. Truntzer[2], M. Hadzagic[1], J.-P. Charrier[3], L. Gerfault, P. Ducoroy[2], B. Lacroix[3], P. Grangeat, P.Roy[4]

ABSTRACT:
Given clinical data regarding a list of proteins for a set of individuals, the tackled problem is to extract a short subset of proteins the concentrations of which are an indicator of the biological status (healthy or pathological). The main contributions of the paper are: (1) a new Bayesian formulation of the biomarker selection problem, (2) the closed-form expression of the posterior probabilities in the noiseless case, and (3) a suitable approximated solution in the noisy case. The methods are numerically assessed and compared on proteins quantified in human serum by mass spectrometry in selected reaction monitoring mode.

SCIENTIFIC COLLABORATIONS: [1] IMS, Univ. Bordeaux, [2] CLIPP Univ. Bourgogne, Dijon, [3] BioMérieux SA, [4] LBBE Hôpitaux Civils de Lyon

Context and Challenges

It is now generally recognized that protein expression analysis is crucial in explaining the changes that occur as a part of disease pathogenesis. Mass spectrometry in selected reaction monitoring (SRM) mode has demonstrated its ability to quantify clinical biomarkers in patient sera. The focus of this publication is on the selection (or discovery) of the “signature profiles,” the so-called biomarkers. They represent, for instance, indicators of normal versus pathogenic biological processes, or positive versus negative pharmacological responses to therapeutic intervention.

Main Results

The paper [1] tackles the problem of biomarker identification by adopting a Bayesian approach to propose the selection of the optimal set of variables. By providing an elegant and mathematically rigorous framework for incorporating the data and the prior information within a joint probabilistic model, the Bayesian setting allows straightforward modeling of both the technical and the biological variabilities of the data.

The identification of biomarkers for diagnosis or prognosis can be classically formulated as a variable selection problem, and this problem has been paid a lot of attention as a specific instance of model choice. Various methodologies exist that can be broadly classified in two categories: the frequentist hypothesis testing and the Bayesian decision-making. Frequentist hypothesis testing consists in deciding between two statements, classically referred to as the null and the alternative hypotheses, by comparing a function of the observed data to a threshold. The Bayesian framework offers an alternative formulation of model selection. The candidate models are assigned prior probabilities that are combined with the likelihood function to yield the so-called posterior probability. The latter summarizes all the available decision information to make the decision. In this context, deciding in favor of the a posteriori most probable model is optimal in the sense that it minimizes the risk associated to the 0/1 cost function. Frequentist methods are designed to test the departure of the data from a pre-defined null hypothesis. In contrast, Bayesian selection procedures evaluate the plausibility of a given hypothesis given a set of candidate hypotheses hence are conveniently well-suited to multiple hypotheses testing.

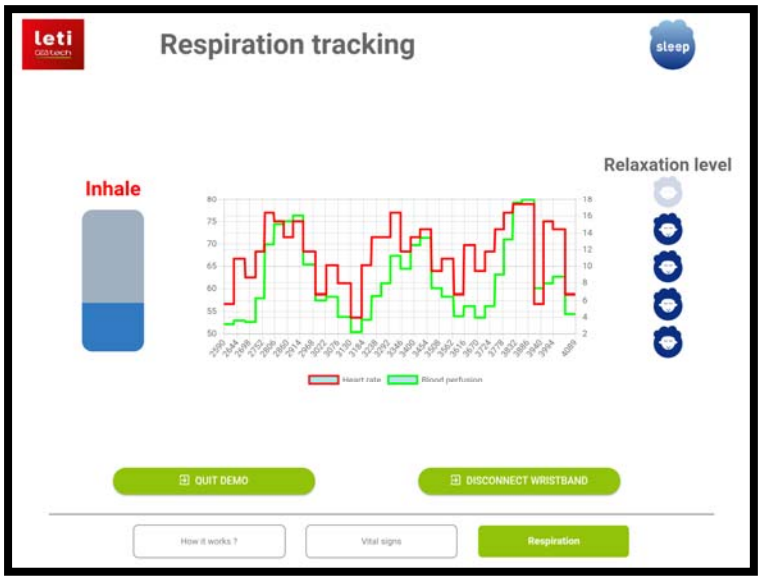
The novelty is that the decision is not made protein by protein. As an alternative, the problem is formulated as directly finding the best partition of the list of proteins into two subsets, namely discriminant and non-discriminant ones, in the sense that it yields the highest posterior probability. In this paper, we have proposed an analytic expression of the evidence rendering the usually complex calculations straightforward. Assuming that all candidate models are equally a priori probable, using this expression, the posterior probability across the 2^P models can be inferred. The selected model is the one which maximizes this probability.

This method has been numerically assessed and compared to the state-of-the-art methods (*t* test, LASSO, Battacharyya distance, FOHSIC) on synthetic and real data from proteins quantified in human serum by mass spectrometry in selected reaction monitoring mode. These clinical data are composed of 206 samples: 105 from healthy volunteers (status *H)* including 76 patients from blood donors and 29 with negative colonoscopy, 101 from pathologic patients with malignant tumor (status *P*). The protein concentrations are obtained using the Bayesian hierarchical inversion method developed on the BHI-PRO project [2] from measurements of SRM spectra. For each sample, the concentrations of 21 proteins has been measured. Only one of the proteins in the sample, named LFABP, was previously identified by SRM as a biomarker. For this data set, the posterior probability has been computed for each of the 221 possible partitions according to the analytic expression we have proposed. By far, the most probable partition (probability 0.9986) was: LFAPB is discriminant and the remaining 20 proteins are non-discriminant. This study has confirmed that our method correctly identifies the valid biomarker. Despite the large number of models to compare (about two millions candidate models), the computation time was just 1 h. This short computation time has been made possible by the analytical calculation of the posterior probability, avoiding the use of extensive numerical integration methods such as for instance MCMC algorithms.

Perspectives

We plan to take advantage of the performance of the method in biomedical fields involving either molecular biomarkers or digital biosignal features such as wearable medical devices.

RELATED PUBLICATIONS:
[1] N. Dridi et al., "Bayesian Inference for Biomarker Discovery in Proteomics: An Analytic Solution", EURASIP Journal on Bioinformatics and Systems Biology, 2017:9, 2017.
[2] A. Klich et al., "Variance component analysis to assess protein quantification in biomarker validation: application to Selected Reaction Monitoring-Mass Spectrometry", BMC bioinformatics, 2018.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Heart rate
Blood perfusion
QUIT DEMO
DISCONNECT WRISTBAND
How it works ?
Vital signs
Respiration

O3 WEARABLE AND IMPLANT DEVICES

- **Real-time neural signal decoding**
- **Energy expenditure monitoring**
- **Artificial pancreas**
- **DRS for skin imaging**
- **DRS for tissue diagnosis**
- **NIR neuroprotection**

REAL-TIME NEURAL SIGNAL DECODING AND SIMULTANEOUS ADAPTIVE DECODER IDENTIFICATION: TOWARD A BRAIN-COMPUTER INTERFACE CLINICAL TRIAL

RESEARCH TOPIC:

Brain Computer Interface, Real-time neural signal decoding, Online adaptive/incremental learning

AUTHORS:

A. Eliseyev, V. Auboiroux, T. Costecalde, L. Langar, G. Charvet, C. Mestais, T. Aksenova, A.-L. Benabid

ABSTRACT:

Accurate real time neural signal decoding is a major challenge for Brain Computer Interface (BCI) clinical applications. In the same time, offline decoder calibration is known to be suboptimal due to difference of open-loop and closed-loop patterns of neural activity. A new recursive algorithm is proposed for adaptive/incremental decoding model learning in real-time, during closed-loop BCI experiments directly. Multi-linear tensor-based regression is integrated to adaptive recursive learning procedure. This work was performed in the context of the CLINATEC® BCI project whose main goal is to open new opportunities to motor disabled subjects to allow them to control effectors with a large number of degrees of freedom such as a 4-limb exoskeleton

Context and Challenges

One of the major challenges in the field of neuroprosthetics is the development of a clinical Brain Computer Interface (BCI) system with high performances. To address this challenge, we are currently conducting a project to develop an ElectroCorticoGram (ECoG)-based BCI platform for chronic use in clinical applications. The goal of our BCI Project is to bring the proof of concept that it will be feasible for a tetraplegic subject to control complex effectors (such as a 4-limb exoskeleton) after training, thanks to his cortical brain electrical activity recording [1] and decoding.

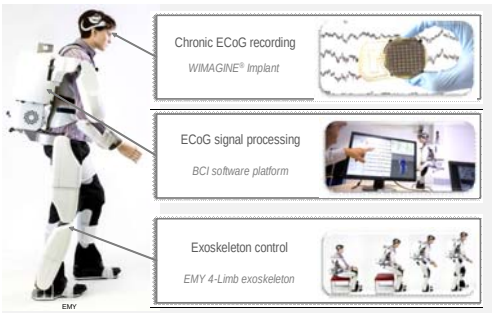

Figure 1: BCI project at CLINATEC.

Neural signal decoding accuracy for complex effector control in real time with a large number of degrees of freedom is a major challenge for BCI clinical applications. To achieve high decoding accuracy user-specific decoders are calibrated from training data set. In the same time, conventional offline decoder identification procedure using the data collected during open loop recording session is known to be suboptimal. Open-loop neural activity patterns differ from closed-loop patterns. To improve the accuracy of neural data decoding and preparing BCI clinical trials adaptive incremental learning algorithm was developed to calibrate BCI system during closed-loop BCI experiment directly

Main Results

The tensor-input/tensor-output Recursive Exponentially Weighted N-Way Partial Least Squares (REW-NPLS) regression algorithm is proposed [2] for high dimension multi-way (tensor) data treatment and adaptive modeling of the complex processes in real-time conditions. The method unites fast and efficient calculation scheme of the Recursive Exponentially Weighted PLS with robustness of the tensor-based approaches. In addition, the Recursive-Validation method for online estimation of the hyper-parameters is proposed instead of the conventional offline Cross-Validation procedure. Innovative algorithm is integrated to the software environment to be used in clinical trial.

