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TECHNOLOGY
RESEARCH
INSTITUTE

TECHNOLOGIES FOR BIOLOGY AND HEALTH

TECHNOLOGY RESEARCH INSTITUTE

LETI AT A GLANCE

Founded in **1967**

Based in **France** (Grenoble)
with offices in **USA** (Silicon Valley)
and **Japan** (Tokyo)

330
industrial partners

1,900
researchers

Committed to innovation, Leti's teams create **differentiating solutions in miniaturization and energy-efficient technologies** for its industrial partners.

Leti is a technology research institute at CEA Tech and a recognized global leader focused on miniaturization technologies enabling energy-efficient and secure IoT. Leti delivers solid expertise throughout the entire IoT chain, from sensors to data processing and computing solutions. Leti pioneered FDSOI low power platform for IoT, M&NEMS technology for low cost multisensors solutions, CoolCube™ integration for highly connected and cost effective devices.

Leti's mission is to pioneer new technologies, enabling innovative solutions to ensure Leti's industrial partners competitiveness while creating a better future. It tackles most current global issues such as the future of industry, clean and safe energies, health and wellness, sustainable transport, information and communication technologies, space exploration and safety & security.

For 50 years, the institute has built long-term relationships with its partners: global industrial companies, SMEs and startups. It tailors innovative and differentiating solutions that strengthen their competitiveness and contribute to creating new jobs. Leti and its partners work together through bilateral projects, joint laboratories and collaborative research programs. Leti actively contributes to the creation of startups through its startup program.

Leti has signed partnerships with major research technology organizations and academic institutions. It is a member of the Carnot Institutes network*.

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

2,760
patents in portfolio

60
startups created

€315
million budget

700
publications each year

IS0 9001
certified since 2000

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 m^2) and surface functionalization/bio probes grafting (100 m^2), biological laboratories with L2 rooms for bacteria, cells and human samples and biological characterization equipment such as PCR, cell microscopy and FACS (100 m^2). We also have a laboratory for synthetic chemistry, electrochemistry and characterization (430 m^2) and a microfluidic laboratory dedicated to technologies and system validation (300 m^2). 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.

TECHNOLOGIES FOR BIOLOGY AND HEALTH

CONTENTS

EDITO

PATRICK CHATON
HEAD OF MICROTECHNOLOGIES FOR BIOLOGY AND HEALTHCARE DIVISION

PR ALIM LOUIS BENABID
CHAIRMAN OF THE CLINATEC BOARD

PR STEPHAN CHABARDES
CLINATEC CLINICAL SECTOR DIRECTOR

"*Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity".* This definition, given by the Worldwide Health Organization when created in 1946, is very inclusive and englobes domains such as environment, feeding, wellbeing and security. Leti-Health addresses the challenge of health at large. Leti-Health regroups 250 researchers, technicians, engineers and clinicians working in the Technology for Biology and Health Division and Clinatec, a novel pre-clinical and clinical platform, allowing accelerated translation of the devices developed with Leti and its partners.

Together with our industrial, clinical and academic partners, we address the following application domains: in vitro diagnostics and monitoring for health, environment, life science, manufacturing and CBRNR (Chemical, Biological, Radiological and Nuclear Risk), therapeutics, nanomedicine and implanted medical devices and imaging systems for health and security. On our medical device projects, we work actively with more than 80 clinicians in Europe and worldwide.

In 2016 Avalun has obtained the CE marking for its Labpad®, a point of care device performing several blood test with the same reader. The 1st measurement is the INR (blood coagulation) with a market launch planned in 2017. The device includes Leti's lensfree technology.
We also have demonstrated in 2016, that near-infrared intracranial illumination can be effective in slowing down neurodegeneration and behavioral decay at the preclinical level, using a non-human primate Parkinson's disease model. The objective now is to perform regulatory tests in order to obtain implantable neurological prototypes compliant with human use. First-in-man clinical trials for Parkinsons's disease will be eventually conducted in a collaboration with CHU-Grenoble Alpes.

Our strategy is to serve the industry and answer societal health challenges, going toward miniaturization, multi-modality and connected devices (m-health, e-health), delivering prototypes "ready to transfer", i.e. compliant with industrial standards and medical regulations.

KEY FIGURES

160 permanent researchers
77 PhDs, Post-docs, and short term contracts

60 book chapters & journals
74 conferences & workshops

230m² clean room for biochip packaging and surface chemistry
100m² biological laboratory
430m² chemical laboratory
300m² microfluidic 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)

46 patents filed in 2016
445 patents portfoliio

SCIENTIFIC ACTIVITY

Publications

60 books chapters and journals, 74 conferences and workshops
Main papers:

> N. Cermak, S. Olcum, F.F. Delgado, S.C. Wasserman, K.R. Payer, M. Murakami, S.M. Knudsen, R.J. Kimmerling, M.M. Stevens, Y. Kikuchi, A. Sandikci, M. Ogawa, V. Agache, F. Baléras, D.M. Weinstock, S.R. Manalis,
> "*High-throughput single-cell growth measurements via serial microfluidic mass sensor arrays*", Nature Biotechnology 34, 1052–1059 (2016) doi:10.1038/nbt.3666.

> F. Darlot, C. Moro, N. El Massri, C. Chabrol, D.M. Johnstone, F. Reinhart, D. Agay, N. Torres, D. Bekha, V. Auboiroux, T. Costecalde, C.L. Peoples, H.D. Anastascio, V.E. Shaw, J. Stone, J. Mitrofanis, A.L. Benabid
> "*Near-infrared light is neuroprotective in a monkey model of Parkinson disease*", Ann Neurol. 2016 Jan;79(1):59-75.doi: 10.1002/ana.24542.

Prize and Awards

European Inventor Award 2016 (European Patent Office):
Pr. Alim-Louis Benabid.

Experts

5 Research Directors, 11 Senior Experts, 18 Experts.

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.

Participation in normalization groups

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

- AFNOR contribution to launch a New Work Item Proposal, the formal document to lead towards an international microfluidic ISO standard.

1.

RADIATION DETECTION

- Scatter correction

- Spectrometry

- Mammography

- Adaptable SPECT

- Digital detector for NDT

EXPERIMENTAL VALIDATION OF A MULTI-ENERGY X-RAY ADAPTED SCATTER SEPARATION METHOD

RESEARCH TOPIC:
Multi-energy X-ray imaging, Scatter correction, Spectral detector

AUTHORS:
A. Sossin, V. Rebuffel, J. Tabary, L. Verger

ABSTRACT:
The emergence of energy-resolved photon counting detectors such as those developed at Leti gives rise to new techniques in X-ray imaging. However, the presence of scattered radiation leads to a loss of spatial contrast, bias in material images, and artefacts, reducing the accuracy of such spectral techniques. We previously proposed a scatter correction method (PASSSA) adapted for multi-energy imaging. The aim of the present study was to carry out an experimental validation of the method, using a prototype X-ray system with a spectroscopic detector and an anthropomorphic thorax phantom. The obtained attenuation images and X-ray spectra visualized after correction proved to be almost scatter free.

SCIENTIFIC COLLABORATIONS: Université de Lyon, CREATIS

Context and Challenges

In X-ray imaging, the signal measured in each pixel is a sum of two components: primary radiation, corresponding to photons attenuated in the object, and scattered radiation, due to Compton and Rayleigh effects. Scattered radiation induces a bias in the image giving rise to a reduction in contrast and quantification accuracy.

New semi-conductor based detectors [1], which can provide an energy resolved signal (spectrum) per pixel, provides new functionalities such as the identification and quantification of individual materials comprising the inspected object by processing a single acquisition image. However X-ray acquisitions with a high level of accuracy are required in order to benefit from the energy-resolved data. Thus, except in the case of high collimated geometries, scattered radiation has to be corrected.

Main Results

The proposed method (PASSSA: Partial Attenuation Spectral Scatter Separation Approach [2]) is based on two X-ray acquisitions of the inspected object, the second one with a mask of attenuators inserted between the source and the inspected object. A dedicated algorithm estimates the scatter image and subtracts it from the original image to obtain a corrected image. Parameters are computed thanks to an experimental calibration, allowing to consider the true response of the detector. This study [3] presents a validation on a thorax phantom in a radiographic geometry (Fig.1).

Fig.1. Schema of the experimental system including a spectrometric detector (32 energy bins).

For comparison purpose, an additional acquisition is performed with a Beam-Stop (BS) system providing a scatter-free image.

A comparison between the original image (a), the scatter-corrected one with PASSSA (b), and the BS scatter-free one is performed. Attenuation images (sum over the full energy range) provided in Fig.2 illustrate the significant reduction of scatter and the increase in contrast from (a) to (b).
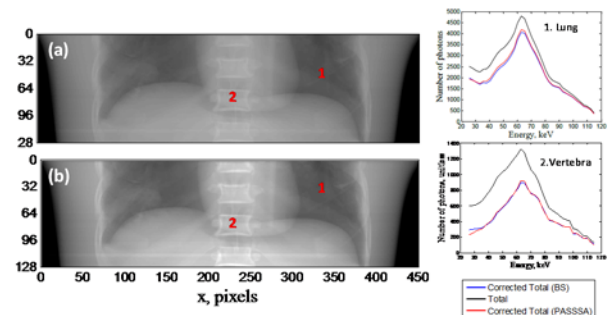

Fig.2. Left: Attenuation images. (a) Original image, (b) after correction with PASSSA. Right: Spectra on control points 1 and 2.

Considering the BS scatter-free image as the reference primary image, the method allows to lower the initial normalized root-mean-square error (NRMSE) of 45% between the uncorrected original and the reference images by a factor of 9, thus reducing it to around 5%. Additionally, two spectra are visualized at two control points, (1) in lung area and (2) at a vertebra area. They indicate an excellent recovery of primary spectra with the scatter induced bias being almost completely reduced.

Perspectives

Future studies will examine the adaptation of the method to a single acquisition protocol, the corresponding attenuator mask optimization and correction method. Tomographic geometry will also be considered.

RELATED PUBLICATIONS:
[1] A. Brambilla, P. Ouvrier-Buffet, J. Rinkel, G. Gonon, C. Boudou, and L. Verger, "CdTe Linear Pixel X-Ray Detector With Enhanced Spectrometric Performance for High Flux X-Ray Imaging," IEEE Trans. Nucl. Sci., vol. 59, no. 4, pp. 1552 (2012).
[2] A. Sossin, V. Rebuffel, J. Tabary, JM. Letang, N. Freud, and L. Verger. "A novel scatter separation method for multi-energy X-ray imaging". In Physics in Medicine and Biology. Vol. 61, Issue 12, pp.4711 (2016).
[3] A. Sossin, V. Rebuffel, J. Tabary, JM. Letang, N. Freud, and L. Verger. "Experimental validation of a multi-energy X-ray adapted scatter separation method". In Physics in Medicine and Biology, Vol. 61, Issue 24, pp.8625 (2016).

BAYESIAN TISSUE DECOMPOSITION METHOD FOR SPECTRAL MAMMOGRAPHY

RESEARCH TOPIC:
X-ray colour imaging, Mammography, Photon Counting Detectors

AUTHORS:
Y. Pavia, A. Brambilla, V. Rebuffel, J.M. Létang[1], N. Freud[1], L. Verger

ABSTRACT:
Quantitative breast imaging using energy sensitive PCD (Photon Counting Dectors) enable to measure breast density and iodine concentration using a single X-ray exposure. We present a Bayesian approach, based on a Poisson maximum likelihood material decomposition method and that includes an adjustable prior, known from the compressed breast thickness during a screening exam. Since some density variations in biological tissues may appear and the measured compressed thickness is not perfectly known, the proposed method moderates the prior upon the confidence on the thickness value.
This simulation study shows that it is possible to simultaneously measure breast density and iodine concentration at a dose of 0.93 mGy. Taking into account the a priori knowledge greatly enhances the accuracy of the measurements.
SCIENTIFIC COLLABORATIONS: [1]INSA

Context and Challenges
Breast density is considered as a risk factor for breast cancer detection [1]. This density is often expressed by the fraction of fibroglandular tissue over the whole breast volume (mainly composed of fibroglandular and adipose tissues). To distinguish those breast components, a material base decomposition can be applied on a dual-energy mammography [2]. Nowadays, Energy Sensitive Photon Counting Detectors (ES-PCD) are emerging and allow to access the spectral information during a single X-ray exposure [3].

Main Results
We defined a test phantom composed of water and PMMA that mimics breast densities ranging from 10 % to 100 % and including a 7 mg/mL iodine concentration (see Figure 1) [4].
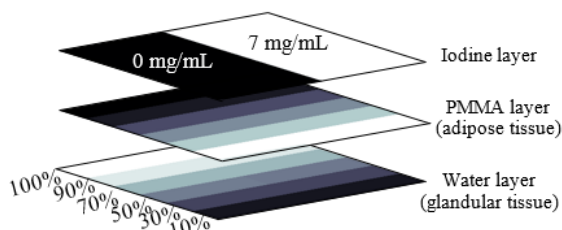

Figure 1: Schematic of the phantom used in this study

Breast density and iodine concentration are estimated in one single exposure 49 kVp (0.8 mm Be + 1.2 mm Al filtration) under 5 mA-s that gives an estimated mean glandular dose (MGD) of 0.93 mGy. The measured absorption of each pixel is decomposed into equivalent lengths of PMMA, water and iodine by maximizing a penalized log-likelihood function:

$$\Psi(S_b(T), S_m) = \sum_{k=1}^{N} S_{m,k} \cdot ln(S_{b,k}(T)) - S_{b,k}(T) + \alpha . F(||T_{tot} - T_{breast}||)$$

The first term of the equation is the standard log-likelihood that compares the measured spectrum Sm to the calibration spectra Sb, measured with different combination of known thicknesses of the 3 materials. The second term is a regularization term that uses the a priori knowledge of the compressed breast thickness T_{breast} . F is a Tukey-lambda distribution that penalize $\Psi(S_b(T), S_m)$ when the total thickness differs from T_{breast}. Breast density and iodine concentration maps are presented in Figure 2 for the MLE (Maximum Lifehood Estimation) (α=0) and Bayesian ($\alpha \neq 0$) methods.
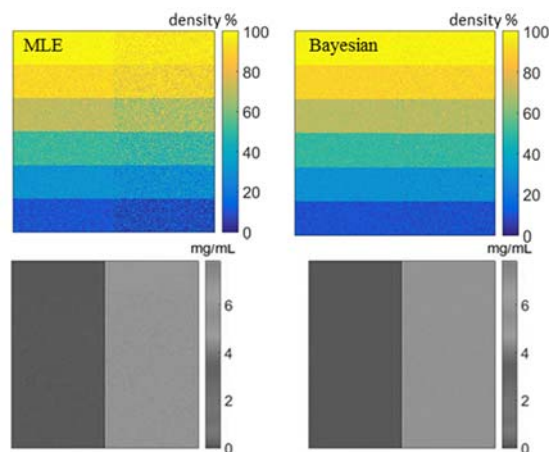

Figure 2: Breast density and Iodine concentration maps with the MLE approach (left) and Bayesian method (right).