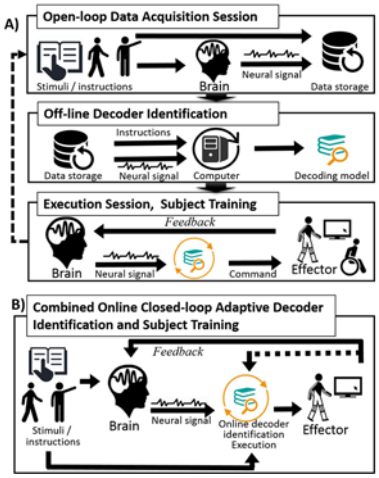

Figure 2: Conventional offline open-loop (A) and online closed-loop user-specific decoder identification with innovative REW NPLS (B) and subject training.

Perspectives

The 5-year clinical research protocol « BCI and Tetraplegia » (Principal Investigator: Professor Benabid) was approved by French authorities (ANSM) and ethical committee. This trial allows, in the frame of patient's training sessions, to test and refine BCI paradigms and decoding algorithms to evaluate the 4-limb exoskeleton ECoG based BCI control. In particular, hybrid discrete/continuous decoding algorithms [3] will address the challenge of multi-limb and self-paced decoding, thus providing Non-Control and Intentional Control periods support for self-paced asynchronous BCI for the clinical trial.

RELATED PUBLICATIONS:

[[1] C. Mestais et al., "WIMAGINE: Wireless 64-Channel ECoG Recording Implant for Long Term Clinical Applications", IEEE TNSRE, 23(1), 10-21, 2015.
[2] A. Eliseyev et al., "Recursive Exponentially Weighted N-way Partial Least Squares Regression with Recursive-Validation of Hyper-Parameters in Brain-Computer Interface Applications". Scientific reports, 7(1), 16281, 2017.
[3] M.-C. Schaeffer et al., "Switching Markov decoders for asynchronous trajectory reconstruction from ecog signals in monkeys for bci applications." Journal of Physiology-Paris 110.4 (2016): 348-360.

AN ORIGINAL PIECEWISE MODEL FOR COMPUTING ENERGY EXPENDITURE FROM ACCELEROMETER AND HEART RATE SIGNALS

RESEARCH TOPIC:

Energy expenditure, Accelerometry, Heart rate, Physical activity

AUTHORS:

H.M. Romero-Ugalde, S. Bonnet, M. Doron, P. Jallon, M. Garnote[1], G. Charpentier[2], S. Franc[2], E. Huneker[3], C. Simon[1]

ABSTRACT:

Energy expenditure (EE) plays an important role in healthcare. This paper proposes an original branched model for computing EE from heart rate (HR) and accelerometer (ACC) signals, which uses HR signals for computing EE on moderate to vigorous physical activities (PAs) and a linear combination of HR and ACC for computing EE on light to moderate PAs. Model was estimated on 53 subjects performing 25 different PAs and validated using leave-one-subject-out. In comparison with linear and nonlinear models, the proposed model leads to more accurate EE estimations (R2=0.84 and R2=0.86 on each validation database).

SCIENTIFIC COLLABORATIONS: [1]CRNH Rhône-Alpes, [2]CERITD, [3]Diabeloop

Context and Challenges

Activity energy expenditure (EE) plays an important role in healthcare for body weight regulation, or hypoglycemia prediction on type 1 diabetes patients. Hence accurate EE measurements are required. Different methods exist to evaluate EE, either directly using doubly labeled water or indirectly using calorimetry. However, methods used in these approaches are usually complex, expensive, uncomfortable, and/or difficult to apply on real time. To overcome these issues, a different approach consisting in estimating EE from other measurements issued from non-intrusive sensors, such as accelerometers and heart rate acquisition systems, is proposed.

Main Results

The proposed model, which consists of an original branched model [1] given by (1), uses heart rate signals for computing EE on moderate to vigorous physical activities (PAs) and a linear combination of heart rate and counts per minute (CPM) for computing EE on light to moderate PAs.

$$EE = \begin{cases} \alpha_1 \mathrm{HR^r} + \beta_1 & if\ \mathrm{HR^r} \geq S_{\mathrm{HR^r}} \\ \alpha_2 \mathrm{LC} + \beta_2 & if\ \mathrm{HR^r} < S_{\mathrm{HR^r}} \text{ and } \mathrm{LC} < S_{\mathrm{LC}} \\ \alpha_3 \mathrm{LC} + \beta_3 & if\ \mathrm{HR^r} < S_{\mathrm{HR^r}} \text{ and } \mathrm{LC} \geq S_{\mathrm{LC}} \end{cases} \quad (1)$$

In eq.(1), $\alpha_1 = 5.45, \beta_1 = -66.09, \alpha_2 = 256.09, \beta_2 = -0.13, \alpha_3 = 85.99$ and $\beta_3 = 82.39$ are the model parameters obtained by linear regression. LC is a linear combination of the normalized values of $\mathrm{HR^r}$ and CPM, computed as $\mathrm{LC} = \theta_1 \mathrm{CPM} + \theta_2 \mathrm{HR^r}$ where $\theta_1 = 0.0000844$ and $\theta_2 = 0.008583$, are normalization terms and $S_{\mathrm{HR^r}} = 40$ and $S_{\mathrm{LC}} = 0.5$ are activity cut-points. All parameter values were estimated from a given database, obtained under lab-conditions, which is composed of 53 subjects performing 25 different PAs (light-, moderate- and vigorous-intensity). Validation is performed either on the same database via leave-one-subject-out (LOSO) approach or on a second semi-controlled in-city circuit database.

Fig.1. shows the performances reached by four different (non)-linear models on the laboratory database. This illustrates the fact that models using $\mathrm{HR^r}$ as input provide better EE estimations than models only using CPM, and the proposed piecewise model yields the best accuracy.

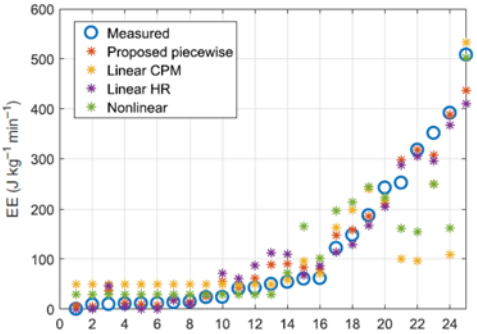

Figure 1: Mean EE measured and estimated by each model sorted by increasing energy demanding activity

Table 1 shows criterion performance obtained by the six models on the semi-controlled in-city circuit data set.

Table 1. Performance measures reached by the six models on the semi-controlled in-city circuit database

Model	r2	R2	RMSE (J kg^{-1} min^{-1})
Proposed piecewise	0.8637	0.9103	51.6074
Nonlinear	0.5503	0.6091	93.5573
Linear CPM	0.4008	0.5848	107.9964
Linear HR	0.7296	0.8525	72.6930

Results show that the proposed piecewise model, which was trained from the lab-experiment database is also accurate on the semi-controlled in-city circuit database. These results also confirm conclusions exposed above, i.e., (1) the models using $\mathrm{HR^r}$ as input provide better EE estimations than the models that only use CPM, and (2) the proposed piecewise model yields the best accuracy.

Perspectives

Results from validation on the two different data sets and comparisons presented in this paper confirm the interest of the proposed model. However, improvements may be achieved by other independent variables, i.e., considering age, sex, and/or weight. Another interesting idea that may improve EE estimations is to use more advanced models, such as linear splines, which allow to consider the continuous nature of the EE.

RELATED PUBLICATIONS:

[1] H. M. Romero-Ugalde et al., "An original piecewise model for computing energy expenditure from accelerometer and heart rate signals", Physiological Measurement 38 (8) (2017) 1599–1615.

DIABELOOP CLOSED LOOP DOES BETTER THAN SENSOR-AUGMENTED PUMP ON BLOOD GLUCOSE DURING 3 DAYS WITH PHYSICAL EXERCISE

RESEARCH TOPIC:

Artificial Pancreas, Type 1 diabetes, Insulin-glycemic model

AUTHORS:

S. Franc[1,2], S. Borot[3], P.-Y.Benhamou[4], M. Doron, B. Guerci[5], H. Hanaire[6], E. Huneker[7], N. Jeandidier[8], E. Renard[9], Y. Reznik[10], I. Xhaard[1,2], A. Penfornis[2], G. Charpentier[1,2], P. Schaepelynck [11]

ABSTRACT:

The Artificial Pancreas objective is to optimize the rate of insulin delivery for Type 1 diabetic (T1D) subjects. Over 180 000 people in France are subject to this chronic disease, which is a daily burden in terms of glycemic control. Most frequent treatment issues are hypoglycemia and hyperglycemia, causing lack of adherence to glycemic objectives. The bio-regulation implemented in Diabeloop's DBLG1 product is based on a Model-Predictive-Control (MPC).

SCIENTIFIC COLLABORATIONS: [1]CERITD, [2]CHSF, [3]CHU Besançon, [4]CHU Grenoble, [5]CHU Nancy, [6]CHU Toulouse, [7]Diabeloop, [8]CHU Strasbourg, [9]CHU Montpellier, [10]CHU Caen, [11]AP-HM Marseille, CRNH-RA

Context and Challenges

Events such as meals, physical activity and stress are the three main sources of glycemic perturbations for people with Type 1 diabetes. These events challenge the artificial pancreas capacity to improve the overall performances of the treatment. In the present study, the focus is on intense physical activity, which drives to immediate and delayed nocturnal hypoglycemia. The primary aim of the study was to compare the glycaemia equilibrium between the Diabeloop artificial pancreas (Diabeloop's DBLG1 close-loop system -CL) and the sensor-augmented insulin pump treatment (SAP) in well- educated patients practicing moderate or intensive PE repeatedly during 3-days [1-4].

Main Results

Fourteen T1D subjects were included in a randomized three-center crossover study, either with Diabeloop or SAP treatment during 72 hours. Each period included standardized meal and physical activity. The intensity of the physical activity was 30 or 45 minutes and intensity was 50% or 75% VO2 max on a fixed scheme for all participants.

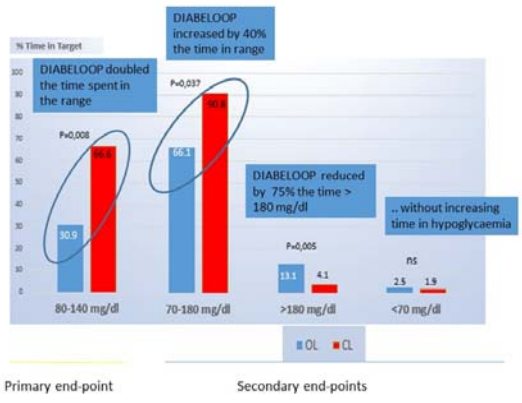

Figure 1: Outcome of the Diabeloop device (CL) versus the SAP treatment (OL) during the three nights.

Fig. 1 shows the main results. The duration spent in the 80-140mg/dL range over three nights monitoring was significantly larger with Diabeloop than with SAP. The time spent in the 70-180mg/dL range during the whole 72hrs was also larger with Diabeloop than with SAP. Three-day mean glycaemia was also significantly lower in CL than in SAP (138.0[129.4;147.1] versus 155.8[142.8;170.0]mg/dL, P = 0.0037). Day and nocturnal times spent in hypoglycemia were significantly lower in CL period.

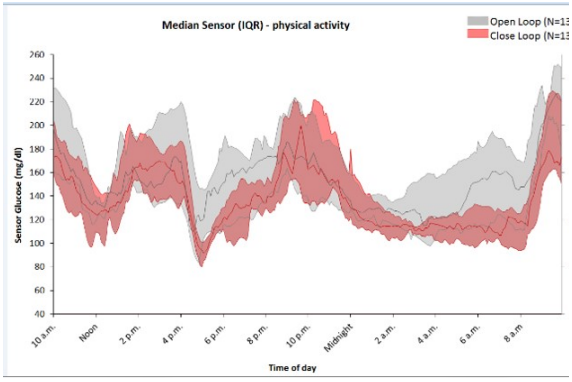

Figure 2: Median glycemic profile after three days of repeated physical exercises with Diabeloop (close loop) or with SAP (open loop).

Fig. 2 shows the glycemic profile with and without Diabeloop. Improvements in glycemic equilibrium, especially during the night.

Perspectives

The results presented here represent one of three axes of the clinical trial of Diabeloop's DBLG1 product. The two other axes, relative to sedentary way of living and rich food, also gave very positive results. The next step deals with the extension of the study to patient in a setting of everyday life, out of hospital.

RELATED PUBLICATIONS:

[1] MA. Quemerais et al., "Preliminary evaluation of a new semi-closed loop insulin therapy system over the prandial period in adult patients with TD1: the WP6.0 Diabeloop study", Journal of Diabetes Science and Technology, 2014.
[2] E. Renard et al., "Multicentre assessment of usability and safety of a model-predictive control algorithm with enhanced hypoglycemia minimizer for closed-loop insulin delivery in patients with TD1: a randomized control cross-over inpatient clinical trial", Diabetes Technology Meeting, 22-24/10/2015, Bethesda, Maryland, USA.
[3] S. Lachal et al., "Personalization of a Nonlinear Glucose-insulin system via a MCMC algorithm for MPC purposes", DTM, 11/2016, Bethesda, Maryland, USA.
[4] P. Jallon et al., "Personalization of a compartmental physiological model for an artificial pancreas through integration of patient's state estimation", EMBC 2017, Jeju Island, Korea.

DEVELOPMENT OF A WEARABLE CMOS-BASED CONTACT IMAGING SYSTEM FOR REAL-TIME SKIN CONDITION DIAGNOSIS

RESEARCH TOPIC:

Diffuse reflectance spectroscopy, Skin analysis, Contact imaging, Wearable device

AUTHORS:

N. Petitdidier, A. Koenig, R. Gerbelot, H. Grateau, S. Gioux[1], J.-M. Dinten

ABSTRACT:

Skin condition diagnosis is a critical issue in a great number of pathologies. We have developed a CMOS-based instrument for the measurement of skin properties in contact with tissue from spatially resolved diffuse reflectance spectroscopy measurements. Our device is based on a commercially available CMOS sensor, LED sources and a dedicated coupling system. This system enables diffuse reflectance imaging with tunable spatial sampling over a 30 mm² area and at source-detector separations as short as 500 µm. Instrument validation was conducted on tissue-simulating phantoms.

SCIENTIFIC COLLABORATIONS: Laboratoire ICube, Télécom Physique Strasbourg, Université de Strasbourg

Context and Challenges

Diffuse reflectance spectroscopy (DRS) has been widely used for biological tissue characterization as skin. In the spatially resolved DRS (srDRS) technique, light is collected at multiple distances from the excitation point. The obtained reflectance decay curve is used to determine scattering and absorption properties of the tissue, which are directly related to tissue content and structure. Existing systems usually use fiber optic bundles to collect light reflected from the tissue and transfer it to a spectrometer [1]. Such devices allow at performing srDRS measurements directly in contact with the tissues. However, they offer poor spatial sampling of the reflectance and low light collection efficiency. We propose to overcome these limitations by using a CMOS sensor placed directly in contact with the tissue.

Main Results

Close to the source, the diffuse reflectance is mainly sensitive to the reduced scattering coefficient μ_s', while further away from the source, both absorption coefficient μ_a and μ_s' contribute to the signal's shape. In consequence, both short (typically below 1 mm) and long source-detector separations (SDS) are needed to separate the contributions from scattering and absorption.

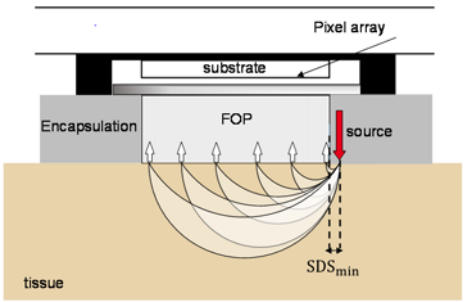

Figure 1: Schematic of the developed contact CMOS-based srDRS system.

A dedicated packaging and wire bonding is generally used on commercially available CMOS sensors for isolation of the sensitive area. Thus, the light source cannot be placed closer than a few millimeters to the pixels border. Therefore, the packaging of CMOS sensors limits the ability to simultaneously recover μ_a and μ_s'. To overcome this limitation, a fiber optic plate (FOP) is inserted between the CMOS sensor and the medium (Fig. 1) [2]. In this configuration, the LED can be placed directly against the FOP side, drastically reducing the shortest accessible SDS in comparison with the configuration where the sensor is put directly against the sample's surface.

In order to validate our approach, we compared the results obtained with the CMOS-based device with those obtained using the DRS fiber-optic probe. Reflectance data were acquired on Intralipide phantoms. Optical coefficients were determined and compared. Results from the CMOS-based system and the fiber-optic probe are in good agreement. Discrepancies between the two devices do not exceed 10%, which shows the potential of our instrument to perform spatially resolved diffuse reflectance measurements in contact with tissues [3].

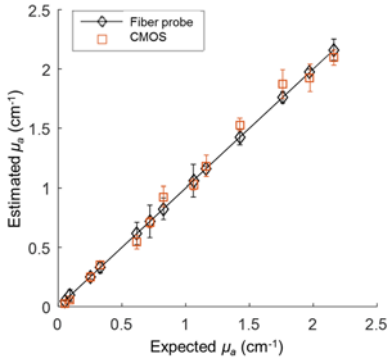

Figure 2: Estimations of µa using the CMOS-based system (red dots) and the fiber optic-probe (black squares).

Perspectives

This preliminary study demonstrates the potential of our approach towards the development of wearable devices for the determination of skin optical properties in real-time. Since only low-cost technology is involved, the described approach is also well suited for general public and point-of-care applications. In future work, we propose to evaluate the potential of our system for the analysis of layered media like skin.

RELATED PUBLICATIONS:

[1] A. Koenig et al., "Diffuse reflectance spectroscopy: a clinical study of tuberculin skin tests reading", Proc. SPIE. 8592, Biomedical Applications of Light Scattering VII 85920S (February 21, 2013).
[2] N. Petitdidier et al., "Dispositif de mesure d'un rayonnement rétrodiffusé par un échantillon et procédé de mesure utilisant un tel dispositif", Patent E.N.:17 63097, 22/12/2017.
[3] N. Petitdidier et al., "Development of a wearable CMOS-based contact imaging system for real-time skin condition diagnosis", 10412:104120F. International Society for Optics and Photonics, 2017.

ASSESSMENT OF THE STATUS OF ONCHOCERCIASIS WORMS WITH DIFFUSE REFLECTANCE SPECTROSCOPY

RESEARCH TOPIC:

Tissue characterization, Spectroscopy, Tissue diagnostic

AUTHORS:

A. Planat-Chrétien, M. Berger, S. Wanji[1], M. Boussinesq[2], B. Pedrique[3], J.-M. Dinten

ABSTRACT:

In the present work, we show the capacity of spatially resolved Diffuse Reflectance Spectroscopy (DRS) to identify live and dead worms in *ex vivo* Onchocerca ochengi nodules. It demonstrates the potential of DRS to diagnose the worm state and to monitor the effect of a drug on macrofilariae. These results have been obtained in constrained conditions, leading to the proposal of a rigorous acquisition and analysis protocol that may contribute to ensuring standardized measures in diffuse optics.