We can visually notice a noise decrease in the right hand side image in all densities (horizontal strips) of the simulated phantom. The Bayesian approach eliminates the bias and reduces the noise by a factor of 1.8 compared to the standard MLE method.

Perspectives
Taking into account the a priori knowledge greatly enhances the accuracy of breast density and iodine concentration estimations. This approach thus provides accurate quantitative information on breast density and vascularization obtained with a single mammogram without additional dose.

RELATED PUBLICATIONS:
[1] N. F. Boyd et al, "Mammographic density and the risk and detection of breast cancer", New English Journal of Medicine, 2007.
[2] J. L. Ducote and S. Molloi, "Quantification of breast density with dual energy mammography: An experimental feasibility study," Medical Physics, vol. 37, n°. 2, 2010.
[3] H. Ding, S. Molloi, "Quantification of breast density with spectral mammography based on a scanned multi-slit photon-counting detector: A feasibility study," Phys Med Biol. 2012 vol. 57:4719–4738.
[4] Y. Pavia, A. Brambilla, V. Rebuffel, J.M. Létang, N. Freud, L. Verger, "Bayesian Tissue Decomposition Method for Spectral Mammography," IEEE Nuclear Science Symposium Conference Record 2016,

MATERIAL-SPECIFIC IMAGING SYSTEM USING EDXRD AND SPATIALLY RESOLVED CDZNTE DETECTORS WITH POTENTIAL APPLICATION IN BREAST IMAGING

RESEARCH TOPIC:
Energy-dispersive X-ray diffraction, X-ray scattering system, Breast imaging, Inverse problem

AUTHORS:
D. Barbes, J. Tabary, C. Paulus, J.-L. Hazemann, L. Verger

ABSTRACT:
To improve detection of breast tumors, the Energy Dispersive X-Day Diffraction (EDXRD) technique is a very promising technique as it reveals the molecular structure of biological tissues and thus precisely distinguishes healthy and cancerous tissues. However, due to the very tight collimations, EDXRD systems generally suffer from poor photon count statistics. The use of pixelated CdZnTe detectors developed at Leti, which are spatially and energy-resolved, allows to significantly improve the tradeoff between resolution and sensitivity. Based on this, we present an EDXRD set-up able to produce 2D image of a representative plastic phantom by translating only one single pixel from our own CZT spectrometric detector.

SCIENTIFIC COLLABORATIONS: Université de Lyon, CREATIS

Context and Challenges
In the field of breast imaging, the classical mammography sometimes fails at determining the cancer presence and the additional exams, such as MRI or biopsy, remain either expensive or very invasive. New techniques improving breast cancer diagnosis are thus constantly being sought. Among them, EDXRD technique is known to provide very specific information of tissues which can be useful for breast cancer diagnosis.

This study proposes a system and a method for material specific medical imaging which might be in the future used as a second-line breast imaging technique.

Main Results
Based on our own developed technologies of spectrometric CZT detectors, we present an original EDXRD system [1] which demonstrates the applicability of this technique for medical applications. Thanks to the sub-pixelation technique implemented in the multi-pixel detectors [2], the collimation can be enlarged to significantly improve the sensitivity without angular resolution degradation. Moreover, the sub-pixelation also enables to segment the inspected volume in several sub-volumes, thus providing spectral imaging in the breast depth direction.
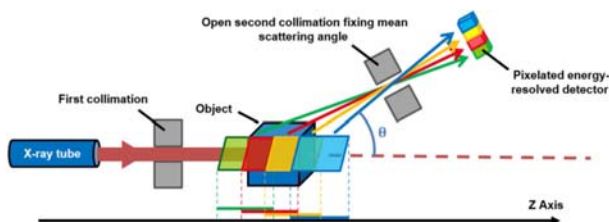

Figure 1: Schema of a multipixel EDXRD system. Each sub-pixel see a different range of positions on the Z axis.

To make the most of this sub-pixelation, an original reconstruction processing has been developed to restore the diffraction profiles of the different tissues from the measured spectra on each subpixels. This reconstruction algorithm enables to remove the degradation factors of the spectra. In particular, the attenuation object is corrected thanks to the transmitted spectrum simultaneously measured with another spectrometric CdTe detectors, dedicated to high x-ray flux.

An experiment with this system [1] has been carried out on a representative phantom. It consists of nylon inserts of different sizes placed into a polystyrene matrix mimic fibrous tissues in an adipose background. By translating in the X direction one single pixel from our CZT based MINIGAMI imager [3], we achieve to perform 2D EDXRD image of the phantom.
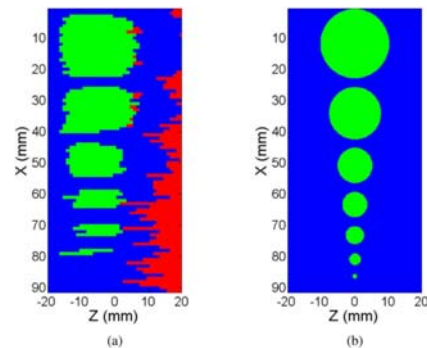

Figure 2: (a) Reconstructed image; (b) Ground truth. Blue, green and red materials are respectively polystyrene, nylon and PMMA

Despite some reconstruction artefacts in areas with few photons, these experimental results are promising. Only the smallest insert is not seen which implies a spatial resolution of at most 0.3 cm.

Perspectives
The long-term purpose of this work is to develop a breast imaging device based on this system. However, to be compatible with in vivo medical examinations, improvements on sensitivity and material identification are required. In a near future, we plan to improve reconstruction and classification algorithm and test our system on real biological tissues. The use of detector with more pixels and new collimator will be advantageous. We also plan to carry out a full dose study.

RELATED PUBLICATIONS:
[1] D. Barbes, J. Tabary, C. Paulus, J-L. Hazemann, L. Verger, "Material-specific imaging system using energy-dispersive X-ray diffraction and spatially resolved CdZnTe detectors with potential application in breast imaging", Nuclear Instruments and Methods in Physics Research A, 848, pp 91–98, 2017.
[2] G. Montemont, S. Lux, O. Monnet, S. Stanchina, and L. Verger. "Evaluation of a CZT Gamma-Ray Detection Module Concept for SPECT." In 2012 IEEE Nuclear Science Symposium and Medical Imaging Conference (NSS/MIC), 4091–97, 2012.
[3] G. Montemont, T. Bordy, V. Rebuffel, C. Robert, L. Verger, "CZT pixel detectors for improved SPECT imaging", in: IEEE Nuclear Science Symposium Conference Record, 2008, NSS '08. pp. 84–89, Oct. 2008.

REAL TIME PROCESSING FOR AN ADAPTABLE SPECT SYSTEM BASED ON CZT DETECTORS

RESEARCH TOPIC:
SPECT Imaging, Real Time, Iterative reconstruction, Adaptivity

AUTHORS:
M. Bernard, G. Montémont, S. Stanchina, S. Mancini[1], L. Verger

ABSTRACT:
Single Photon Emission tomography (SPECT) is mainly limited by the trade-off between spatial resolution and sensitivity. In this context, adaptable systems are investigated to adjust the acquisition parameters to the object to be imaged. Adjustment of acquisition parameters to the object has to be made at real time, however the reconstruction process for such flexible system is complex because the multiple possibilities of measurements geometries. The classical method consisting in using a binned representation of the model is then not possible, furthermore in a short time. This work aims at proposing algorithmic solutions to handle the reconstruction in real time.

SCIENTIFIC COLLABORATIONS: [1]TIMA laboratory (Université Grenoble-Alpes)

Context and Challenges

In this work [1, 2], we studied the case of a system usable for cardiac imaging illustrated on Fig. 1, with ten detection heads placed on a 120-degrees-wide arc around the patient. Each head can rotate on itself independently from the others to focus on a given area of the region of interest (ROI). Each head is composed of a CZT detector and a parallel holes collimator. This configuration has been simulated using different phantom shapes, in order to validate the reconstruction method.
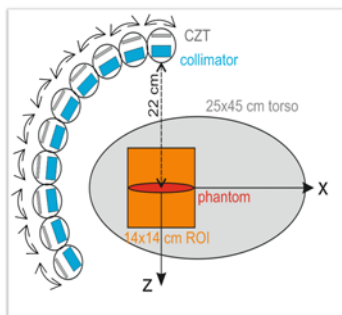

Figure. 1: Adaptable configuration [1]

Main Results

The MLEM iterative reconstruction technique is widely used in SPECT imaging. Nevertheless, its binned implementation (using voxel and pixel representation) is not possible because of the size of the numerical representation of the model (about 10^{13}). The first optimization proposed by Barrett consists in dealing with the measurements as a list of events instead of as a sinogram. This is efficient because of the sparsity of the measurement space. Then, the size of the model representation is reduced by separate the model into three sub models: detector, collimator, and geometry. Coefficients are computed on the flight, using ray tracing. We thus avoid the matrix representation of the model. Finally, events can be processed by groups. Each group is used

for one iteration of MLEM algorithm. By this way, intermediate updates of the estimation are available to be used to adapt the system configuration.

Simulations on this reconstruction method shows that some trade-offs between accuracy and computation time can be applied. First, the sampling of back-projections through the model can be adjust up to a certain limit to make the process faster (MCx on Fig. 2). Then, the computation time can be shorten by reducing the size of measurements groups used for partial update (PU), but too small groups lead to a poor image quality. The performances showed in Fig. 2 are given for the total process.
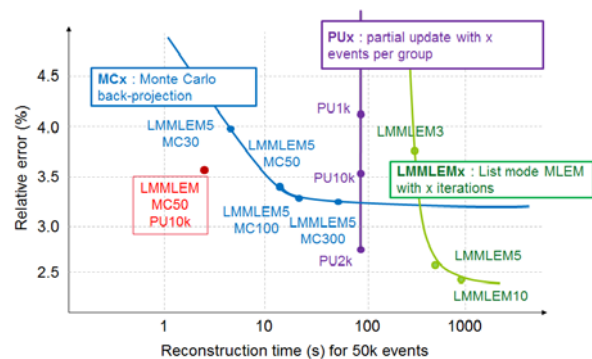

Figure. 2: Time/accuracy performances depending on the adjustments on the algorithm [1]

Thus, this algorithmic solution enables a correct intermediate estimation of the object much faster than classical list-mode MLEM. The reconstruction process is fast enough to dynamically adapt the configuration during the examination.

Perspectives

The next step of this work consists in computing a criteria to determine the best configuration for a given object. Criteria such as the DQE and Fisher information are investigated.

RELATED PUBLICATIONS:
[1] M. Bernard, G. Montémont, S. Stanchina, S. Mancini, and L. Verger, "Real-Time Processing for an Adaptable SPECT System Based on CdZnTe Detectors", IEEE NSS-MIC-RTSD conference, 2016, paper submitted to IEEE TRPMS in December 2016.
[2] M. Bernard, G. Montémont, S. Stanchina, S. Mancini, and L. Verger, "Enabling real time reconstruction for high resolution SPECT systems", IEEE 20th real time conference record, 2016.

PERFORMANCE COMPARISON BETWEEN DIRECT CONVERSION DIGITAL DETECTORS AND COMPUTED RADIOGRAPHY FOR NDT APPLICATIONS

RESEARCH TOPIC:

Non Destructive Testing, CdTe detector, Computed Radiography, Digital Radiography, Weld inspection

AUTHORS:

J.-M. Casagrande, E. Gros d'Aillon, T. Goursolle, A. Chandelle, E. Romero, L. Verger

ABSTRACT:

In the trend to replace the current photostimulable phosphor screen used for Computed Radiography (CR) by detectors able to achieve Digital Radiography, a new generation of detectors based on semiconductor material, running in direct conversion X-ray photon to electron and operating at room temperature, seems promising. Two types of CdTe array detectors operating either in counting mode or in energy integration mode have been investigated for performance comparison with CR. Experimental results in weld inspection application show a better or equivalent defects detectability when using CdTe detectors and a significant (around 10 times) X-ray exposure time reduction.

Context and Challenges

For Non Destructive Testing applications, Computed Radiography (CR) is largely used in the industry but needs an image readout and an erasing operation of the photostimulable plate. As this additional operation is not needed when using digital detectors, the trend is to replace CR by DR (Digital radiography). The current digital detectors are based on a scintillator layer coupled to a photodiode array to achieve the X-ray photon conversion to electron through visible photon. Semiconductor materials such as cadmium telluride (CdTe) are able to convert X-rays directly into electric signal leading the way to direct conversion digital detector.

This study deals with the performance comparison between these semiconductor digital detectors and CR system for NDT applications such as weld inspection.

Main Results

Two CdTe-based digital detectors have been experimented: PIXIRAD, a commercial detector [1] and IRIS, a CEA prototype detector [2] operating respectively in photon counting mode with two thresholds and in energy integration mode.
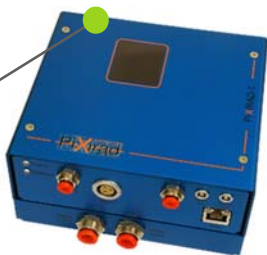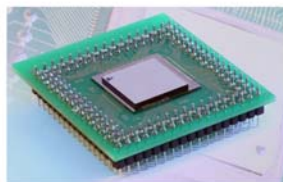

Fig 1 PIXIRAD detector   IRIS sensitive component

PIXIRAD and IRIS feature respectively a 31x25 mm² sensitive area at 60 μm pixel pitch and 15x15 mm² at 75 μm pixel size (Fig 1). CdTe material thickness is 0.65 mm for PIXIRAD and 1 mm for IRIS, well adapted for the X-ray range used in this application (40-160 kV).

Basic digital detector performance characteristics have been measured using standardized Image Quality Indicators (IQI): ASTM E2797 standard for spatial resolution (SRb), ASTM E 1647-98a standard for Contrast to Noise Ratio (CNR). For spatial resolution, the measured SRb is in accordance with the pixel size of each semiconductor detector, i.e. 60 μm and 75 μm whereas it is around 100 μm for CR. CNR performance is very comparable between direct conversion detectors and CR. Other characteristics such as linearity and Detection Quantum Efficiency show good results for CdTe detectors. Dynamic range is significantly larger for CdTe detectors than for CR [3].
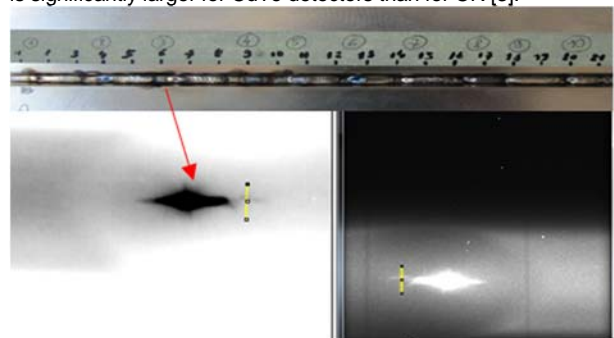

Fig 2 Weld test sample picture (top), CR image (bottom left) and PIXIRAD image (bottom right) of welding zone n°3

A real case comparison has been done on weld defects within a Ni-alloy plate (Fig 2). CdTe-based detectors feature a better or equal weld defect detectability, depending on weld defect, than the one obtained with CR plate, at significantly reduced X-ray exposure time (factor of 10) and without need of further readout operation like for CR. These results are very encouraging to propose an alternative to scintillator-photodiode digital detector for the replacement of CR systems in the industrial field.