SCIENTIFIC COLLABORATIONS: [1]REFOTDE, University of Buea, Cameroon, [2]Institut de Recherche pour le Développement (IRD), Montpellier, [3]Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland

Context and Challenges

Onchocerciasis is a filarial disease that currently affects 37 million people, mainly in sub-Saharan Africa. Onchocerciasis is an eye and skin disease caused by the worm Onchocerca volvulus. It is transmitted to humans when bitten by a black fly of the genus Simulium. Inside the human body, the adult female worm produces thousands of larvae (microfilariae) that migrate to the skin and eyes. Massive drug delivery programs have been in place for over 20 years to treat and control filarial diseases. However, although current treatments with ivermectin may kill microfilariae, they must be repeated for at least 12 to 15 years each year in onchocerciasis, i.e. during the entire fertility period of adult female worms. There is therefore a need for new drugs, and preferably a macrofilaricide that will kill adult worms (macrofilaria). Work is underway at DNDi to develop this new drug. To assess the ability of this new drug to kill adult worms, a new measurement tool needs to be developed.

In this context, the objective is to show that an optical measurement can be used to qualify and to quantify the presence of live or dead adult worms in the nodules of patients receiving treatment, to monitor the treatment of a patient over time, and to evaluate the efficacy of treatment using a simple, non-invasive method. First, a feasibility *ex vivo* study on the bovine model of onchocerciasis is carried out.

Main Results

The Leti has developed a planar optical probe capable of characterizing biological tissues via their endogenous optical properties (absorption and diffusion) [1,2]. The instrumentation that has been developed is based on the use of a white light excitation source covering the 450 to 1050nm light spectrum, an exploration probe comprising an excitation fibre and six rings of detection fibres coupled to a spectrometer that detects the spectrum emerging from the tissues. A diffuse reflectance spectroscopy (DRS) analysis of the multi-spectral data acquired identifies changes in the optical properties of the tissues and quantifies their composition or variation. This device has been adapted and optimized for tropical conditions and environment (Fig.1). It involved a specific acquisition protocol and analysis method to ensure the robustness of the optical measures.

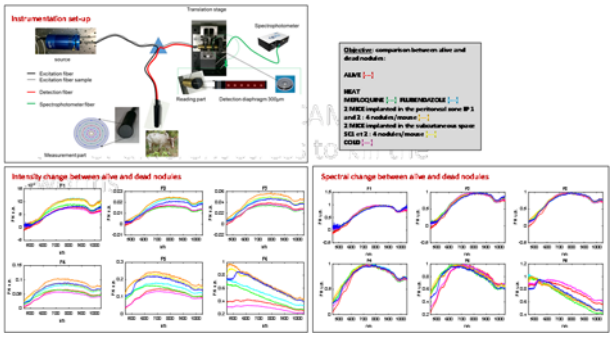

Figure 1: Schematic of the experimental setup. Reflectance signals obtained during the *ex vivo* campaign: test of different stress to kill the worms. Intensity (left) and spectral (right) changes between alive (red) and dead worms (other).

A phase of evaluation was carried out during *ex vivo* experiments on the bovine model of onchocerciasis. The nodules were stressed by different stimuli to cause worm death; the different groups obtained were measured by DRS and compared (Fig1). It showed that (1) the DRS measures a modification of the optical signature of the nodule due to its degradation. It may be used *to monitor* its degradation along time. The signal modification consists in both spectral and intensity variations. (2) It measures a modification of optical properties of the worm *and* its local environment. Thus it is an *indirect measurement* of the worm death. (3)Moreover, the optical signature is specific of the stress applied. (4) It provides a specific signature of alive and dead nodules showing its capacity *to distinguish* dead and alive worms in the *ex vivo* campaign.

The results *in vivo* are mode difficult to interpret because of artefacts induced by the surgery protocol, but remains encouraging.

Perspectives

A campaign is scheduled for 2018 to validate the relevance of optical measurement on phototype 6 skin, before defining a clinical validation campaign.

RELATED PUBLICATIONS:

[1] A. Koenig et al., "Diffuse reflectance spectroscopy: a clinical study of tuberculin skin tests reading", In SPIE BiOS, Proc. SPIE 8592, Biomedical Applications of Light Scattering VII, 85920S (February 21, 2013); doi:10.1117/12.2002314.

[2] V. Sorgato et al., "ACA-Pro: Calibration Protocol for quantitative diffuse reflectance spectroscopy: Validation of Contact and Non-Contact probe- and CCD- based systems", J. Biomed. Opt. 21(6), 2016.

TOWARDS A BETTER UNDERSTANDING OF PHOTOBIOMODULATION-INDUCED MEDCHANISMS

RESEARCH TOPIC:

Parkinson's disease, Infrared light, Neuroprotection, Active implantable medical device

AUTHORS:

D. C. Moro, F. Darlot, J.Molet, N. Torres, F. Reinhart, D. Agay, C. Chabrol, S.Renault, AL. Benabid, J.Mitrofanis[1].

ABSTRACT:

Photobiomodulation, or PBM, (Intracranial application of red to infrared light) is a new therapeutic strategy for diseases such as neurodegeneration. We evaluated changes induced by intracranial PBM in terms of toxicity and benefits using a non-human primate model. We did not observe any histological basis for any major biosafety concerns, and identified Glial Derived Neurotrophic Factor (GDNF) as a main actor of beneficial effects mechanisms, such as axonal regrowth and reinnervation of cellular targets in the striatum. These results open the way to future clinical trials to evaluate neuroprotective properties of near-infrared light in Parkinson's disease.

SCIENTIFIC COLLABORATIONS: [1] Sydney University, Australia

Context and Challenges

Red to infrared light (λ=600–1000nm), or photobiomodulation (PBM), is an innovative treatment for a wide range of neurological and psychological conditions. Our previous studies on preclinical models of Parkinson disease demonstrate a neuroprotective potential of intracranial PBM. To this aim we developed an intracranial chronically implantable device delivering near infra-red light and evaluated consequences of chronic illumination into brain tissue of normal non-human primates, or parkinsonian animals.

Main Results

In a first study [1], five naïve adult macaque monkeys were implanted with intracranial devices, developed at Clinatec, in a similar way than what is planned for a human clinical trial. Optical fiber device was turned on, and left on throughout the remaining survival period, for up to 12 weeks. We limited our explorations to MRI scans (to reveal the location and extent of the implant site), and histological analysis.

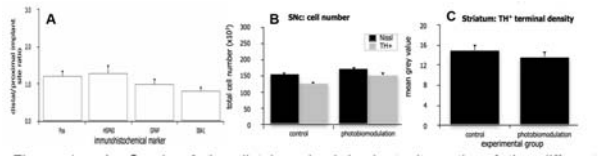

Figure 1: (A) Graph of the distal proximal implant site ratio of the different immunohistochemical markers used in this study. (B) Graph of the estimated number of nigral Nissl stained (black columns) and TH+ (grey columns) cells in PBM treated monkeys. (C) Graph of the density of the striatal TH+ terminals in PBM-treated and control monkeys. The column show the mean ± standard error.

The distal/proximal ratio in implant site area indicates little or no impact of PBM on the size of implant site, i.e. no evidence for a cavitation caused by PBM. The optical fiber implant site was also not associated with a major inflammatory response in surrounding regions. We found no evidence of toxicity in the brain: there were few, if any, differences in the appearance and cellular organization surrounding the proximal, as compared to the distal implant sites. We also found no major differences in the number of nigral Nissl stained and TH+ cells, and density of striatal TH+ terminations between the PBM-treated and control animals.

In another study [2], we examined on parkinsonian animals whether PBM had any effect on the number of striatal TH+ cells and GDNF expression, as parkinsonian lesions are related to a loss of dopaminergic inputs into the striatum. In addition, parkinsonian animals treated with GDNF usually show an increase in the number of striatal TH+ cells and an improvement in motor behavior. In our monkey model, where there was a marked loss of striatal TH+ terminations in animals, there was a clear increase in the number of striatal TH+ cells. We found that, while the monkeys in the MPTP group had more TH+ cells and GDNF expression than the controls, the MPTP-PBM group had even more striatal TH+ cells (~60%) and GDNF expression (~50%) than the MPTP group

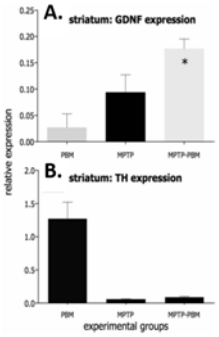

Figure 2: (A) Graph showing GDNF expression normalized against the loading control GADPH (relative expression) in the three groups; *in MPTP-PBM group column represents significant difference ($p<0.05$) to MPTP group. (B) Graph showing TH expression normalized against the loading control GADPH (relative expression) in the three groups analyzed. SEM are indicated for each column.

Perspectives

We found no evidence of pathology in the Substantia Nigra pars compacta (SNc) or striatum after PBM, indicating no impact on the morphology and cytoarchitecture of the dopaminergic cell system. Our results suggest that the PBM-induced GDNF expression stimulated the expression of TH in new cells and has a trophic function. Next steps will include performing a clinical trial in Parkinson's disease to evaluate PBM effects in a first-in-human trial.

RELATED PUBLICATIONS:

[1] C. Moro et al., "No evidence for toxicity after long‐term photobiomodulation in normal non‐human primates", Exp Brain Res, 235:3081–3092, 2017

[2] N. El Massri et al., "Photobiomodulation-induced changes in a monkey model of Parkinson's disease: changes in tyrosine hydroxylase cells and GDNF expression in the striatum", Exp Brain Res, 235:1861-1874, 2017.