Perspectives

The main next step is to obtain larger sensitive areas for direct conversion detector by butting unit components in order to cover more NDT applications.

RELATED PUBLICATIONS:

[1] www.pixirad.com

[2] M. Arques, S. Renet, A. Brambilla, G. Feuillet, A. Gasse, N. Billon-Pierron, M. Jolliot, L. Mathieu, and P. Rohr, "Dynamic X-ray direct conversion detector using a CdTe polycrystalline layer coupled to a CMOS readout chip," Nucl. Instrum. Meth. Phys. Res., Sec. A: Accel.,Spectrom., Detect, vol. 633, pp. S55–S58, May 2011.

[3] JM. Casagrande, E. Gros d'Aillon, T. Goursolle, A. Chandelle, E. Romero, L. Verger, "Comparaison de performances entre capteurs numériques à conversion directe et écrans photo-stimulables pour la radiographie industrielle. Application au contrôle de soudures." Communication aux Journées COFREND 2017 Strasbourg 30 Mai-1er Juin 2017.

2.

OPTICAL IMAGING

- **Diffuse reflectance spectroscopy**

- **Diffuse optical tomography**

- **Lensfree microscopy**

- **Optical Elastic Scattering**

ADAPTIVE CALIBRATION ALGORITHM AND PROTOCOL (ACA-PRO) FOR QUANTITATIVE DIFFUSE REFLECTANCE SPECTROSCOPY

RESEARCH TOPIC:

Spectroscopy, Diffuse reflectance, Optical properties, Calibration, Quantification

AUTHORS:

V. Sorgato, M. Berger, C. Emain, C. Vever-Bizet, J.-M. Dinten, G. Bourg-Heckly, and A. Planat-Chrétien

ABSTRACT:

We have developed an Adaptive Calibration Algorithm and Protocol (ACA-Pro) that corrects from the instrumental response of various Spatially-resolved Diffuse Reflectance Spectroscopy (DRSsr) systems to enable the quantification of absorption and scattering properties based on a Monte Carlo based Look-Up-Table (LUT) approach. The protocol involves the use of a calibration reference base built with measurements of a range of different diffusive intralipid phantoms. Moreover, an advanced strategy was established to take into account the experimental variations with an additional measurement of a common solid material, allowing the use of a single calibration reference base for all experiments. The approach is validated on contact and non-contact probe-based DRSsr systems, as well as on a CCD-based DRSsr System

SCIENTIFIC COLLABORATIONS: Laboratoire Jean Perrin, UPMC - CNRS Paris,

Context and Challenges

Spatially-resolved Diffuse Reflectance Spectroscopy (DRSsr) is an optical spectroscopic technique that provides quantitative estimations of optical properties from measurements of diffuse reflectance at multiple source-detector (SD) distances [3]. To obtain absolute quantitative estimations of absorption and scattering properties, many research groups rely on the instrumental calibration through phantom measurements. This calibration considers the various instrumental responses, detector geometry, and measurement modalities (contact/non-contact). Thereupon, it is possible to solve the inverse problem in which the calibrated reflectance measurements are compared to the theoretical reflectance of the forward model. The forward model is set by the Radiative Transfer Equation (RTE) which is approximated by an analytical diffusion model or numerically solved by a Monte Carlo simulation. Results of both procedures can be saved under a Look-Up Table (LUT). Alternatively, the forward model and the instrumental calibration can be combined in a LUT built beforehand with reflectance measurements of a comprehensive set of characterized phantoms and used for direct comparison with further experimental measurements. In this work [1,2], we have developed an Adaptive Calibration Algorithm and Protocol (ACA-Pro) that allows optical properties estimation with measurements taken with different DRSsr setups (contact/non-contact systems) and a single Monte Carlo-based LUT under contact conditions.

Main Results

The ACA-Pro algorithm is a μ_s'-based two-step calibration approach. The first step makes use of a reference base built with measurements of a few reference intralipid phantoms covering a large range of reduced scattering coefficients proper to biological tissues (Black-part Fig1). The second step integrates an interpolation strategy to reduce the number of reference intralipid phantoms needed to build the reference base (Purple - part Fig1). Besides, we extend the calibration capacity of ACA-Pro to the correction of experimental variations that are common between measurements taken at different time periods and degrade the estimation of optical properties.

The approach relies on the single measurement of a common optically stable solid material that characterizes individual experimental conditions (Green part – Fig1).

With this, all measurements are adapted to the experimental conditions of a unique reference base. One of the new advantages of this strategy comprises the exemption of manufacturing the various reference intralipid liquid phantoms, subject to temporal optical instability and tedious handling, for each experiment.
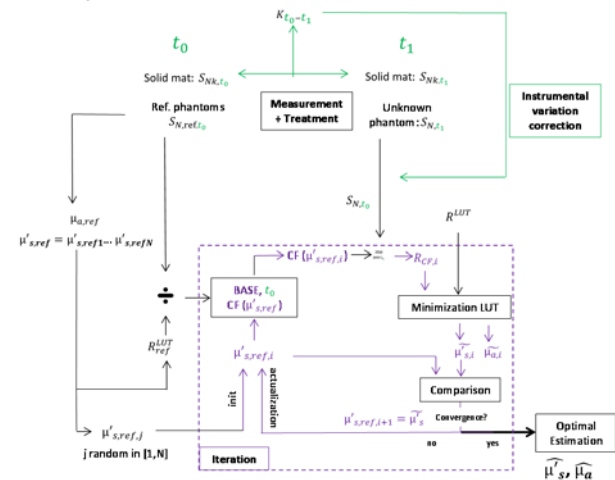

Fig.1. Summary of ACA-Pro execution with the Principle and Interpolation approaches in purple and the Correction of Instrumental Variations in green.

Another advantage of ACA-Pro lies in the use of a unique Monte Carlo-based LUT with which measurements, taken with the different systems, are compared to derive optical properties. Errors for contact and non-contact probe-based DRSsr setups remained below 4% and 8% for μ_s' and μ_a, respectively.

Perspectives

The ACA-PRO approach will be used in wide-field multispectral imaging to achieve 2D quantitative μ_a - μ_s' maps for the 1st time.

RELATED PUBLICATIONS:

[1] 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.
[2] V. Sorgato et al., "Wide-Field Absolute Quantification of Absorption in Turbid Media", Biomedical Optics 2016, OSA Technical Digest (online) (Optical Society of America, 2016), paper JM3A.32 (2016).
[3] V. Sorgato et al., "Non-contact quantitative diffuse reflectance spectroscopy", ECBO Munich, Proc. SPIE 9538, Diffuse Optical Imaging V, 95380U (2015).

QUANTIFICATION IN TIME-DOMAIN DIFFUSE OPTICAL TOMOGRAPHY USING MELLIN-LAPLACE TRANSFORMS

RESEARCH TOPIC:
Imaging through turbid media, Time-domain measurements, Time Resolved Diffuse Optical Tomography (TR-DOT), Reconstruction

AUTHORS:
J. Zouaoui, L. Di Sieno[1], L. Hervé, A. Pifferi[1], A. Farina[1], A. Dalla Mora[1], J. Derouard[2], and J.-M. Dinten

ABSTRACT:
Time-domain diffuse optical tomography has attracted a great interest in the field of medical imaging. Simulations and phantom measurements are used here to evaluate the ability of TR-DOT to quantify the absorption perturbation of centimetric objects immersed at depth 1-2 cm in turbid media. We found that the estimated absorption coefficient varies almost linearly with the absorption change in the range of 0-0.15 cm^{-1} but is underestimated by a factor that depends on the inclusion depth (~2, 3 and 6 for depths of 1.0, 1.5 and 2.0 cm respectively). For larger absorption changes, the variation is sublinear with ~20% decrease for $\delta\mu a$ = 0.37 cm^{-1}. By contrast, constraining the absorption change to the actual volume of the inclusion may considerably improve the accuracy and linearity of the reconstructed absorption.

SCIENTIFIC COLLABORATIONS: [1]POLITECNICO DI MILANO, [2]Université Grenoble Alpes (UGA)

Context and Challenges

We aim at characterizing in-vivo and non-invasively the optical properties (absorption and scattering) of biological samples since it could image pathologies like breast cancer, osteoarticular diseases, brain ischemia or hemorrhage, image brain functions or allow post-surgery follow-up (for reconstruction surgery). Measurements are performed in the reflection geometry (see Fig.1) in the perspective to build a hand-held diagnostic tool. The medium is probed by Infrared light injected sequentially at (30) source locations and by collected at (2) detector locations. The set of measurements is processed [1] to give 3D reconstructions of optical parameters. Time-domain measurement setup (pico-second pulsed laser source and very fast time-resolved detectors) is used so as to probe deeper in the medium, since late arrival photons (which are more prone to have propagated deeper) are discriminated.

Quantification performance of DOT (i.e accuracy of the reconstruction of the optical properties) is seldom addressed in the literature, and never for the reflection geometry with time-domain measurements. The goal of this paper [2] is to perform such assessment on simulations and phantom measurements.

Main Results

We found in the simulations and in the measurements campaign that the technique is sufficiently sensitive to correctly detect and localize inclusion up to 20 mm. Quantification is correct for inclusion at a depth to 10 mm but suffer a 4-fold discrepancy for z=20 mm. The reconstructed absorption of the inclusion δμa (Fig.2) is fairly linear with respect to the increase in the real δμa up to around 0.15 cm^{-1}. For higher absorption changes, a deviation from linearity with a tendency to saturation is observed. The adoption of a constrained approach, where the perturbation location and volume are fixed a-priori, completely cures depth- and absorption- reduction in the reconstructed δμa on simulations, and greatly improves the outcome on experiments. Taken as a whole, these results are quite encouraging since they demonstrate that for a fixed depth – e.g. in brain functional imaging at the brain cortex – absorption linearity for limited, yet realistic absorption changes is preserved. This feature is important for instance in functional brain imaging or in the study of brain connectivity, since it permits to follow temporal evolutions of the signal during the exercise, or to perform spectral analysis with low distortion.
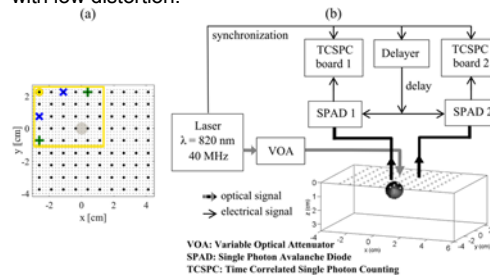

Fig.1 a) Geometry of source-detector distances (source = yellow circle, inclusion = grey disk, crosses = couple of detectors), b) Instrumental set-up
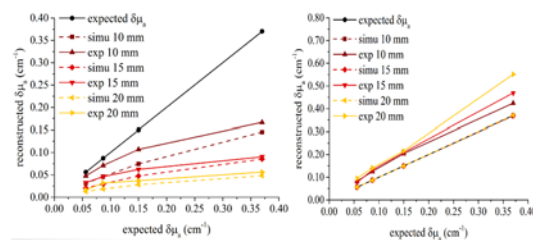

Fig.2 Quantification results for various inclusion absorbance and depth, without (left) or with (right) localization priors.

Perspectives

The results showed the adequateness of time-domain DOT for medical applications and paves the way to actual design of medical imaging systems based on the technique.

RELATED PUBLICATIONS:
[1] L. Hervé, A. Puszka and A. Planat-Chrétien, J.-M. Dinten, "Time-domain diffuse optical tomography processing by using the Mellin--Laplace transform," Applied Optics, vol 51, no 25, pp 5978-5988, 2016.
[2] J. Zouaoui, L. Di Sieno, L. Hervé, A. Pifferi, A. Farina, A. Dalla Mora, J. Derouard, and J.-M. Dinten, "Quantification in Time-Domain Diffuse Optical Tomography using Mellin-Laplace Transform," Biomedical Optics Express., vol. 7, no. 10, pp. 4346-4363, 2016.

WHOLE SLIDE IMAGING OF UNSTAINED TISSUE USING LENSFREE MICROSCOPY

RESEARCH TOPIC:
Lensfree imaging, Wide-field imaging, Digital pathology slides, Unstained tissue slide imaging

AUTHORS:
S. Nhu An Morel, L. Hervé, T. Bordy, O. Cioni, A. Delon[1], C. Fromentin[2], J.-M. Dinten, C. Allier

ABSTRACT:
We present a simple cost-effective lensfree imaging method to record 2-4μm resolution wide-field (10 mm² to 6 cm²) images of unstained tissue slides. The sample processing time is reduced as there is no need for staining. A wide field of view (10 mm²) lensfree hologram is recorded in a single shot and the image is reconstructed in 2s providing a very fast acquisition chain. This technique is much cheaper and compact than a conventional phase contrast microscope and could be made portable. In sum, we present a new methodology that could quickly provide useful information when a rapid diagnosis is needed, such as tumor margin identification on frozen section biopsies during surgery.

SCIENTIFIC COLLABORATIONS: [1]Lab. Interdisciplinaire de Physique, Grenoble, [2]Centre Hospitalier Dr. Schaffner, Lens

Context and Challenges

Pathologist examination of tissue slides provides insightful information about a patient's disease. Traditional analysis of tissue slides is performed under a binocular microscope, which requires staining of the sample and delays the examination. Since the field of view under the microscope is limited, the pathologist must scan the whole tissue slide, which can be cumbersome. Besides, no image is recorded. New lensfree imaging methods provide wide field multiscale images of stained tissue slides [1,2]. Although spatial features down to 250 nm are reconstructed with these methods, they require hundreds of acquisitions for reconstructing 20.5mm², and processing time is about 90 min./mm². Here we present a new imaging method based on lensfree imaging that allows fast (less than 20 minutes) wide-field (up to 6.25 cm²) image recording of unstained tissue slides [3]. This method provides phase-contrast like images of transparent samples, and therefore does not require any staining step during the sample preparation.

Main Results

Our lensfree imaging method enables whole tissue slide fast imaging at low cost and complexity, with single shot RGB holograms recording. The multispectral illumination is provided by a multi-quadrant RGB LED associated with a diffuser and a pinhole. The RGB sensor records three RGB holograms that are extracted from the sensor Bayer filter. 80 single shot 9.7 mm² RGB holograms are recorded within 12 minutes. Reconstruction time is about 20 minutes for 500 mm² phase image area, using Matlab on an Intel Xeon 3.2 GHz processor, and 99 seconds on GPU with a Nvidia Quadro K4200 graphics card. Figure 1 shows the reconstructed phase of a whole unstained human colon slide. The reconstructed area is 484mm². Details down to 2-3μm size are resolved, enabling individual cell visualization (Fig. 1d). Structural information of the whole tissue slide is enhanced by computing the local variance over a 100 pixels window, over the whole reconstructed phase (Fig.2). Tissue slices from the same biopsy were HES (Hematoxylin Eosin Safran) stained and scanned with a digital slide scanner microscope for comparison.
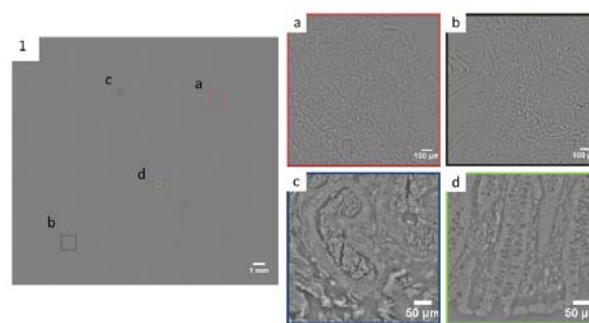

Figure 1. 484 mm² wide-field reconstructed phase of a human unstained colon slide. (a-d) Details showing (a) intestinal glands from the mucosa layer, (b) sub-mucosa layer, (c) zoom on blood vessel (d) zoom on intestinal glands.
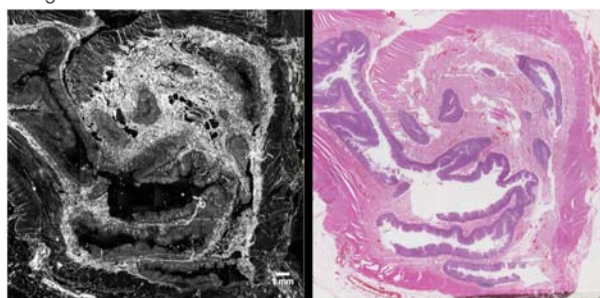

Figure 2. Left: 484 mm² wide-field reconstructed phase with applied variance filter, showing the tissue structure over the whole slide. Right: Comparison with a digital slide scanner image of a HES stained tissue slide that was cut from the same colon biopsy.

Perspectives

To our knowledge, our method is the first technique that enables fast wide-field lensfree imaging of transparent tissue slides. This simple technique is cheaper than a phase contrast microscope, and may be compatible with telepathology applications in remote areas.

RELATED PUBLICATIONS:
[1] A. Greenbaum, Y. Zhang, A. Feizi, P. Chung, W. Luo, S. R. Kandukuri, and A. Ozcan, "Wide-field computational imaging of pathology slides using lens-free on-chip microscopy," Sci. Transl. Med. 6(267), (2014).
[2] W. Luo, A. Greenbaum, Y. Zhang & A. Ozcan, "Synthetic aperture-based on-chip microscopy," Light Sci. Appl. 4, e261 (2015).
[3] SNA Morel, L. Hervé, T. Bordy, Ol. Cioni, A. Delon et al. "Whole slide imaging of unstained tissue using lensfree microscopy", Proc. SPIE 9711, Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues IX, 97111L (April 6, 2016); doi:10.1117/12.2210831.

CEREBROSPINAL FLUID LENSFREE MICROSCOPY: A NEW TOOL FOR THE LABORATORY DIAGNOSIS OF MENINGITIS

RESEARCH TOPIC:
Infectious diseases, Microbiology, Lens-free microscopy

AUTHORS:
R. Delacroix[1], S. Nhu An Morel, L. Hervé, T. Bordy, J.-M. Dinten, M. Drancourt[1], C. Allier

ABSTRACT:
Cerebrospinal fluid cytology is performed by operator-dependant light microscopy as part of the routine laboratory work-flow diagnosis of meningitis. We evaluated lens-free microscopy for the cytological diagnosis of meningitis. Lens-free microscopy algorithms were adapted for counting cerebrospinal fluid cells and discriminating leukocytes from erythrocytes. A blind lens-free microscopic analysis of 116 cerebrospinal fluid specimens, including six cases of microbiologyconfirmed infectious meningitis, yielded a 100% sensitivity and a 79% specificity. Adapted lens-free microscopy is thus emerging as an operator-independent technique for the rapid numeration of leukocytes and erythrocytes in cerebrospinal fluid. In particular, this technique is well suited to the rapid diagnosis of meningitis at point-of-care laboratories.

SCIENTIFIC COLLABORATIONS: Aix Marseille Univ, INSERM, CNRS, IRD, URMITE, [1]CHU la Timone Marseille, France

Context and Challenges

Performing the cytological analysis of the cerebrospinal fluid (CSF) and enumerating leukocytes and erythrocytes is a routine first step in the laboratory diagnosis of meningitis. Indeed, meningitis is diagnosed if more than 10 leukocytes/µL are counted, in the absence of erythrocytes. CSF cytology and cell counting are routinely performed by optical microscopy. Optical microscopy observation is an operator-dependent task and the subsequent reporting is subject to variability; this may indeed result in the erroneous classification of the CSF specimen as meningitis/non-meningitis. Accordingly, cytological analysis of the CSF with optical microscopy can hardly be incorporated into the point-of-care (POC) laboratory for the rapid diagnosis of meningitis. Here, we established the proof-of-concept that an adapted lens-free microscopy protocol could be used in the laboratory instead of the manual cell-counting diagnosis of meningitis [1]. The CSF cell count method using the lens-free technology should have the same level of reliability as optical microscopy, though it should achieve greater reproducibility, with a significant time-saving capacity. This method will have another advantage, that of being applicable in the POC laboratory and "in low-resource settings," unlike previously developed apparatus [2].

Main Results

We evaluated lens-free microscopy numeration of erythrocytes and leukocytes for the cytological diagnosis of meningitis. In a first step, prospective optical microscopy counts of leukocytes done by five different operators yielded an overall 16.7% misclassification of 72 cerebrospinal fluid specimens in meningitis/non-meningitis categories using a 10 leukocyte/µL cut-off. In a second step, the lens-free microscopy algorithm adapted for counting cerebrospinal fluid cells and discriminating leukocytes from erythrocytes was modified step by-step in the prospective analysis of 215 cerebrospinal fluid specimens. The definite algorithm yielded a 100% sensitivity and a 86% specificity compared to confirmed diagnostics. In a third step, a blind lens-free microscopic analysis of 116 cerebrospinal fluid specimens, including six cases of microbiology confirmed infectious meningitis, yielded a 100% sensitivity and a 79% specificity.
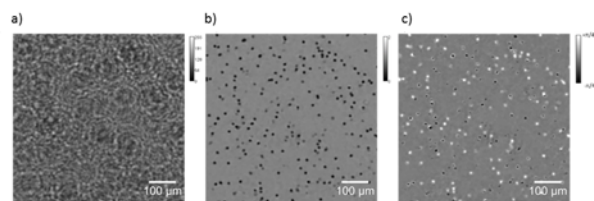

Figure 1. Acquisition of a CSF sample by means of lens-free microscopy. (a) Raw data. (b-c) Reconstructed module and phase image showing leukocytes and erythrocytes.
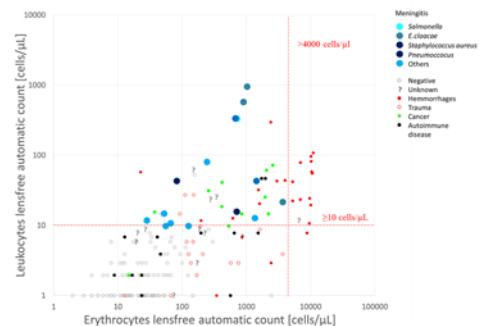

Figure 2. Scatterplot of the automatic lens-free count of leukocytes and erythrocytes resulting from the analysis of the first datasets featuring 215 clinical specimens. A color code has been defined with respect to the different established diagnosis. The infectious meningitis of interest are plotted in large blue dots. The criteria for the diagnostics of meningitis is depicted by a vertical red dotted line. With this criterion, lens-free microscopy achieved a sensitivity of 100% and a specificity of 86%.

Perspectives

Adapted lens-free microscopy is thus emerging as an operator-independent technique for the rapid numeration of leukocytes and erythrocytes in cerebrospinal fluid. In particular this technique is well suited to the rapid diagnosis of meningitis at point-of-care.

RELATED PUBLICATIONS:
[1] R. Delacroix, S. N. A. Morel, L. Hervé, T. Bordy, J.-M. Dinten, M. Drancourt, and C. Allier, (2017), "Cerebrospinal fluid lens-free microscopy: a new tool for the laboratory diagnosis of meningitis". Nature Scientific Reports, 7.
[2] M. Drancourt, A. Michel-Lepage, S. Boyer, & D. Raoult, "The Point-of-Care Laboratory in Clinical Microbiology". Clin. Microbiol. Rev. 29, 429–447 (2016).

OPTICAL ELASTIC SCATTERING FOR EARLY LABEL-FREE IDENTIFICATION OF CLINICAL PATHOGENS

RESEARCH TOPIC:
Clinical microbiology, Label-free bacteria identification, Optical elastic scattering, Recognition of Fresnel diffraction patterns

AUTHORS:
V. Genuer, O. Gal, C. Belafdil, J. Méteau, P. Marcoux, E. Schultz, E. Lacot[1], M. Maurin[2], J.-M. Dinten

ABSTRACT:
We report on an innovative, fast and accurate label-free method for identification of microorganisms directly on agar media at a very early stage of growth (microcolonies sizing between 30 and 300µm). This method is based on the analysis of the scattering pattern (scatterogram) generated by a microcolony growing on agar when placed in the path of a laser beam. This technique is non-invasive (measurements are directly performed on closed Petri dishes), low cost and requires neither skilled operators nor reagents. It is therefore fully compatible with today's lab automation requirements. Scatterograms are acquired on transparent and opaque blood-supplemented agar media. The developed system allows the identification of different species of bacteria and yeasts.

SCIENTIFIC COLLABORATIONS: [1]Labo. interdisciplinaire de physique (Liphy, UGA), [2]Labo. de bactériologie (CHU Grenoble), CEA-LIST

Context and Challenges

While MALDI-TOF spectrometry is becoming the gold standard for identification of pathogens as it is a fast and accurate method, many efforts aim at developing new identification techniques that would be both cheaper and non-destructive. That is why elastic scattering is a promising method studied by several groups. [1]

Up to now, most of the studies about elastic scattering were limited to large colonies (24h incubation) growing on transparent media (i.e. forward scattering). Our results pave the way towards collecting relevant information on small colonies using a forward or a backward geometry. The latter mode offers added value for the technique by enabling the analysis of the scattering patterns on all types of nutrient agar media.

Fig. 1. Photograph of the integrated MICRODIFF instrument for fast and accurate identification of clinical pathogens using elastic forward scattering

Main Results

In order to validate the ability of the technique to discriminate bacteria and yeasts, two large databases were performed in the forward mode on transparent media (6h of incubation) with the Microdiff prototype (Fig. 1). The first one gathers 1900 scatterograms acquired on 15 strains (8 strains of yeasts, 7 strains of bacteria). A very high classification rate (95%) was achieved on the Gram+/Gram-/yeasts discrimination [2]. A second thorough database was collected on *Staphylococci* in order to investigate the screening on *S. aureus* carriers in a much faster way (6h) than today's culture-based methods (24h). We collected 5459 scatterograms (Fig. 2) on 38 strains of *Staphylococci* (1/3 of *S. aureus*, 2/3 of coagulative-negative strains). Our home-made pattern recognition algorithms provide a 91.4 % discrimination rate between *S. aureus* and non-aureus *Staphylococci*. As a comparison, color reading of colonies after 24h of incubation on chromogenic medium ChromID *S. aureus* (SAID bioMérieux) yields in average 93% of correct classification. These results open the way to an innovative, fast and label-free identification of pathogens at an early stage of growth.

ATCC BAA-44
Staphylococcus aureus subsp. aureus

ATCC 27851
Staphylococcus simulans

ATCC 29970
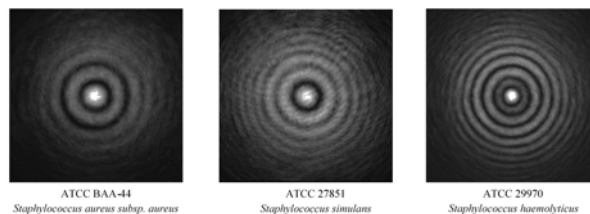*Staphylococcus haemolyticus*

Fig. 2. Scatterograms of micro-colonies acquired after 6h of growth at 37°C with the forward scattering prototype at the Grenoble's hospital laboratory of bacteriology.

In clinical microbiology, a majority of cultures are performed on opaque media, especially on blood-supplemented media such as Columbia or "chocolate" agars. Developing an equipment for backward-scattering was therefore crucial [2]. We considered a database of four strains of E. Coli (400 scatterograms) at 6h of incubation for the validation of the newly developed backward system [1]. An overall good classification rate of 87% was demonstrated with the backward system, which is close to the 90% obtained with the forward system.

Perspectives

Clinical studies are ongoing on nasal swabs, so as to challenge the database collected for the screening on *S. aureus* carriers. At the same time, we have initiated intensive studies to find more efficient algorithms of pattern recognition, involving neural networks and/or texture descriptors.

RELATED PUBLICATIONS:

[1] P. R. Marcoux, M. Dupoy, A. Cuer, J.-L. Kodja, A. Lefebvre, F. Licari, R. Louvet, A. Narassiguin, F. Mallard, "Optical forward-scattering for identification of bacteria within microcolonies", Appl. Microbiol. Biotechnol. **2014**, 98, 2243-2254.

[2] V. Genuer, O. Gal, J. Méteau, P. Marcoux, E. Schultz, E. Lacot, M. Maurin, J.-M. Dinten, "Optical elastic scattering for early label-free identification of clinical pathogens", Conference Proceedings, **2016**, 9698, 96980A-96980A-13.

3.

LAB ON CHIP

- Spontaneous capillary flows

- Acoustofluidic chip

- Biology protocol and microfluidic

- Microfluidic in polymeric foam

- Microfluidic standardization

- Gas sampling and analysis

- Mass spectrometry analysis

SPONTANEOUS CAPILLARY FLOWS IN PIECEWISE VARYING CROSS SECTION MICROCHANNELS

RESEARCH TOPIC:

Microfluidic, Spontaneous capillary flow, Capillary wetting, Lucas–Washburn–Rideal (LWR) law

AUTHORS:

J. Berthier, D. Gosselin, A. Pham[1], F. Boizot, G. Delapierre, N. Belgacem[2], D. Chaussy[2]

ABSTRACT:

The study of the dynamics of capillary wetting has started in the years 1920s with the studies of Lucas, Washburn, and Rideal. The LWR law states a square root dependency with time for the penetration distance. This property was shown to be valid for arbitrary cross section microchannels. However, the dynamics of capillary wetting in non-uniform cross section channels is still a subject of investigation. In this work an analytical model for piecewise varying cross section channels is developed. It is shown that the model compares favorably to experiments.

SCIENTIFIC COLLABORATIONS: [1]Louisiana State University (LSU), [2]INPG/LGP2

Context and Challenges

Studying the dynamics of capillary wetting was started in the 1920s with the studies of Lucas, Washburn, Rideal and others, for the filling of cylindrical tubes [1,2]. This property was also proved to be valid for the capillary filling of channels of arbitrary cross section, open or closed [3,4]. However microfluidic chips often contain reaction chambers which induces a change of the microchannel dimensions. In this work, we develop an analytical approach to the problem of piecewise varying cross-sectional dimensions based on the balance between the drag and capillary forces [5]. We present experimental results obtained in different geometries. We show that the model results compare favorably to that of the experiments.