04 MATERIALS: FROM FUNCTIONALIZATION AND BIOMATERIALS TO SENSORS AND VECTORIZATION

- **Bacteria capture and release**
- **Aerogels for drug delivery**
- **Drug nano-carriers**
- **Polysaccharide hydrogels**
- **Bacteria secretion electrochemistry**

β-CD-FUNCTIONALIZED MICRODEVICE FOR RAPID CAPTURE AND RELEASE OF BACTERIA

RESEARCH TOPIC:
Bacterial concentration, Cyclodextrin, Host−guest interaction, Microfluidic chip, POC

AUTHORS:
A. Perez-Anes, A. Szarpak-Jankowska[1], M. Alessio, R. Auzély-Velty, D. Jary

ABSTRACT:
In the field of pathogens detection with miniaturized and autonomous systems, concentration of the sample is a key step. Here we demonstrate that functionalization of surfaces by β-cyclodextrin (β-CD) enables rapid and efficient capture of bacterial cells in liquid but also subsequent facile elution with an aqueous solution of a selectively methylated β-CD derivative used as a competitive binding molecule. This capture/elution strategy is performed in physiological conditions, and is so compatible with many analysis techniques. This concentration step has been successfully integrated in a micropillar microfluidic device.

SCIENTIFIC COLLABORATIONS: [1]CNRS, Centre de Recherches sur les Macromolecules Végétales (CERMAV)

Context and Challenges

Rapid and sensitive detection of pathogenic organisms at very low concentrations is crucial to safeguard public health from food- and water-borne pathogens as well as from infectious diseases. Among the rapid pathogen detection technologies, PCR detection is highly specific, can facilitate the identification of microorganisms that are difficult to culture, and has the potential to reduce the overall cost of testing. Rapid preconcentration of pathogens is essential to take full advantage of PCR for detecting bacteria especially when using environmental or food samples due to the mismatch between high sample volumes (>25 mL) and small amplification volumes (10–100 µL).

It is known that surface exposed proteins and lipopolysaccharides in bacteria are responsible for important functions, including adhesion and virulence. This fact, together with the ability of cyclodextrins to selectively interact with cellular membranes by virtue of either their complexation ability and/or their surface activity, prompted us to use these cage molecules as synthetic receptors to design a simple and cost-effective bacterial capture platform [1].

Main Results

To evaluate the bacterial capture and elution capabilities of β-cyclodextrin modified surfaces, we first studied the binding of *E. coli* to β-CDs using quartz crystal microbalance with dissipation monitoring. Fast adsorption of bacterial cells onto the functionalized sensor crystal was observed following bacteria suspension injection. Adsorbed bacteria were fully released from the surface when rinsing the quartz with the highly soluble cyclodextrin DIMEB, owing to interactions competition. These experiments proved that bacteria are bound to the β-CD-coated in a strong but reversible fashion through multivalent interactions.
As a proof of concept, we studied bacterial cell capture and subsequent release using a micropillar-integrated microfluidic device. The bacteria capture and elution yields were measured by qRT-PCR, for both gram+ (*B. subtilis*) and gram- (*E. coli*). Playing with the concentration of β-CD, the best results were obtained with *E. coli*, for which the relative elution yield reached up to 85% whereas relative elution yield is significantly lower for *B. subtilis*, around 30%.
After optimization, using this surface chemistry, our microdevice could be used for gram- bacteria enrichment, which is an original feature compared to literature.

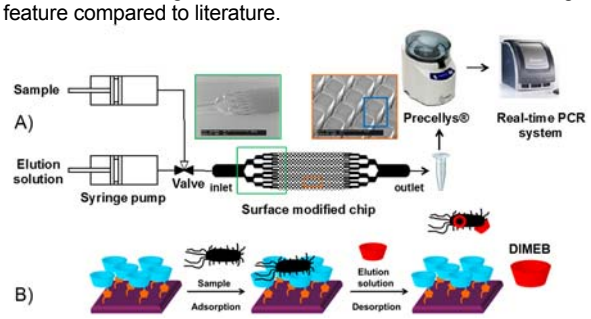

Figure 1: (A) Experimental setup for real-time qRT-PCR detection of bacterial cells from aqueous samples. (B) Scheme of bacterial capture/elution using β-CD-modified substrates and a methylated β-CD derivative.

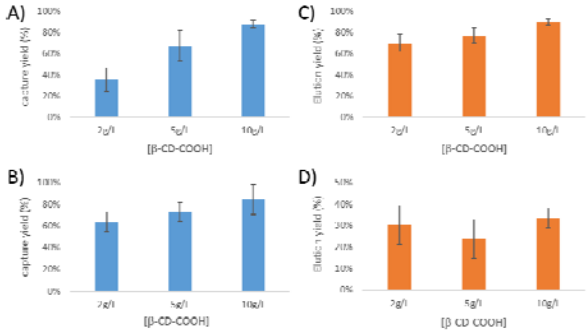

Figure 2: Quantitative evaluation by qRT-PCR of the bacterial capture and relative elution yields for β-CD-coated surfaces prepared with [β-CDCOOH]=2, 5, and 10 g/L. (A, B) E. coli and (C, D) B. subtilis.

Perspectives

Performances of our microdevice may be optimized by modifying the design parameters of the microchip, like the pillars spacing, entries design, and also microfluidic parameters like flow rate.

RELATED PUBLICATIONS:
[1] A. Perez-Anes et al., "β-CD-Functionalized Microdevice for Rapid Capture and Release of Bacteria", ACS Appl. Mater. Interfaces 2017, 9, 13928−13938.

DESIGN ON INTERPENETRATING CHITOSAN/POLY(ETHYLENE GLYCOL) AEROGELS FOR DRUG DELIVERY APPLICATIONS

RESEARCH TOPIC:

Drug delivery systems, Biomaterials, Polymer, InterPenetrated Network, Chitosan, PEG

AUTHORS:

L. Racine, G. Costa, E. Bayma-Pecit[1], R. Auzély-Velty[1], I. Texier

ABSTRACT:

Taking advantage of its haemostatic action together with its ability to activate macrophages, chitosan (CS) has received much attention as a functional biopolymer for designing materials for medical applications. CS was therein combined with poly(ethylene glycol) (PEG), a bio-compatible and bio-degradable safe polymer, to achieve innovative aerogels with a semi-interpenetrating polymer network design. Obtained materials combined both highly porous structure suitable for wound healing, and tough mechanical structure. Such materials with process-tunable pore-size are of high interest for rate-controlled drug delivery systems.

SCIENTIFIC COLLABORATIONS: [1]Université Grenoble Alpes, CERMAV-CNRS, Grenoble

Context and Challenges

As a bio-sourced highly biocompatible polymer, chitosan (CS) is a material of choice for biomedical applications. Depending on the preparation process, a wide range of materials can be obtained, from thin films to highly porous hydrogels or aerogels [1]. However, CS materials should be cross-linked, preferentially using chemical methods, to prevent their very fast degradation in contact with biological fluids and to improve their mechanical resistance [1]. A different strategy was here proposed to obtain CS-based robust aerogels, relying on the interpenetration of a cross-linked poly(ethylene glycol) (PEG) network within the un-modified CS matrix [2].

Main Results

Semi-interpenetrating CS/PEG aerogels were obtained by crosslinking PEG in the CS matrix via nucleophilic thiol-yne addition (Fig.1). This reaction does not require the use of any potentially cytotoxic catalytic species and offers possibilities to prepare materials with tunable properties.

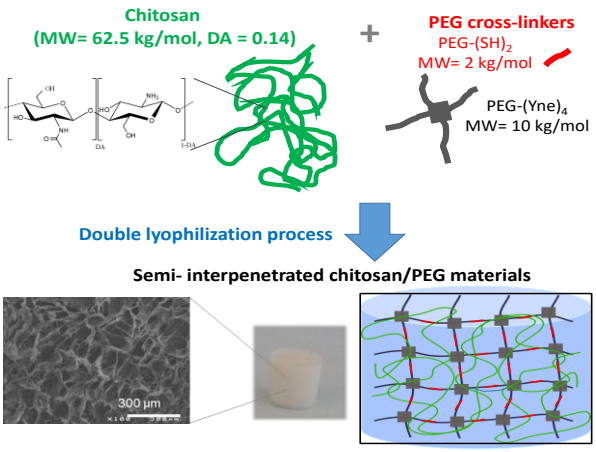

Figure 1: Process of fabrication of semi-interpenetrating CS/PEG aerogels, and their porous structure as assessed by SEM.

The molecular structure of the sponges was analyzed by FTIR spectroscopy, which provided evidence of intermolecular interactions between PEG and CS, and the presence of a cross-linked PEG network in the CS matrix. The crosslinked CS/PEG aerogels displayed a structure with large interconnected pores (tens of micrometers) as demonstrated by scanning electron miscoscopy (Fig.1), in comparison to blend CS/PEG materials with irregular and smaller pores. The crosslinked PEG/CS aerogels also exhibited improved mechanical properties (higher Young's modulus, Fig.2) and stability at physiological pH.

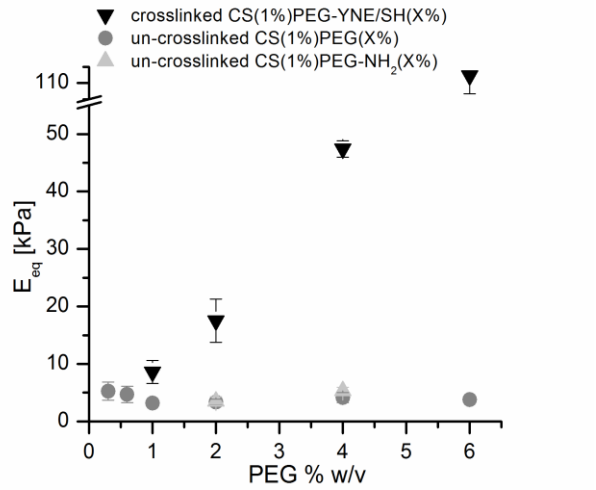

Figure 2: Young modulus of cross-linked semi-interpenetrating CS/PEG aerogels in comparison to blended un-crosslinked CS/PEG materials.