Main Results

The velocity of spontaneous capillary flow in a uniform cross section channel is usually obtained by balancing the capillary force and the wall friction, and neglecting the inertial forces. Such a theoretical approach is proposed in this article while considering a capillary channel with piecewise uniform cross sections as sketched in Fig. 1
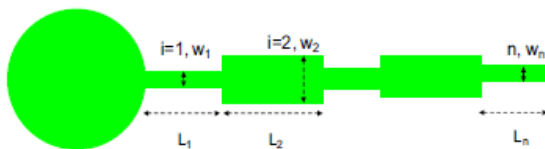

Figure 1: Sketch of the channel with the different dimensions of the channel.

Closed form expressions for the penetration distance versus time, and for the average velocity versus time have been derived. The predictions of the analytical model are shown to be in agreement with experimental results in the geometry of rectangular open channels, milled as well in PMMA (see Fig 2) or silicon.

As reported in the literature, it is confirmed that the flow is locally accelerated at the entrance of a constricted section, while it is decelerated at the entrance of an enlarged section.
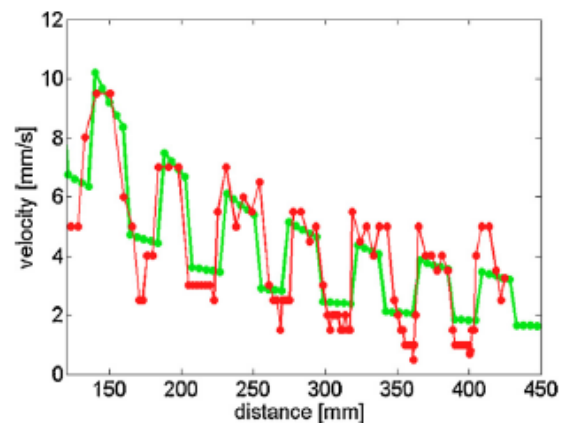

Figure 2: Average velocities in the open channel: Comparison between experiments (red) and analytical model (green).

Calculation of the velocity jump at a constricted/enlarged section entrance has been derived from the model. Numerous alternate changes of cross-sectional areas produce successive acceleration and decelerations. Globally, a square root law for the penetration distance is respected if the successive changes of section continuously alternate, and the flow behavior is similar to a channel with an equivalent cross section.

Perspectives

In this work, the effect of cross sectional changes have been investigated and well clarified. This understanding will help to the design of future microchips. For example, it can lead, with a careful definition of the geometry, to the precise control of the fluid velocity within the capillary device.

RELATED PUBLICATIONS:

[1] R. Lucas, "Ueber das Zeitgesetz des Kapillaren Aufstiegs von Flussigkeiten", Kolloid Z 23 (1918) 15.
[2] E.W.Washburn, "The dynamics of capillary flow", Phys. Rev. 17 (1921) 273–283.
[3] J. Berthier, D. Gosselin, E. Berthier, "A generalization of the Lucas–Washburn–Rideal law to composite microchannels of arbitrary cross section", Microfluid. Nanofluid. 19 (3) (2015) 497–507.
[4] L. Gervais, N. de Rooij, E. Delamarche, "Microfluidic chips for point-of-care immunodiagnostics", Adv. Mater. 23 (24) (2011) H151–H176.
[5] J. Berthier et al., "Spontaneous capillary flows in piecewise varying cross section microchannels". Sensor Actuat B-Chem. (2016) 223:868-77.

SPONTANEOUS CAPILLARY FLOWS IN CURVED, OPEN MICROCHANNELS

RESEARCH TOPIC:
Microfluidics, Spontaneous capillary flows, Capillary wetting, Contact angle, Curved channels

AUTHORS:
J. Berthier, K. A. Brakke[1], D. Gosselin, F. Navarro, N. Belgacem[2], D. Chaussy[2],

ABSTRACT:
Capillary flows are increasingly used in biotechnology, biology, chemistry, energy and space applications. Motivated by these new developments, designs of capillary channels have become more sophisticated. In particular, capillary microsystems often use "zig-zag" or winding channels for compactness, or mixing. The behavior of spontaneous capillary flows in curved channels is still underdeveloped. This type of behavior is investigated in the work. In the case of suspended capillary flows, it is shown that the flow profile in the curved section is approximately the same as in a rectilinear section. On the contrary, in the case of open U-grooves where inner corners are present, the importance of the turn sharpness and of the presence of capillary filaments is pointed out.

SCIENTIFIC COLLABORATIONS: [1]Susquehanna University (Pennsylvania, USA), [2]INP/LGP2

Context and Challenges

Capillary microsystems are now widely used for the development of point-of-care (POC) systems for biology and biotechnology [1,2]. Accompanying these new developments, the geometry of up-to-date capillary channels has become increasingly sophisticated. Very often, in order either to fit a sufficient length on a miniaturized chip, or to perform fluidic functions, winding serpentine channels are used. So far, the shape, position and dynamics of the advancing meniscus in a turn of a channel have not been reported in the literature. Winding capillary networks have been examined globally for their usefulness as capillary pumps [3]. In this work, we propose a first investigation of the capillary behavior of liquids in curves [4]. We focus on open, rectangular U-grooves and suspended channels.

Main Results

The behavior of the capillary flow in a turn is different depending on the geometry of the channel, on the contact angle and on the turn aspect ratio, i.e., the ratio between the inner and outer turn radius.

In the case of suspended channels, thus devoid of interior corners no special effect of the contact angle or turn aspect ratio has been found, and the flow is similar to that in a rectilinear channel. Figure 1A shows a picture of the liquid flowing through a suspended turn. On figure 1B, locus of the center of the circles auscultating the advancing interface has been drawn, for both the experimental observations and the theoretical calculations. A good agreement between the two loci is found suggesting that the contact angles are identical on both walls, and nearly equal to the static Young contact angle.

The case of rectangular open U-grooves is more complicated: Turning corners curve the interfaces depending on the radius of the turn. If the contact angle is relatively high (60°–90°), the curvature effect is small, and there is a great similarity with a rectilinear capillary flow. In the case of smaller contact angles, the footprint of the contact line on the bottom floor becomes asymmetric. Below 45°, capillary filaments form, and the inner filament advances ahead of the outer filament. This effect can be important in spiraling channels, for example. It is also noticeable in the case of small turn aspect ratio, as shown in Fig 2.
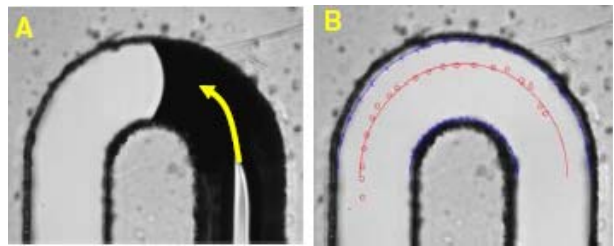

Figure 1: A: Top view of the incoming liquid (in black). B: the red line corresponds to the locus of the center of the osculating circle of the interface, and the red dots to the experimental observations.
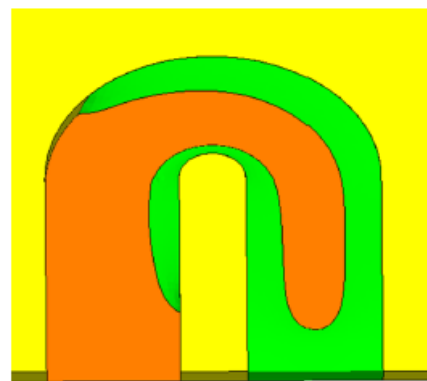

Figure 2: View of the capillary flow in a turning U-groove with a small turn aspect ratio

Perspectives

This work brings a better understanding of the behavior of spontaneous capillary flows which can lead to a better design of future microchips.

RELATED PUBLICATIONS:
[1] C.D. Chin, S.Y. Chin, T. Laksanasopin, S.K. Sia, Chapter 1: Low-cost microdevices for point-of-care testing. In: Issadore D, Westervelt RM (eds) Point-of-care diagnostics on a chip. Springer, Berlin, 2013
[2] L. Gervais, E. Delamarche, "Toward one-step point-of-care immunodiagnostics using capillary-driven microfluidics and PDMS substrates". Lab Chip 9:3330, 2009.
[3] R. Safavieh, A. Tamayol, D. Juncker, "Serpentine and leadingedge capillary pumps for microfluidic capillary systems", Microfluid Nanofluid 18:357–366, 2015.
[4] J. Berthier, K.A. Brakke, D. Gosselin, F. Navarro, N. Belgacem, D. Chaussy, "Spontaneous capillary flow in curved, open microchannels", Microfluid Nanofluid, 20, 100, 2016.

USE OF ACOUSTIC EVANESCENT WAVES: A NEW TOOL FOR ACOUSTICS ON A CHIP

RESEARCH TOPIC:
Acoustic waves, Sample preparation, Microfluidics

AUTHORS:
V. Aubert, C. Poulain, D. Rabaud, R. Wunenburger[1], T. Valier-Brasier[1], J.P Kleman[2]

ABSTRACT:
Acoustofluidics, which is the use of sound waves at microscale, is now well-known in biotechnology as a powerfull tool for contactless manipulation of living cells or bacteria. To date, most techniques rely on the use of propagating (leaky) acoustic waves which radiate sound in the fluid bulk and require dedicated piezoelectric substrate materials and rather complicated packaging. In a recent paper, we have shown that, exactly like in the field of optics and optical tweezers, evanescent waves are very promising for a very simple and effective cell manipulation on a chip. We show how to generate this type of waves on a simple thin glass plate, and how these confined waves can be controlled to achieve cell or whole blood patterning, and even individual cell rotation.

SCIENTIFIC COLLABORATIONS: [1]UPMC, [2]CEA-IBS

Context and Challenges

For about 10 years, acoustofluidics has shown to be a very powerful tool for Lab on Chip manipulation. Massive research have been carried out in this field and acoustofluidics is now mature enough to address concrete biotechnological issues.

It is for instance now possible to sort living cells upon their size or elasticity, isolate CTC (Circulating Tumoral Cells), concentrate or pattern cells, use bubbles or micro-swimmers to capture cells or deliver a targeted drug, mix two components, to mention but a few. Despite impressive capabilities, the most widely used technology (SAW: surface acoustic waves) at an industrial scale remains limited due to cost effective fabrication processes and an intrinsic difficult packaging.

Different groups are working hard to circumvent these difficulties by either making surface waves more reliable and easy to package, or developing new meta material offering also very promising perspectives.

Main Results

Recently, we have proposed a new approach based on the use of subsonic and hence evanescent waves [1]. Several examples of key lab on Chip applications have been demonstrated by taking advantage of a very simple and low-cost device. We have shown that a very thin plate on which a piezo-transducer is glued consists in a very simple evanescent wave emitter capable to exert strong and well defined force or torque fields upon micro-particles or cells. The evanescence of the field in the direction orthogonal to the plate confines the acoustic energy close to the channel walls where the particles to be moved are located instead of radiating it into the whole bulk fluid or through the chip. In particular, we have shown how to use this device for efficiently forming lines or arrays of living cells in a few seconds which could simplify Lab-on-Chip 3D cell printing for tissue engineering applications or cell identification.
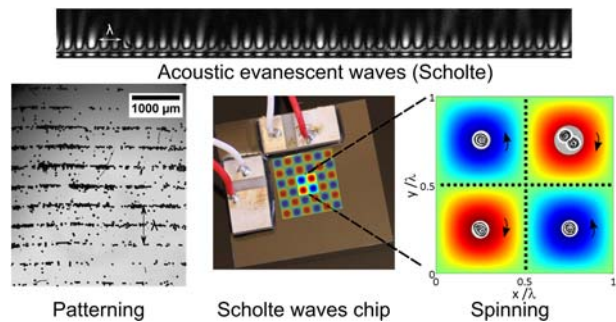

Fig 1: Simple application of a low-cost device using evanescent acoustic waves for acoustorotation.

We have also demonstrated single cell acoustorotation of individual cells in an array by superimposing two orthogonal out of phase evanescent waves. Finally, using whole blood, we have shown how to create plasma enriched regions in a few micro liters whole blood opening the route towards very simple plasma characterization.

Perspectives

This work opens the route towards a new mean for manipulating the matter at small scale by evanescent field.
We hope this approach will stimulate new point of care applications for which a low-cost manufacturing remained an obstacle.
In the future and like in plasmonic optics, it might be possible to form non diffracting beams (Bessel beam) and hence new kinds of traps on a chip.

RELATED PUBLICATIONS:
[1] V. Aubert, R. Wunenburger, T. Valier-Brasier, D. Rabaud, J.P. Kleman and C. Poulain, "A simple acoustofluidic chip for microscale manipulation using evanescent Scholtes waves" Lab Chip.,2016,16, 2532-2539.

REAL TIME OBSERVATION AND AUTOMATED MEASUREMENT OF RED BLOOD CELLS AGGLUTINATION INSIDE A PASSIVE MICROFLUIDIC BIOCHIP CONTAINING EMBEDDED REAGENTS

RESEARCH TOPIC:

Agglutination assay, Passive microfluidic, Embedded reagents Automated image processing, Real time detection, ABO blood typing

AUTHORS:

M. Huet, M. Cubizolles, A. Buhot

ABSTRACT:

The process of agglutination is commonly used for the detection of biomarkers like proteins or viruses. The multiple bindings between micrometer sized particles, either latex beads or red blood cells (RBCs), create aggregates that are easily detectable and give qualitative information about the presence of the biomarkers. In this study, we address the development of a real-time time observation of RBCs agglutination. Specific reagents were dried inside the microchannel of a passive microfluidic chip designed to enhance capillary flow. A blood drop deposit at the tip of the biochip established a simple biological protocol. *In situ* agglutination of autologous RBCs was achieved by means of embedded reagents and real time agglutination process was monitored by video recording. Using ABO blood typing as a proof-of-concept, we developed i) an integrated biological protocol suitable for further use as point-of-care (POC) analysis and ii) two dedicated image processing algorithms for the real-time and quantitative measurement of agglutination.

SCIENTIFIC COLLABORATIONS: Avalun (Grenoble)

Context and Challenges

Hemagglutination assays (HA) are widely employed to characterize viruses and bacteria that naturally agglutinate red blood cells (RBCs), especially for influenza and veterinary diagnosis. In addition, HA can also be used to detect the presence of antigens on RBCs by specific probes. Agglutinates can be generated using various protocols, usually performed manually. In most cases, the detection of the agglutination is made by simple naked-eye observation In order to obtain absolute quantitation of a biomarker, qualitative or semi-quantitative end point measurements may not be sufficient. In this context, we address the development of a real-time observation of RBCs agglutination [1] [2], using ABO typing as a proof of concept.

Main Results

We focused our approach on passive microfluidics. The system comprises of a biochip [3], an optical instrument and a computational unit, used to perform an optical measurement of a red blood cells agglutination process [4]. Commercial anti-A and anti-B blood typing reagents were reformulated and then separately embedded in microfluidic chips (Fig. 1) by drying. Then 6.5 µL of 1:5 diluted blood is deposited at the curved tip of the biochip and capillary forces drained the blood inside the microchannel. The dried reagent is dissolved in the blood and provokes the agglutination process of the RBCs in case the corresponding antigens are present at their surface. Therefore, the agglutination is triggered *in situ* without requiring action from the operator apart from depositing the blood drop.
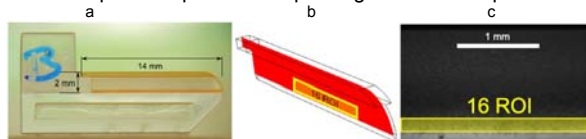

Figure 1: (a) microfluidic chip with embedded reagent. (b) 3D view of the microchannel: the observed plane (red) and location of the 16 regions of interest (ROI). (c) picture camera.of chip.

Two image processing algorithms were specifically created and developed to automatically measure the agglutination process in real-time. The measurement relies on two different indicators: one is based on a correlation value between different pixel lines and the other is based on a variance of the gray level in small areas. The image processing was performed on the whole picture width (1296 pixels) divided in 16 ROI. The average of the indicators on each of the 16 ROI serves as the agglutination indicators and their error estimation is performed from the standard deviation. Both indicators allow for the successful determination of the blood type characterization within 90s (Fig. 2). We demonstrated the possibility to follow in real-time the agglutination process.
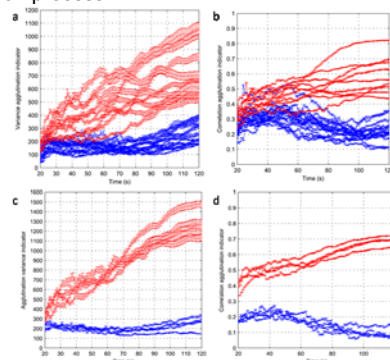

Figure 2 - Real-time agglutination measurement. (a) variance & (b) correlation indicators for the 24 reference experiments (c) variance & (d) correlation indicators for the 8 experiments of the validation set. Red curves: positive and blue curves: negative agglutinations.

Perspectives

This study opens the door to a large set of potential POC applications besides ABO blood typing. Further work could lead to efficient biomarkers detection and quantification by hemagglutination techniques or even latex agglutination assays.

RELATED PUBLICATIONS:

[1] M. Huet, M. Cubizolles, A. Buhot, "Real time observation and automated measurement of red blood cells agglutination inside a passive microfluidic biochip containing embedded reagents", Biosens Bioelectron. Available online 20 September 2016 (2016).
[2] V. Poher, M. Cubizolles, P. Pouteau, C. Allier, J. Spiaczka, "Method and system for characterisation of the agglutination of particles contained in a liquid", WO2013178777 (2013).
[3] P. Pouteau, J. Berthier, V. Poher, "Device for collecting a liquid sample by capillary action and related analysis method", WO2014135652 (2014).
[4] M. Huet, J.-G. Coutard, "Method for determining the particle agglutination level in a sample", EP3076156 (2016).

MICROFLUIDICS IN POLYMERIC FOAM: A NEW TECHNOLOGY FOR BANDAGES AND LOW-COST POINT-OF-CARE DIAGNOSTIC DEVICES

RESEARCH TOPIC:
Microfluidics, Point-of-care, Low-cost, Diagnostic, Polymeric foam, Hyperelastic polymer, Peristaltic pump, Bandages

AUTHORS:
G. Gropplero, Y. Fouillet, F. Revol-Cavalier, L. Davoust

ABSTRACT:
A new way of producing low-cost microfluidic devices based on the joint-use of open-cell polyurethane foam and a hyperelastic silicon rubber [1] is presented. Such devices are found to be completely flexible and to provide original ways for fluid handling that do not rely on external devices. Peristaltic pumping allows effective monitoring of the microfluidic flows. Lumped element model enables a reliable modelling of the unsteady flows inside foam-based channels. Filtration of objects a few tens of micrometers sized is achievable thanks to the structure of the foam. Its chemical functionalization is also made possible. DNA amplification is achieved inside the foam devices for colorimetric or fluorescent detection of target DNA. A blood typing prototype is developed demonstrating the capabilities of the foam technology to handle blood, to store liquids and dried reagents with no compromise on data accessibility, from imaging for instance.

SCIENTIFIC COLLABORATIONS: SIMaP (Grenoble)

Context and Challenges

The need for low-cost microfluidic devices in point-of-care applications keeps growing. Well known examples can be found relying on paper or fiber-based approaches [2]. Open-cell polyurethane foams are currently used in bandages with interesting properties (elasticity, porosity, high fluid retention…) [3] and fabrication processes are well established permitting worldwide low-cost availability especially for microfluidic devices. The peculiar mechanical and structural properties of a polymeric foam are unique compared to current materials used in microfluidics (PDMS, paper, plastic, glass, silicon...).

Main Results

The new microfluidic foam device production process relies on the combined use of a polymeric foam and an elastomer with the aim to produce highly elastic microfluidic systems essentially characterized by the initial structural properties of the foam. Based on a controlled and repeatable embossing technique, the manufacturing process is compatible with mass production. Thus, foam microfluidic devices offer, besides capillarity, a decisive advantage: the use of either a manual compression or an external peristaltic actuation for contamination-free monitoring of microfluidic flows (Fig. 1). The peristaltic actuation can behave as a pump as well as a valve. Lumped element model enables a reliable modelling of the unsteady flows inside foam-based channels [4]. The model considers the viscoelastic behavior of the foam and its ability to retain fluids depending on the deformation rate. One decisive benefit of using this model is its ability to quickly simulate dynamic phenomena by providing a macroscopic view of the flow pattern with no attempt for computing the microflows at pore scale.

To ensure proper integration in low-cost portable devices, the feasibility of performing a diagnostic test (liquid sample preparation, deposition or removal, biological detection) is demonstrated. Filtration of objects a few tens of micrometers sized is made possible.

Foam devices can also be chemically functionalized to enhance

the capture of biological targets. The fluorescent or colorimetric detection of biological elements is equally possible by means of isothermal DNA amplification.
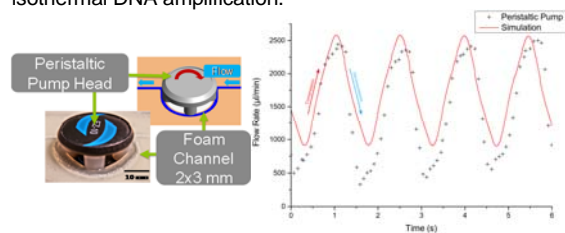

Figure 1: Flow induced by the rotation of the peristaltic pump head compared to the simulation issued from the electric/fluidic analogy (angular velocity: 10 RPM).

Finally, a blood typing prototype gives access to blood type of a whole blood sample within a few minutes (Fig. 2). This last test is carried out on an integrated foam-based device which highlights the following benefits: robustness, user-friendly, embedded reagents, multiple materials combination, motion of a biological sample from an external compression, live access to a bioassay result within few minutes.

Figure 2: Blood typing prototype for effective identification of the blood type of a whole blood sample.

Perspectives

The mechanical and structural properties of the foam devices are unique in microfluidics. The possible integration of key biological functions opens the way for high technology point-of-care devices and bandages.

RELATED PUBLICATIONS:
[1] G. Gropplero, F. Revol-Cavalier, Y. Fouillet & L. Davoust, "Procédé de fabrication d'un élément fluidique et élément fluidique fabriqué à l'aide dudit procédé", FR1651116.
[2] D. R. Ballerini, X. Li, & W. Shen, "Patterned paper and alternative materials as substrates for low-cost microfluidic diagnostics", Microfluidics and Nanofluidics 13, 769–787 (2012).
[3] F. Revol-Cavalier, M. Lamoise, M. Messaoud & J.-M. Pernot, Article for absorbing a physiological liquid, such as a dressing - WO2015092702 (2015).
[4] G. Gropplero, L. Davoust, S. Arnoux, Y. Fouillet & F. Revol-Cavalier, "Foam-based microfluidics: experiments and modeling with lumped elements", Microfluidics and Nanofluidics, 20(12):170, 2016.

EUROPEAN INITIATIVE FOR THE STANDARDIZATION OF MICROFLUIDIC DEVICES

RESEARCH TOPIC:
Microfluidics, standardization, MicroFluidic Building Block, Fluidic Circuit Board

AUTHORS:
M. Alessio, F. Baleras, F. Boizot, O. Constantin, M. Gellie, S. Maubert, B. Icard and N. Verplanck

ABSTRACT:
The MFManufacturing project, granted by ECSEL-JU, aims to structure the European manufacturing through a Distributed Pilot Line and to initiate the standardization process of interconnects. We describe here the different steps to reach an ISO standardization of microfluidics interconnects.

SCIENTIFIC COLLABORATIONS: Axxicon, Blacktrace, CfBI, eMNT, MICRONIT, MICRONIT, NPL, TNO, U. Twente

Context and Challenges

There is a clear need for microfluidics devices. However, "the general adoption for microfluidics will only be possible with an agreement on standardized interconnects between chips and systems", concluded the Microfluidic Consortium in 2011.

The MFManufacturing project, granted by ECSEL-JU, is a consortium of 21 European partners, including the whole microfluidics value chain. Its objectives are to structure the European manufacturing through a Distributed Pilot Line and to initiate the standardization process of interconnects.

Main Results

The project consortium has reached a consensus on design guidelines to be standardized and has published two whitepapers on the project Website [1]. A guideline example is given Fig.1. It represents the recommended pitch spacing between two holes on a microfluidic component. Indeed, one of the main objectives of these guidelines is to allow a reliable and easy connection of a MicroFluidic Building Block (MFBB), integrating a fluidic function (e.g. a pneumatic valve), to a Fluidic Circuit Board (FCB).
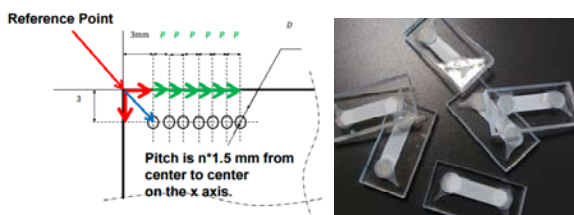

Fig.1. a) Design guideline for top connection pitch spacing [1], b) Example of MFBB for concentration

Thanks to these guidelines, the microfluidic designer will have access to design kits library and off-the-shelf reliable components and will save time in product development.

Based on the whitepapers, an International Workshop Agreement (IWA) has been held in London in April 2016. This opened workshop was the opportunity to present our guidelines to international microfluidic players, outside the project and to list 7 resolutions, described in an ISO document [2].

A strong dissemination is important to get the acceptance of the microfluidic community. In 2016, we have participate to several major international conferences and workshops [3,4].

To illustrate the impact of the guidelines, Leti has designed a generic test bench to test in specific conditions all the MFBBs either developed within the project or described in the whitepapers (Fig.2.).
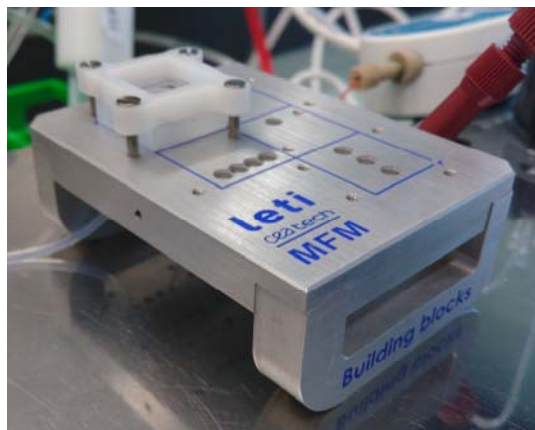

Fig.2. MFBB generic test bench

Perspectives

Leti, as AFNOR member, has launched with some MFM partners, a New Work Item Proposal, the formal document to get an ISO standard. This standardization should be followed through the ISO Technical Committee TC48 and led to the first international microfluidic standard.

The project consortium will continue to implement the standards through the Distributed Pilot Line.

RELATED PUBLICATIONS:
[1] H. van Heeren, T. Atkins, N. Verplanck, C. Peponnet, P. Hewkin, M. Blom, W. Buesink, J.E. Bullema, S. Dekker, "Design Guideline for Microfluidic Device and Component Interfaces (part 1) v2", WhitePaper, http://www.mf-manufacturing.eu, May 2016.
[2] "Interoperability of microfluidic devices: Guidelines for pitch spacing dimensions and initial device classification", IWA 23:2016, London, April 2016.
[3] H. van Heeren, T. Atkins, M. Blom, J.E. Bullema, R. Tantra, D. Verhoeven and N. Verplanck, "Microfluidic standardization : past, present and future",
20th International Conference on Miniaturized Systems for Chemistry and Life Sciences 557-558, Dublin, October 2016 Workshop Microfluidic Standardization.
[4] H. van Heeren, T. Atkins, M. Blom, J.E. Bullema, R. Tantra, D. Verhoeven and N. Verplanck, "Microfluidic standardization, status overview", MicroNanoConf., 2016.

MICROFLUIDIC GENERATION AND STUDY OF HIGHLY VISCOUS BIOPOLYMER CAPSULES FOR BIOLOGY APPLICATIONS

RESEARCH TOPIC:

Cell therapy, Microencapsulation, Microflow focusing, Microfluidic, Biopolymer

AUTHORS:

F. Bottausci, C. Authesserre, P.Y. Benhamou[1], F. Rivera

ABSTRACT:

We report for the first time the production of ~200 µm-size monodispersed droplets using highly viscous newtonian and non-newtonian fluids (up to 3.6Pa.s) in a pressure actuated device. Droplet size and production frequency have been characterized regarding flow rates and fluid properties (viscosity of the dispersed phase, interfacial tension, shear-thinning properties) and scaling laws are presented. Shear-thinning behavior results in smaller droplet size and higher production frequency than droplets produced with a newtonian fluid having the same zero-shear rate viscosity.

SCIENTIFIC COLLABORATIONS: [1]Department of Endocrinology (Grenoble University Hospital, 38043 Grenoble, France)

Context and Challenges

Cells encapsulation is a promising field for drug screening, therapeutic and bioengineering applications. Droplet based microfluidics systems are widely used to encapsulate a large variety of cells or chemicals. We have been developing a microfluidic platform to address a range of applications among the cure of diabetes mellitus by encapsulating Langerhans islets. The biopolymers used as extracellular matrix for the living cells is non Newtonian and can be highly viscous based on its intrinsic properties and concentration (up to 4Pa.s) making the capsule formation impossible with traditional technologies. Using microfluidic plastic cartridge-based technology, micro-capsules can be generated, manipulated and characterized.

Main Results

First the non-Newtonian biopolymer (alginate) is rheologically characterized showing a shear-thinning behavior compared with Newtonian fluid of same viscosity at zero shear. The viscosity varies from 0.1 to 3.6Pa.s for an alginate concentration of 1 to 3%.