Perspectives

The possibility to modulate the porosity of such interpenetrating CS/PEG aerogels is of high interest for rate-controlled drug delivery systems. These interesting material properties open the way for their biomedical application, particularly in the field of topical drug delivery.

RELATED PUBLICATIONS:

[1] L. Racine et al., "Chitosan-based hydrogels: recent design concepts to tailor properties and functions", Polym. Int., vol. 66, pp. 981-998, 2017.
[2] L. Racine et al., "Design of interpenetrating chitosan and poly(ethylene glycol) sponges for potential drug delivery applications", Carbohydrate Polym., vol. 170, pp. 166-175, 2017.

NEW NANOTHERAPEUTIC AGAINST *M. tuberculosis*: BEDAQUILINE ENCAPSULTION IN LIPID NANOPARTICLES

RESEARCH TOPIC:

Nanocarrier, Bedaquiline, *M. tuberculosis*

AUTHORS:

D. Jary, A. Lucía[1], D. Pérez[2], J.A. Ainsa[1], N. Redinger[1], U. Schreibe[1], F.P. Navarro

ABSTRACT:

Nowadays, new strategies must be set up to improve the treatment of infectious diseases. The development of effective and safe nanotechnology-based methods can be particularly relevant to increase antimicrobial concentration at the site of infection, to reduce doses in the general circulation, which in turn reduces adverse effects. In this work bedaquiline was encapsulated in lipid nanoparticles (NP) with high encapsulation efficiency. The efficacy of the drug-encapsulating nanocarrier has been demonstrated in vitro against *Mycobacterium tuberculosis*, with an excellent compatibility of the carrier with animal cells, which makes it promising for improvement of bedaquiline based treatment of tuberculosis (TB).

SCIENTIFIC COLLABORATIONS: [1]Universidad de Zaragoza; [2]Nanoimmunotech, Vigo, Spain

Context and Challenges

Bedaquiline, the first FDA approved anti-tuberculosis drug in four decades, is a very effective drug, nonetheless it shows serious side effects including induction of life-threatening cardiac arrhythmias. Therefore, this drug is to be prescribed only when no other treatment options are available, justifying the development of effective and safe nanotechnology-based methods to decrease these side effects. The main challenge is to obtain nanocarriers combining several different properties such as high stability in storage conditions, kinetics of in vivo drug release compatible with a therapeutic effect, and surface properties enabling the penetration of the NP deep in the lungs. To this aim, we have assessed the behavior of slightly negatively and positively charged lipid nanopaticles (LNP) that encapsule bedaquiline in order to evaluate their performances as novel nanotherapeutics to cure tuberculosis [1].

Main Results

The physico chemical properties of LNPs have been characterized. Both types of LNPs exhibited, as expected, a small size (below 100nm) enabling to exacerbate interactions at the cellular level and to penetrate in tissues, especially in the lungs. They are weakly polydisperse with polydispersion index below 0.15 and zeta potential were in agreement with the differences on nanocarrier, indicating the presence of a strong positive charge in the case of LNPs(+). Finally, as expected, the LNPs are very stable in storage conditions, without significant drug release during 6 months.

	DL, %	EE, %	Hydrody-namic diameter, nm	PDI	Z-potential, mV	Six-month stability, DL (6 months)/ DL (t0),%
LNP(−)	2.8 ± 0.15	93 ± 6	86 ± 1.3	0.148	−10 ± 1	97 ± 5
LNP(+)	2.8 ± 0.15	93 ± 7	83 ± 3.1	0.123	+28 ± 3	98 ± 4

Table 1: Nanocarriers characterization after bedaquiline encapsulation.

The Minimum Inhibitory Concentration has also been compared between free drug and loaded LNP. No difference have been found, showing that the drug is still active after encapsulation. The profile of the release of bedaquiline from nanocarriers was determined and media selected on the basis of conditions used in the in vitro assays. LNPs loaded with bedaquiline were found to be very stable in PBS, RPMI and Middlebrook 7H9 medium (in all cases less than 10% drug was released after one week) indicating again a very good colloidal stability of these carriers. Release in human plasma was also studied in the case of LNPs for future intravenous injection applications and data are reported in Fig. 1. Results indicated that the kinetics of the release is fully compatible with a potential therapeutic effect as a circulating time of a few hours should allow distribution of the LNPs to the lungs.

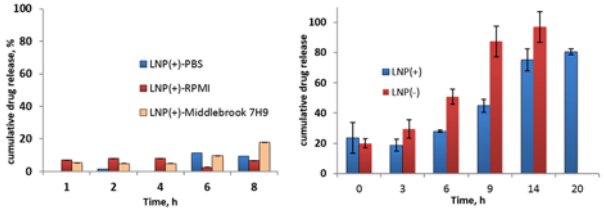

Figure 1: Cumulative drug release at 37°C of (A) LNP(+) in storage buffer, PBS, and in vitro culture media, (B) LNP(+) and LNP(-) in human plasma.

In addition, in vivo drug LNP biodistribution has been performed with TB infected mice, to evaluate the ability of these carriers to accumulate in the lungs. Results showed that both kinds of LNPs accumulated in the lungs during a 2 weeks administration of the bedaquiline loaded carriers, indicating that these carriers are well designed for the intended application.

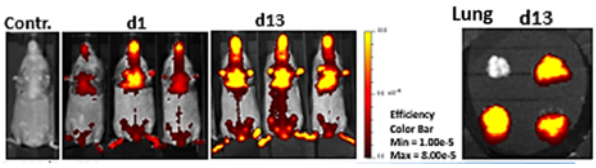

Figure 2: Biodistridistribution by IVIS imaging of bedaquiline-LNP(+) in C3HeB/FeJ mice treated from day 30 to 43 after M. tb. exposure by aerosol exposure to the strain H37Rv.

Perspectives

From the obtained results, it can be concluded that both kinds of carriers represent very good candidates to be tested for in vivo activity and toxicity hopefully in view of a safer administration of this drug.

RELATED PUBLICATIONS:

[1] L. De Matteis et al., "New active formulations against M. tuberculosis: Bedaquiline encapsulation in lipid nanoparticles and chitosan nanocapsules", Chemical Engineering Journal 340 (2018) 181–191.

TIME-CONTROLLABLE LIPOPHILIC-DRUG RELEASE SYSTEM DESIGNED BY LOADING LIPID NANOPARTICLES INTO POLYSACCHARIDE HYDROGELS

RESEARCH TOPIC:
Drug delivery systems, Biomaterials, Cellulose, Lipid nanoparticles

AUTHORS:
L. Racine, A. Guliyeva[1], I. Wang[2], V. Larreta-Garde[3], R. Auzély-Velty[1], I. Texier

ABSTRACT:
"Double encapsulation" drug delivery systems for loading hydrophobic active ingredients inside a hydrophilic hydrogel matrix is proposed. CarboxyMethyl Cellulose (CMC)-based materials were loaded with lipid nanoparticles (LNPs) encapsulating a lipophilic fluorescent dye acting as a hydrophobic drug model. The diffusion of drug-loaded lipid nanoparticles form the CMC matrix was shown to depend on the particle diameter and surface charge, as well as the cross-linking density of the cellulose hydrogel.

SCIENTIFIC COLLABORATIONS: [1]Université Grenoble Alpes, CERMAV-CNRS, [2]Université Grenoble Alpes/CNRS, LIPhy, [3]Université Cergy-Pontoise, ERRMECe

Context and Challenges

Hydrogels are polymeric materials constituted of a highly hydrophilic backbone which confers them the ability to hold large amounts of water in their three-dimensional network. Hydrogels based on biopolymers such as cellulose derivatives (nanocellulose, CMC), chitosan or hyaluronic acid, are particularly interesting for medical applications, such as drug delivery systems or tissue engineering [1]. However, many active ingredients, such as anti-inflammatory or chemotherapeutic drugs, present hydrophobic structure which impedes their efficient loading in the water-swollen hydrogel matrices. In parallel, lipid nanoparticles (LNP) able to encapsulate important payloads of lipophilic molecules have been developed at Leti and tested for different drug delivery and imaging applications [2]. A hybrid hydrogel composed of solid lipid nanoparticles (LNPs) entrapped within chemically crosslinked carboxymethylcellulose (CMC) was therefore developed to achieve localized and sustained release of lipophilic drugs [3].

Main Results

Hybrid CMC/LNP hydrogels were obtained by photo-crosslinking pentenoate-modified CMC (CMC-ene) with poly(ethylene glycol) -bis-thiol (PEG(SH)$_2$) in the presence of LNPs (Fig.1).

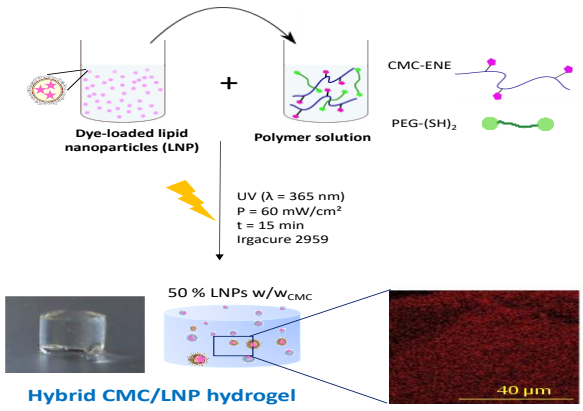

Figure 1: Process of fabrication of hybrid CMC/LNP hydrogels.

The analysis of LNP stability as well as the hydrogel swelling and mechanical properties confirmed the successful incorporation of particles up to a concentration of 50 %w/w CMC.

LNP release from the hydrogel matrix was quantified using dye-loaded LNP as drug model, and compared with the results achieved with a mathematical diffusion model based on Fick's second law. The initial LNP release rate could be prolonged by increasing the particle diameter from 50 nm to 120 nm, while the amount of long-term release could be adjusted by tailoring the particle surface charge (Fig.2) or the crosslinking density of the polymer matrix. After 30 days, 58% of 50 nm diameter negatively charged LNPs escaped from the matrix while only 17% of positively charged nanoparticles were released from materials with intermediate crosslinking density (Fig.2).