Droplet formation

The droplets generation is done using the plastic cartridge (Fig1). The microflow focusing device (MFFD) is composed of a T junction with a divergent chamber to better focus the shear stress at the junction [1,2]. Soybean oil is used as continuous phase to pinch the biopolymer flow (alginate) and form the droplets.
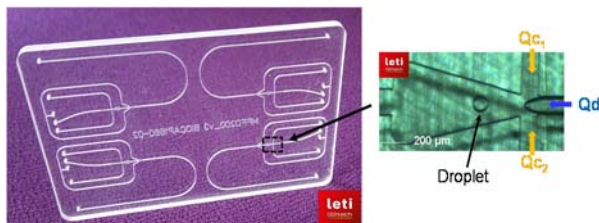

Figure1: Plastic cartridge (size of credit card) comprising four fluidic circuitries. Right, zoom in on the MFFD during a droplet formation where the continuous phase (Qc) pinches the dispersed phase (Qd).

Characterizations

The droplet volume scales with the flow rate ratio as a power law (Fig. 2a) with a power $0<\alpha<0.5$. The power depends on the viscosity but does not seems to depend on the non-Newtonian property of the continuous phase. Two regimes appear. For V>8nl, there is a geometry-controlled breakup mechanism [3] whereas for V<8nl, there is more a shear-rate-controlled breakup mechanism.
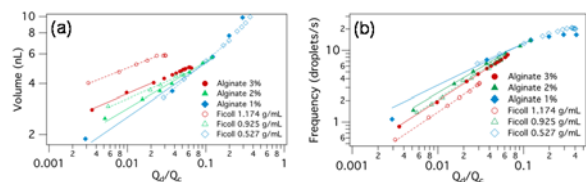

Figure 2: (a) Volume of the droplets versus the flowrate ratio for three viscosities for non-Newtonian (alginate) and Newtonian (Ficoll) fluids. (b) Frequency of the droplets production.

Fig. 2b shows that when the flow rate ratio increases, the droplet frequency increases until a critical flow rate ratio $Q_d/Q_c=0.2$ where droplet frequency becomes constant. This is in accordance with the two domains observed in Fig. 2a. When $Q_d/Q_c>0.2$, as droplet volume rapidly increases with Q_d/Q_c, frequency stops increasing to satisfy mass conservation during breakup. For $Q_d/Q_c<0.2$, droplet production frequency scales with the flow rate ratio as a power law, with a power $0.5<\beta<1$ depending on viscosity.

Conclusion

The droplet formation has been characterized for the first time for both newtonian and non-newtonian polymers with a viscosity up to 3.6 Pa.s, showing that shear-thinning behavior results in smaller droplet size and higher production frequency.

Perspectives

The defined scaling laws will be helpful for the future development of microfluidic droplet generation systems, in particular to optimize the production frequency.

RELATED PUBLICATIONS:

[1] S. L. Anna and H. C. Mayer, "Microscale tipstreaming in a microfluidic flow focusing device," Phys.Fluids, vol. 18, no. 12, 2006.
[2] Y. Tan, V. Cristini, and A. P. Lee, "Monodispersed microfluidic droplet generation by shear focusing microfluidic device," vol. 114, pp. 350–356, 2006.
[3] P. Garstecki, I. Gitlin, W. Diluzio, G. M. Whitesides, E. Kumacheva, and H. a. Stone, "Formation of monodisperse bubbles in a microfluidic flow-focusing device", Appl. Phys. Lett., vol. 85, no. 13, pp. 2649–2651, 2004.

REVISITING GAS SAMPLING AND ANALYSIS WITH MICROTECHNOLOGY FEASABILITY OF LOW COST HANDHELD GAS CHROMATOGRAPHS

RESEARCH TOPIC:
MEMS, Sensor, Gas analysis, Gas chromatography, Sampling

AUTHORS:
B.-A. Pham-Ho, F. Ricoul, T. Chappuis, A. Bellemin Comte, O. Constantin, J.-F. Bêche, B. Truong, B. Icard and B. Bourlon

ABSTRACT:
We report the feasibility of handheld systems that can both sample gas for further laboratory analysis as well as provide first field analysis by gas chromatography. In particular the systems include a micro silicon preconcentrator that enables concentration of Volatile Organic Compounds (VOCs) by a factor of 1000, as well as a micro silicon thermal conductivity detector with a limit of detection in the parts-per-million level. Regarding sampling, the system is compared to state of the art technique using sorbent tube and thermodesorption: comparable results are obtained on a 500 ppb BTEX (Benzene, Toluene, Ethylbenzene, Xylene) mixture. Regarding analysis, the separation and detection of VOCs down to 20 ppb is demonstrated, without the need of a carrier gas cylinder. Such systems, compatible with low cost development in the future, could find applications in air pollution monitoring as well as in security and health.

Context and Challenges

Gas chromatography (GC) is a first choice laboratory technique for complex gas mixture analysis. Following the development of microtechnologies and microelectronics, efforts have been made towards the development of lab-on-a-chip analysis systems. Research is motivated in particular by the increasing need for handled low cost, low power, highly selective systems with detection in the ppb range.

Main Results

The first system "PrimoSamp", allows to both sample gas into a removable cartridge and inject it in laboratory GC instruments.
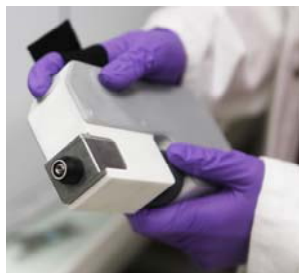

Figure 1: PrimoSamp Sampling/Injection system

It uses mainly a Leti micro-preconcentrator (µPC) chip [1], a 12V battery and a commercial miniaturized gas pump. The preconcentrator chip is packaged into the removable cartridge with a gas inlet and outlet and four electrical connectors for the chip thermal management (Joule heating). After sampling, the cartridge is removed from PrimoSamp and placed in a laboratory GC-MS system between the carrier gas inlet and the chromatography, while remaining electrically connected to PrimoSamp (dedicated cable). The low thermal capacity µPC chip is heated to 210°C during 15s in order to desorb and inject the sampled gas into the laboratory gas chromatography column. Tests in figure 2 show the results obtained in comparison to sampling/thermodesorption laboratory instruments.
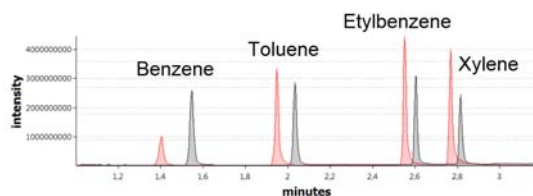

Figure 2: Comparison of sampling and injection of 500 ppb of BTEX with PrimoSamp (red) and the laboratory pump/thermodesorber instrument (gray)

The second system "PrimoSens" allows as well to do a direct field handheld GC analysis [2]. In addition to a Leti µPC, it uses mainly a GC column, a Leti micro thermal detector (µTCD), a 12V battery and a commercial miniaturized gas pump. After sampling into the µPC, the sampled gas is thermally desorbed and pumped through the column and µTCD, using air as carrier gas. Figure 3 illustrates results obtained on BTEX mixture down to 20 ppb, after baseline correction.
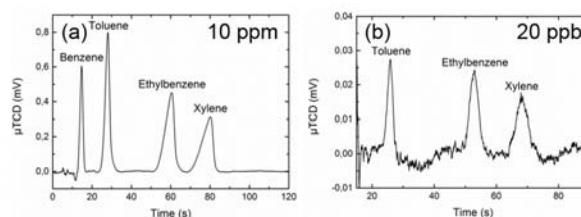

Figure 3: BTEX mixture analysis with PrimoSens. air pumped through the system is used as carrier gas. Chromatograms obtained (a) for 10 ppm concentration, 30s sampling; (b) for 20 ppb concentration, 10 minutes sampling

Perspectives

Applications range from air quality monitoring to chemical hazards detection, breath analysis and process monitoring.

RELATED PUBLICATIONS:
[1] B. Bourlon et al. "Silicon based micro-preconcentrators for portable gas analysis systems" (2014), MicroTAS 2014, pp. 2381-2383.
[2] B. Bourlon, B.-A. Pham-Ho, F. Ricoul, T. Chappuis, A. Bellemin Comte, O. Constantin, B. Icard, "Revisiting gas sampling and analysis with microtechnology: Feasability of low cost handheld gas chromatographs" (2017) Proceedings of IEEE Sensors, 7808672.

ALLOWING FOR TECHNOLOGICAL VARIABILITY IN BIOMARKER QUANTIFICATION

RESEARCH TOPIC:

Mass spectrometry, Bayesian estimation, Spectrum deconvolution, Technological variability, Biostatistics

AUTHORS:

C. Mercier[1], A. Klich[1], C. Truntzer[2], V. Picaud[3], J.F. Giovannelli[4], P. Ducoroy[2], P. Grangeat, D. Maucort-Boulch[1], P. Roy[1]

ABSTRACT:

Mass-spectrometry technologies are widely used for biomarker discovery and biomarker validation. However, technological variability on the analytical chain should be taken into account to improve protein quantification. Signal processing algorithms have been developed on the BHI-PRO project for the MALDI-TOF analytical chain applied to biomarker discovery and for the SRM (Selective reaction Monitoring) analytical chain applied to biomarker validation. To assess the performances of those algorithms, a biostatistical analytical protocol has been studied to quantify the biological and technological components of the variance for each analytical chain.

SCIENTIFIC COLLABORATIONS: [1] Service de Biostat.-Bioinfo. (HCL, Univ. Lyon 1, UMR 5558, Lyon), [2] CLIPP (Pôle de Recherche Université de Bourgogne, Dijon), [3] CEA List (Gif-sur-Yvette), [4] IMS (Univ. Bordeaux, UMR 5218, Talence)

Context and Challenges

Controlling technological variability is essential for biomarker discovery and validation [1]. A specific biostatistical methodology should be used to assess this control. Signal processing algorithms have been developed on the BHI-PRO project for the MALDI-TOF analytical chain applied to biomarker discovery [2] and for the SRM analytical chain applied to biomarker validation [3] in order to reduce the technological variability.

The main sources of technological variability in a MALDI-TOF chain are:
- Equalisation (ProteoMiner) which limits the relative abundance of proteins
- Purification (C8 magnetic beads) which selects proteins according to their chemical properties
- Spotting which lays out the proteins on the MALDI support
- Signal processing to estimate the list of peaks and their intensities.

The classical signal processing approach is based on denoising and baseline removal before the final peak picking procedure. The BHI-PRO algorithm for MALDI-TOF [2] is based on deconvolution using an additive model combining a smooth baseline part and a sparse peak list convolved with a, a priori known, peak shape under a Gaussian noise hypothesis.

To assess the performances of those algorithms, a biostatistical analytical protocol including an experimental design and a statistical plan of analysis has been studied by SBB to quantify the biological and technological components of the variance both for MALDI-TOF and SRM analytical chain [4,5].

Main Results

For MALDI-TOF, the experimental design presented in [4,5] generated the biological variance by controlling the relative abundance of proteins via dilution (5, 10, 20, 40, 80, 120 and 160 µl) and allowed for the estimation of the components of the technological variance via replicates at each step of the protocol. The statistical plan of analysis included modelling of the relationships between protein quantities and signal intensities and variance decomposition. We selected specific nested models to estimate the specific contribution of biological versus technological factors of the total variance.

The performance of the classical and BHI-PRO signal processing algorithms for MALDI-TOF were compared on the part of the variance explained by biological factor, the part of the variance explained by technological factors, and the modelling error. Statistical analysis was achieved considering one linear regression per peak.

There was a relationship between the peak intensity and the relative protein concentration in 4 peaks out of 9 (see peaks 1348.642, 1620.86, 3149.574, 5734.52 on Fig. 1). In these four peaks, the parts of the variance attributable to the technical process were smaller with BHI-PRO algorithm than with the classical algorithm: the technological variance was divided by 2 to 3 (according to the peak) and the model error was divided by 2.
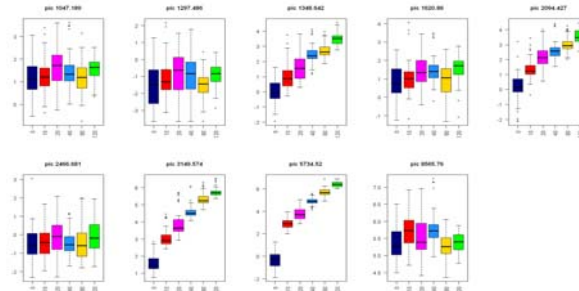

Fig. 1: Peak intensity according to the relative protein concentration

Perspectives

An original experimental design and a model-based variance decomposition method are now available to evaluate technological variability of measurement with MALDI-TOF technology or with SRM technology. This global approach can be applied to other technologies and used to compare biomarker quantification algorithms.

RELATED PUBLICATIONS:

[1] P. Grangeat et al. (2013), " Convergence entre l'analyse biostatistique et les méthodes d'inversion hiérarchique bayésienne pour la recherche et la validation de biomarqueurs par spectrométrie de masse ", XXIVème Colloque GRETSI, 3-6 septembre 2013, Brest, France.
[2] V. Picaud et al. (2016), Spectrum simultaneous deconvolution and baseline removal, BHIPRO report.
[3] P. Szacherski et al. (2014), "Classification of proteomic MS data as Bayesian solution of an inverse problem", Access, IEEE, Vol. 2, 1248 – 1262.
[4] C. Mercier et al. (2016), "Prise en compte de la technologie dans la quantification des biomarqueurs", EPICLIN, 25 – 27 mai 2016, Strasbourg, France.
[5] C. Mercier et al. (2016), "Experimental design and statistical analysis to assess biomarker quantification ", ISCB, August 21st- 25th 2016, Birmingham, UK.

4.

WEARABLE & IMPLANTABLE DEVICE

- **EEG analysis**

- **Closed loop therapy**

- **Organic electronic**

- **Sub-retinal prosthesis**

- **Impedance tomography**

- **BCI signal analysis**

- **NIR implant**

EFFICIENT MENTAL WORKLOAD ESTIMATION USING TASK-INDEPENDENT EEG FEATURES

RESEARCH TOPIC:
EEG, Mental Workload, Brain-Computer Interface

AUTHORS:
R.N. Roy, S. Charbonnier[1], A. Campagne[2] and S. Bonnet

ABSTRACT:
Mental workload is frequently estimated by EEG-based mental state monitoring systems. This study compared the performance of spectral markers and event-related potentials (ERPs) for mental workload assessment, and evaluated their stability in time. This study demonstrates that an efficient and stable in time workload estimation can be achieved using task-independent spatially filtered ERPs elicited in a minimally intrusive manner.

SCIENTIFIC COLLABORATIONS: [1]GIPSA-LAB, [2]LPNC (Université Grenoble Alpes)

Context and Challenges

Mental state monitoring (MSM) through physiological computing, or **neuroergonomics**, is an actively growing research field, for it possesses numerous human factor applications, ranging from safety to smart technology development. Those systems make use of an operator's neural activity, measured with EEG (Electro Encephalo Graphy) device, in order to implicitly enhance human–machine interaction. Several mental states are currently the focus for research, including mental fatigue, mental workload, attention, and affective states. Challenge comes from the real-time estimation of mental workload from its neural correlates to the processing chains.