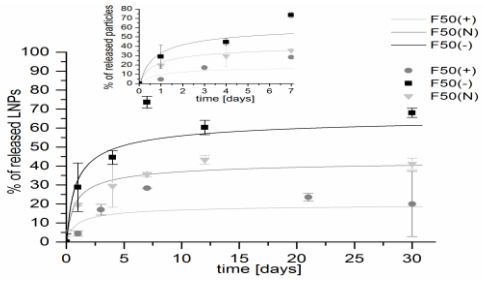

Figure 2: Influence of the particle charge on the kinetics of release of 50 nm diameter LNPs loaded in CMC hydrogels. (line: model values; symbols: experimental values).

Perspectives

The incorporation of LNPs in crosslinked CMC/PEG hydrogels represents a promising strategy for sustained and controlled release of lipophilic drugs. These biomaterials processed in a dried aerogel form could be dedicated to post-surgery implantation to promote wound healing, or presented as skin patches.

RELATED PUBLICATIONS:
[1] L. Racine et al., "Chitosan-based hydrogels: recent design concepts to tailor properties and functions", Polym. Int., vol. 66, pp. 981-998, 2017.
[2] Q. Cabon et al., "Evaluation of intraoperative fluorescence imaging–guided surgery in cancer-bearing dogs: a prospective proof-of-concept phase II study in 9 cases", Translational Res., vol. 170, pp. 73-88, 2016.
[3] L. Racine et al., "Time-controllable lipophilic-drug release system designed by loading lipid nanoparticles into polysaccharide hydrogels", Macromol. Biosci. vol. 17, no. 9, 1700045, 2017.

ELECTROCHEMICAL CHARACTERIZATIONS OF FOUR MAIN REDOX–METABOLITES OF PSEUDOMONAS AERUGINOSA

RESEARCH TOPIC:

Electrochemistry, Metabolites detection, Pseudomonas aeruginosa, Quorum Sensing

AUTHORS:

J. Oziat, M. Gougis, G.G. Malliaras[1], P. Mailley

ABSTRACT:

Nowadays, some researches focuse on the electrochemical detection of the opportunistic pathogen Pseudomonas aeruginosa (PA) because of its production of the Pyocyanin toxin which has an electrochemical case study behavior. However, PA secretes other molecules that are less or poorly studied. This work deals with the systematic electrochemical characterization of 4 main metabolites of PA in the view of multispecies detection of PA in bacteria supernatants.

SCIENTIFIC COLLABORATIONS: [1]Department of Bioelectronics, ENS Mines de Saint-Etienne, Gardanne

Context and Challenges

PA is a gram-negative bacterium that focuses tremendous attention these last decades. Such opportunistic human pathogen is one of the major microorganism responsible for nosocomial infections in Europe. Patients in intensive care units or suffering from burning, cancer or immunodeficiency present high risk of PA infection. In addition to its pathogenicity, PA exhibits high resistance to antibiotic making its eradication complicated.

According to the aforementioned statements, some works were dedicated to the early detection of PA using complex biochemical and/or physicochemical methods. Interestingly, PA secretes small signaling molecules including Pyocyanin (PYO) that has a strong redox signature. PYO was then early implemented to characterize the presence of PA in bacterial. Away from PYO, PA (as all bacteria) secrete a range of (bio)molecules in their culture medium. These (bio)molecules include components of the bacterial quorum sensing (QS), virulence factors or small metabolites issued from bacterial function. In this work [1], we focused on the electrochemical characterization of four of these signaling units (Fig.1) in the view of establishing/understanding the electrochemical fingerprint of bacteria culture supernatants [2].

Main Results

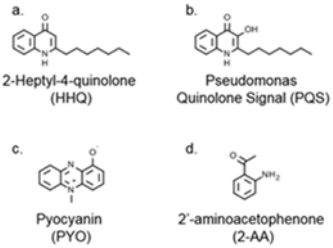

Figure 1: The four PA metabolites studied in this work.

The electrochemical behavior of the four main metabolites of PA (PYO, PQS, HHQ and 2-AA) were studied in aprotic solvent and aqueous media buffered at different pH. This extensive study enabled for the first time to propose electrochemical mechanisms of the four metabolites. Moreover, it allowed understanding the redox comportment of the different metabolites in terms of electrochemical reversibility, number of exchanged electrons or nature of the by-products generated by electrolysis and their fouling characteristics in case of irreversible behavior.

Otherwise, the electrochemical response of the four species (Fig.2) was assayed in phosphate buffer saline solution (PBS 1X, pH7) in concentration ranges coherent with bacteria culture conditions. This study shown that the electrochemical response of the four metabolites is easy to identify with minimum overlap except between 2-AA and HHQ. PYO and PQS represent interesting target due to their well-defined reversible response in the electrochemical stability window of water. Interestingly, we also shown a nice dependency of PYO oxidation peak potential with pH. Such behavior would be beneficial to estimate pH evolution of bacteria culture supernatant along PA growing process [2].

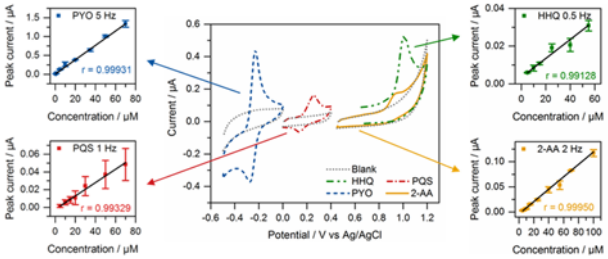

Figure 2: Central figure: Cyclic voltametries (50mV/s) of HHQ, PQS, PYO and 2AA (100µM in PBS 1x) on glassy carbon electrode. Around: calibration curves of the 4 metabolites issued from square wave voltametry.

Perspectives

These first fundamental insights will further help i. identifying the different responses of PA electrochemical fingerprint and ii. quantifying PA growth in bacteria culture media. Complementary works will be carried out to improve the electrochemical detection using nanostructured electrodes to provide in situ PA identification during growth.

RELATED PUBLICATIONS:

[1] J. Oziat et al., "Electrochemical characterizations of four main redox-metabolites of Pseudomonas aeruginosa", Electroanalysis, 29 (2017) 1-10.
[2] J. oziat et al., "Electrochemistry provides a simple way to monitor Pseudomonas aeruginosa metabolites" Conf Proc IEEE Eng Med Biol Soc. (2015) 7522-7525.


HR
89
bpm
NIPB
108/68
mmHg
SpO2
99
%
RR
16
rpm
BT
36.8
°C

05 PHD DEGREE AWARDED

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VIVIAN AUBERT

ACOUSTOFLUIDICS EVANESCENT WAVES: APPLICATION TO ADHERENT CELLS PATTERN IN CULTURE CONDITIONS

École doctorale Sciences mécaniques, acoustique, électronique et robotique de Paris

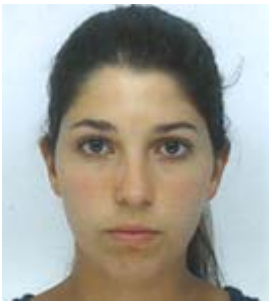
It has been shown that the use of acoustic waves enables nanoparticles, microbubbles, drops or microbeads, living cells and fluids to be moved, sorted, or mixed in a contactless and label-free manner. Here, we take advantage of the acoustic radiation force to manipulate living cells. Most of the applications and their associated techniques rely on the use of the so-called SAW (Rayleigh Surface Acoustic waves). This technique is powerful but requires piezoelectric substrates and suffers from a high damping due to radiation losses in the supersonic regime. Here, we work instead in the subsonic regime of propagation which allows us to generate an evanescent field ("Scholte" waves) thanks to a thin substrate. This wave presents very interesting characteristics since acoustic energy is concentrated in the vicinity of the substrate where objects are located. Moreover, the propagation is lossless and doesn't require any substrate or particular medium. We then showed the potential of this new approach through key-applications in microfluidics. This device enables to establish patterns and to concentrate cells in a flow. We have also designed a rotating acoustic field and shown the possibility of trapping and spinning of individual cells. We also describe a blood plasma characterization method by acoustic "centrifugation" within a drop. In a second part, we have designed a network of switchable acoustic traps compatible with living cells in order to study its effect on a population of adherent cells in culture. It reveals a change of cells behaviour depending on the phenotype. This work opens the way to the use of acoustics in the study of mechanotransductive effects on cells population.

ANTHONY BERDEU

3D LENS-FREE IMAGING OF 3D CELL CULTURE

Ecole Doctorale Ingénierie pour la Santé, la Cognition et l'Environnement, Grenoble

This PhD work was at the interface of two fields: 3D cell culture and lens-free imaging.
Providing a more realistic cell culture protocol, switching from single-layer (2D) cultures to three-dimensional (3D) cultures, via the use of extracellular gel in which cells can grow in three dimensions, was at the origin of several breakthroughs in several fields such as developmental biology, oncology and regenerative medicine. These new objects to study have created a need in terms of 3D imaging.
On another side, 2D lens-free imaging provides a robust, inexpensive, non-labeling and non-toxic tool to study cell cultures in two dimensions over large scales and over long periods of time. This type of microscopy records the interferences produced by a coherent light scattered by the biological sample. Knowing the physics of the light propagation, these holograms are retro-propagated numerically to reconstruct the unknown object. The reconstruction algorithm replaces the absent lenses in the role of image formation.
The aim of this PhD was to show the possibility of adapting this lens-free technology for imaging 3D cell culture. New lens-free microscopes were designed and built in parallel with the development of dedicated tomographic reconstruction algorithms. Biological samples were reconstructed with volumes of several tens of cubic millimeters, inaccessible in standard microscopy. 3D time-lapse data successfully obtained in incubators have also shown the relevance of this type of imaging by highlighting large-scale cell interactions between cells or between cells and their three-dimensional environment.



MELANIE BERNARD

MODULAR PROCESSING SYSTEM FOR CDZNTE DETECTORS EMISSION-TOMOGRAPHY

Ecole Doctorale Mathématiques, Sciences et Technologies de l'Information, Informatique, Grenoble

In the last decades, new CdTe/CdZnTe based detectors enabled enhanced performances on medical imaging systems, especially in SPECT imaging. These improvements are achieved by developing simultaneously the precise localization of interactions in detectors, the collimator, matching between the system geometry and the specificities of the examination, reconstruction algorithms, and data processing architectures. In addition to their interesting intrinsic performances, CdZnTe detectors benefit from their compactness compared to indirect conversion detectors. This compactness enables more flexible systems. New designs are thus proposed, especially in cardiac imaging. In order to go further the compromise between resolution, sensitivity, and field-of-view imposed by SPECT imaging, this work focuses on the possibilities for online adaptation offered by flexibles designs.
Adaptation on a system composed of several independent detection heads was under study. We proposed to adapt the angular sampling of heads, enabling acquisition protocols more accurate for different patient morphologies and examination protocols. The purpose of this work was to propose acquisition and adaptation protocols enabling the reconfiguration of the angular sampling of detection heads depending on an estimation of the imaged object. Algorithmic solutions were proposed in order to compute the reconstruction in real time from more complex data because of additional degrees of freedom and detector precision. The resulting estimation has to enable the identification of informative areas in order to focus detection heads on it. Simulations approved theoretically the use of our rapid algorithmic solution on a modular system carrying a reliable estimation of the object. Some experimental results validated the system model and the reconstruction algorithm. Some adaptation strategies were investigated using simulations.

VALENTIN GENUER

ELASTIC LIGHT SCATTERING FOR FAST IDENTIFICATION OF PATHOGENS

École Doctorale Electronique, Electrotechnique, Automatique & Traitement du signal, Grenoble

There is an urgent need for novel and innovative diagnostic methods that would speed up accurate treatments decisions and be of significant utility for public health in the fight against antibiotic resistance. This work aims to better understand the Elastic Light Scattering (ELS) method for microbial identification. This phenotypic technique is based on the ELS of a coherent light beam by a microorganism colony growing on its culture plate. The resulting scattering pattern can be considered as the phenotypic signature of the microorganism. Then this image is translated using mathematical descriptors so that it can be compared to a database previously obtained using learning algorithms. Part of this work was dedicated to the improvement of the optical design so that the instrument can handle opaque culture media widely used in clinical diagnosis. Then two approaches were proposed to model the interaction between light and bacterial colonies. A 1st geometrical approach could help us, using ray tracing algorithms, to estimate the numerical aperture needed for the acquisition depending on the colonies morphologies. The 2nd approach, based on scalar diffraction theory, highlighted the importance of the biomass distribution inside the colonies. Macro-structures resulting from cells arrangement play a great role in the scattering patterns formation. In addition, the features extraction step from images using a Bessel-Fourier basis significantly improved the description accuracy. A systematic approach comprising the optimization of the learning algorithm and a dimensionality reduction technique was proposed. Great improvements of classification rates were achieved. Among them: a Gram+/Gram-/Yeasts discrimination at 98.1% was obtained over 15 species. Finally, the use of coherent lighting for the reading of antibiotics susceptibility test by means of dynamic speckle analysis was introduced and showed promising results.

DAVID GOSSELIN

TOWARDS AN INTEGRATED DEVICE FOR POINT-OF-CARE DIAGNOSTICS: USE OF CAPILLARITY WITH THERMOFORMING AND SCREEN-PRINTING PROCESSES

Ecole Doctorale Ingénierie - Matériaux, Mécanique, Environnement, Energétique, Procédés, Production, Grenoble

Towards an integrated device for point-of-care diagnostics: use of capillarity with thermoforming and screen-printing processes independent detection heads was under study. We proposed to adapt the angular sampling of heads, enabling acquisition protocols more accurate for different patient morphologies and examination protocols.
The purpose of this work was to propose acquisition and adaptation protocols enabling the reconfiguration of the angular sampling of detection heads depending on an estimation of the imaged object. Algorithmic solutions were proposed in order to compute the reconstruction in real time from more complex data because of additional degrees of freedom and detector precision.
The resulting estimation has to enable the identification of informative areas in order to focus detection heads on it. Simulations approved theoretically the use of our rapid algorithmic solution on a modular system carrying a reliable estimation of the object. Some experimental results validated the system model and the reconstruction algorithm. Then, some adaptation strategies were investigated using simulations.

HADRIEN LAMOTTE

RELEASE OF INTRACELLULAR COMPOUNDS BY SPARK DISCHARGES BETWEEN IMMERSED ELECTRODES

École Doctorale Ingénierie - Matériaux, Mécanique, Environnement, Energétique, Procédés, Production, Grenoble

This thesis focuses on the study of an innovative technology for microorganisms lysis, based on high voltage pulses generated in an aqueous medium. This technology is different from electroporation which operates thanks to the electric field for damaging cell membranes; in our study high voltage pulses generate an electric arc leading to various physicochemical phenomena supposed to lyse microorganisms. The technology efficiency is evaluated with the following microorganism: some lipid producting microalgae (Nannochloropsis gaditana and Phaeodactylum tricornutum) and classical laboratory model bacteria (Escherichia coli and Bacillus subtilis). In this work, we found that generated shock waves are mainly responsible of the cells lysis. At the end, the development of self-functioning devices is investigated either for bioproduction or for cell analysis.

EMILIE PERRET

ARTIFICIAL NOSE WITH OPTICAL TRANSDUCTION BASED SOL-GEL NANOPOROUS TECHNOLOGY

Ecole Doctorale Ingénierie pour la Santé, la Cognition et l'Environnement, Grenoble

In the fields of industrial and clinical microbiology, the detection of microorganisms through the emission of volatile metabolites rises unprecedented interest. Indeed, since volatile metabolites detection is effected in gas phase, this non-invasive methodology can be implemented for the control of complex samples such as food matrices and blood samples. Xerogels are used here to capture these microbial Volatile Organic Compounds (VOC) thanks to their nanoporosity and large and chemically tailored specific surface. Three approaches have been studied. First, an innovative approach was considered, based on the analysis of the global olfactory profile emitted by bacteria. The objective was to design an artificial opto-chemical nose that allows to characterize complex gas mixtures. Arrays of xerogels were modified through the incorporation of non-specific molecular probes that enable optical transduction of VOC, through a range of low energy chemical interactions. In presence of bacterial VOC emission, the xerogel array will generate a complex optical-response pattern (fluorescence and absorbance responses) that may be bacteria-specific. The feasibility study of this approach was done on a range of artificial VOC and on real bacterial metabolites. Secondly, targeted detection of VOC of interest via specific sensors was considered. Xerogel incorporate here molecular probes that specifically react in situ with targeted VOC to form a coloured compound. This method was coupled with a third detection methodology based on bacteria enzymatic pathways. Thus, enzymatic substrates are added to culture media and are specifically cleaved by bacterial enzymes, to generate selected VOC that provide convenient optical detection.

MARIE-CAROLINE SCHAEFFER

ECOG SIGNAL PROCESSING FOR BRAIN COMPUTER INTERFACE WITH MULTIPLE DEGREES OF FREEDOM FOR CLINICAL APPLICATION

École Doctorale Ingénierie pour la Santé, la Cognition et l'Environnement, Grenoble

Brain-Computer Interfaces (BCI) are systems that allow severely motor-impaired patients to use their brain activity to control external devices, for example upper-limb prostheses in the case of motor BCIs. The user's intentions are estimated by applying a decoder on neural features extracted from the user's brain activity. Signal processing challenges specific to the clinical deployment of motor BCI systems are addressed in the present doctoral thesis, namely asynchronous mono-limb or sequential multi-limb decoding and accurate decoding during active control states. A switching decoder, namely a Markov Switching Linear Model (MSLM), has been developed to limit spurious system activations, to prevent parallel limb movements and to accurately decode complex movements. The MSLM associates linear models with different possible control states, e.g. activation of a specific limb, specific movement phases. Dynamic state detection is performed by the MSLM, and the probability of each state is used to weight the linear models. The performance of the MSLM decoder was assessed for asynchronous wrist and multi-finger trajectory reconstruction from electrocorticographic signals. It was found to outperform previously reported decoders for the limitation of spurious activations during no-control periods and permitted to improve decoding accuracy during active periods.

2017

Annual research report

TECHNOLOGIES FOR BIOLOGY AND HEALTH

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PHOTOS

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2017

Annual research report

TECHNOLOGIES FOR BIOLOGY AND HEALTH

Contacts

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

Pascal MAILLEY
Chief Scientist
pascal.mailley@cea.fr

Adrienne PERVÈS
European Affairs
adrienne.perves@cea.fr

Patrick BOISSEAU
Nanomedicine program, European Commission interface
patrick.boisseau@cea.fr

Olivier FUCHS
Business development - implantable medical device, agriculture, food
olivier.fuchs@cea.fr

Anca-Nicoleta GALATANU
Business development - X-ray and gamma ray for NDT & security
anca-nicoleta.galatanu@cea.fr

Francis GLASSER
Business development - medical imaging
francis.glasser@cea.fr

Gilles MARCHAND
Business development - chemistry, cosmetics, environment & process monitoring, medical device & connected health
gilles.marchand@cea.fr

Nadège NIEF
Business development - in vitro diagnostic & vaccine, pharmacy
nadege.nief@cea.fr

Alexandre THERMET
USA market, based at San Francisco
alexandre.thermet@cea.fr

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

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

Peggy REMATIER
Clinatec business development - industrial alliances
peggy.rematier@cea.fr

Simon BACONNIER
Clinatec business development - clinical alliances
simon.baconnier@cea.fr

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