Main Results

This study proposes a comparison of two processing chains, one based on the power in 5 frequency bands, and one based on event-related potentials (ERPs), both including a spatial filtering step, an FLDA classification and a 10-fold cross-validation.

To get closer to a real life implementation, spectral markers were extracted from a short window (i.e. towards reactive systems) that did not include any motor activity and the analyzed ERPs were elicited by a task-independent probe that required a reflex-like answer.
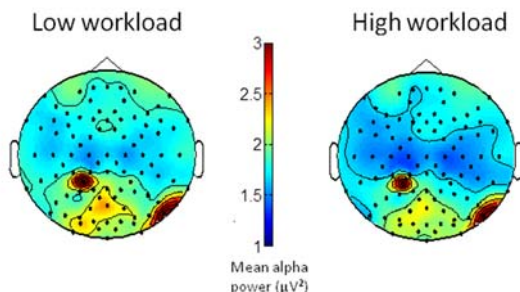

Figure 1: Topographical representation of the mean alpha power on the 800 ms window depending on

workload condition (average across participants)

The data were acquired from 20 participants who performed a Sternberg memory task for 90 min (i.e. 2/6 digits to memorize) inside which a simple detection task was inserted. The results were compared both when the testing was performed at the beginning and end of the session.

Both chains performed significantly better than random; however the one based on the spectral markers had a low performance (60%) and was not stable in time. Conversely, the ERP-based chain gave very high results (91%) and was stable in time.
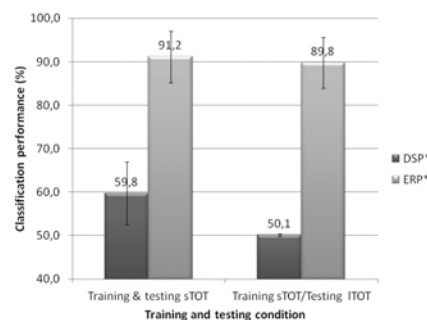

Figure 2: Classification results depending on the training and testing condition.

Perspectives

It stems from this study that ERPs appear to be more efficient for mental workload estimation in a close to real life implementation than spectral markers, given that they provide better classification accuracies and are stable in time both at the marker level and at the estimation level. However, the use of these markers requires an external stimulation that can be disturbing for the participants. In order to render the use of the ERPs less intrusive, a way would be to use *infrequent* task-independent probes that are *ignored* by the participants, i.e. do not require any overt or covert response.

RELATED PUBLICATIONS:
[1] R.N. Roy, S. Charbonnier, A. Campagne, S. Bonnet, "Efficient mental workload estimation using task-independent EEG features", J. Neural Eng., 13 (2016) 026019 (10pp).
[2] R.N. Roy, S. Bonnet, S. Charbonnier, A. Campagne, "Enhancing single-trial mental workload estimation through xDAWN spatial filtering", Proc. IEEE Conf. Neur. Eng., 2015, pp 360–363.
[3] R.N. Roy, S. Bonnet, S. Charbonnier, P. Jallon, A. Campagne, "A comparison of ERP spatial filtering methods for optimal mental workload estimation", Proc. IEEE Conf. Eng. Med. Biol. Soc., 2015 7254–7257.

PERSONALIZATION OF A NONLINEAR GLUCOSE-INSULIN SYSTEM VIA A MARKOV CHAIN MONTE CARLO ALGORITHM FOR MODEL PREDICTIVE CONTROL PURPOSES

RESEARCH TOPIC:

Artificial Pancreas, Type 1 diabetes, Insulin-glycemic model

AUTHORS:

S. Lachal, C. Franco, M. Doron, E. Huneker[1], S. Franc [2,3], Charpentier[3], P. Jallon

ABSTRACT:

The Artificial Pancreas objective is to optimize the rate of insulin delivery for Type 1 diabetic 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 is based on a Model-Predictive-Control (MPC).

SCIENTIFIC COLLABORATIONS: [1]Diabeloop SAS, [2]CHSF (Centre Hospitalier SUd-Francilien), [3]CERITD

Context and Challenges

The Type 1 diabetes treatment is based on daily injections of insulin. The state-of-the-art treatment is based on functional insulinotherapy based on carbohydrate counting, insulin boluses associated with meals and estimation of the daily insulin basal rates for physiological needs unrelated to meals.

Despite large improvements in the glycemic control, there are still numerous issues due to hypoglycemia (short-term effects) and hyperglycemia (long-term comorbidities). Half of the French subjects glycemic equilibrium leads them to comorbidities. The improvement of the temporal pattern of insulin delivery should improve the medical conditions of the bio-regulated diabetic people.

The artificial pancreas Diabeloop [1] is based on Model Predictive Control (MPC). MPC is a widespread control design approach particularly suitable for long time delay systems such as glucose-insulin ones. Such control design techniques strongly rely on a model that needs to be accurate enough in order to predict patient glycaemia several hours later. To address the issue of model identification, a Bayesian approach has been developed and is compared to a conventional global nonlinear optimization approach

Main Results

The model used is the one developed by Hovorka et al. (2002) which is a high order, complex nonlinear model containing 16 parameters, from which only 7 are estimated. The algorithm has been evaluated on 25 real patients, on a 8-hour period containing at least one meal and one bolus. The identification method used is a custom MCMC (Markov Chain Monte Carlo) based algorithm called Metropolis within Gibbs that aims at finding the Expectation A Posteriori (EAP) estimator on model parameters. These choices were made to ensure convergence. Mean Square Error (MSE) was used as a performance index for comparison with the benchmark nonlinear optimization method [2, 3].

Better reconstructions of real data were obtained using the Bayesian approach (MSE = 0.59) instead of the conventional one (MSE = 0.89), leading to a MSE reduction of 33% on average (p<0.01).

Fig. 1 shows an example of the comparison between the Bayesian method and the conventional approach.

These results encourage the use of Bayesian methods to go further into model personalization. Furthermore, the obtained parameters distributions could also be used as prior information for subsequent model updates.
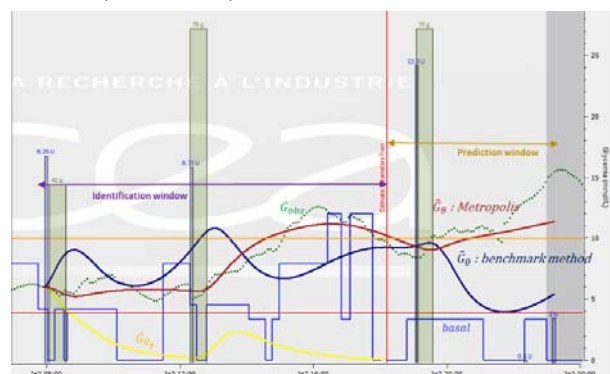

Figure 1: Estimated glycaemia curves obtained with Bayesian approach and benchmark algorithm - patient # 18

Perspectives

The development of the Diabeloop artificial pancreas progresses at a steady pace. A set of clinical trials were conducted in 2016 with 9 hospitals and 40 subjects. Three arms represented respectively a sedentary behavior, ingestion of heavy meal and practising physical. They show good performances. Next clinical trials will focus on outpatient use of the artificial pancreas.

RELATED PUBLICATIONS:

[1] M.A. Quemerais, M. Doron, et al., "Preliminary evaluation of a new semi-closed loop insulin therapy system over the prandial period in adult patients with type 1 diabetes: the WP6.0 Diabeloop study", Journal of Diabetes Science and Technology, 2014.
[2] E. Huneker et al., "Comparaison in silico de modèles physiologiques et de leur implémentation pour un pancréas artificiel pour le traitement du diabète de type 1 via la création d'un simulateur", Congrès 2016 de la Société Francophone du Diabète, 22/03/2016 - 25/03/2016, Lyon, France.
[3] S. Lachal, C. Franco, M. Doron, E. Huneker, S. Franc, G. Charpentier, P. Jallon, "Personalization of a Nonlinear Glucose-insulin system via a Markov Chain Monte Carlo algorithm for Model Predictive Control purposes", Diabetes Technology Meeting, 10/11/2016 - 12/11/2016, Bethesda, Maryland , Etats-unis.

REFERENCE-LESS PH SENSOR USING ORGANIC ELECTROCHEMICAL TRANSISTORS

RESEARCH TOPIC:

Organic Electrochemical Transistor, PEDOT, pH sensor, Sweat

AUTHORS:

G. Scheiblin, R. Coppard[1], R.M. Owens[2], P. Mailley, G.G. Malliaras[2]

ABSTRACT:

Organic electrochemical transistors were integrated in a differential bridge (Wheatstone bridge) using screen printing process. The gate electrode of each transistor was further functionalized with electrodeposited Iridium oxide, used here as pH sensitive layer owing to its redox properties. The output potential of the Wheatstone bridge was directly proportional to the difference of transconductance of each transistor channel, the latter being modulated by its gate potential that is fixed by the pH of the solution in contact with the grid. This circuit allows then pH sensing without the use of a reference electrode. This reference-less device was assayed through the pH determination of a real biological sample (sweat).

SCIENTIFIC COLLABORATIONS: [1]CEA-Liten, [2]Bioelectronics Lab. (ENSMSE in Gardanne)

Context and Challenges

pH is an extremely important parameter in all biological and chemical reactions. So, pH sensing has been specifically shown to be of paramount importance in many fields such as healthcare, environmental science, or food industry as the acidosis or alkalinity of a solution is a marker of a physiological or physicochemical state.

Commercially available electrochemical pH sensors rely on the potentiometric measurement of an active working electrode compared to a reference electrode. Upon variation of pH, the potential of the working electrode change whereas the potential of the reference is expected to remain stable. However, reference electrodes are never so stable thus requiring pH sensors to be punctually calibrated. Thereby, continuous monitoring appears challenging due to this need in regular calibration steps.
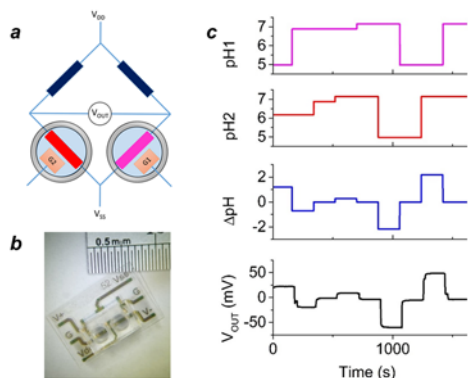

Figure 1: a. Schematic view and wiring of the differential bridge. b. Photograph of the printed device and *c*. potential response (VOUT) in black of the alimented bridge (VSS = -1 V, VDD = 0 V) upon pH solutions added in the right (pink) and left (red) compartments, and the resulting variation (blue).

Main Results

Organic electrochemical transistors (OECT) represent a new class of electrochemical transducers in which the channel is made of a conducting polymer, PEDOT-PSS and the grid dielectric is an electrolyte. By carefully choosing the grid material to be sensitive to a physicochemical parameter of interest, one can modulate the channel transconductance through grid interfacial potential change. To fabricate (bio)electrochemical sensors, the grid metal is then modified by a sensitive layer. Each change in the grid potential arising from a physicochemical Interaction or reaction in the sensitive layer will thus generate change in the channel transconductance leading to efficient transduction. In such a way we designed lactate biosensors for which the OECT grid is modified by the lactate oxidase enzyme [1]. Interestingly, conversely to classical electrochemical sensors, OECT can be associated in more complex circuits such as Wheatstone bridge or logic gates. In a recent work [2], we associated two pH sensitive OECT (using pH sensitive Iridium Oxide grid) in a Wheatstone bridge. Each OECT is then soaked in a different pH solution as exemplified in the figure **a** and **b**. Figure **c** shows the pH evolution imposed in each OECT compartment, pH1 in pink and pH2 in red, and the resulting difference of pH, ΔpH in blue, between the 2 compartments. In such a configuration, the measured V_{out} potential of the bridge is directly dependent on the difference of pH within the two compartment as demonstrated by the V_{out} measurement trace in figure **c** (blackline). This work shown for the first time a reference-less detection of pH in which the measured pH is referred to a reference solution instead of using unstable reference electrode in classical potentiometric measurements. This approach was further successfully applied to the pH determination of a real biological sample, sweat.

Perspectives

Further works are currently on the way to associate these OECT in different logic gate configurations (NOR, NAND…). The idea here is to develop a complex biosensor that generate a threshold response to the concomitant presence of few metabolites of interest. Such works open the way to the design of fully integrated diagnostic systems for in field applications.

RELATED PUBLICATIONS:

[1] "Screen-printed organic electrochemical transistors for metabolite sensing" G. Scheiblin, A. Aliane, X. Strakosas, V.F. Curto, R. Coppard, G. Marchand, R.M. Owens, P. Mailley, and G.G. Malliaras, MRS Comm. 5 (2015) 507.
[2] "Reference-less pH sensor using organic electrochemical transistors" G. Scheiblin, R. Coppard, R.M. Owens, P. Mailley, and G.G. Malliaras, Advanced Material Technologies, (2016), DOI : 10.1002/admt.201600141.

PROBING THE FUNCTIONAL IMPACT OF SUB-RETINAL PROSTHESIS

RESEARCH TOPIC:
Medical devices, Electrical stimulation, Visual implant, Bionic eye

AUTHORS:
S. Roux[1], F. Matonti[3], F. Dupont, L. Hoffart[3], S. Takerkart[1], S. Picaud[2], P. Pham & F. Chavane[1]

ABSTRACT:
By comparing, at the rodent, the activity of the visual cortex generated artificially by electrical stimulation to that produced by light, researchers of the CNRS, CEA and Inserm were able to improve the precision of the prosthetic activation. This multidisciplinary work, published on August 23rd, 2016 in the eLife Journal [1], opens the way towards high performing retinal prostheses, with the aim of improving the quality of life of the implanted patients.

SCIENTIFIC COLLABORATIONS: Institut des Neurosciences de la Timone (INT, [1]CNRS - INSERM - [3]AP-HM - Aix-Marseille University), [2]Institut de la Vision (UPMC)

Context and Challenges

Retinitis Pigmentosa (RP) is a slow, progressive genetic disease that affects the retina's ability to respond to light. RP inexorably leads to blindness. Currently, no RP treatment exists and retinal prostheses, based on electrical stimulation of surviving retinal neurons, remain the unique alternative. These 'bionic eyes' restore visual perception in tens of patients worldwide but still offer very poor gains in visual acuity which does not allow autonomous locomotion. Theoretically, the retinal implant spatial resolution results from the number of microelectrodes and their size. In practice, the increase of the number of microelectrodes does not allow to obtain a finer vision in the implanted patients. Improving spatial focality of retinal implants is thus a key therapeutic challenge.

Main Results

Conjugating their skills in ophthalmology and in physiology of the visual system, INT scientists compared the answer of the visual system of a rodent to natural visual stimuli and to stimuli produced by the sub-retinal prosthesis developed by Leti (Fig. 1). They established a precise primary visual cortex (V1) mapping as a functional benchmark to demonstrate that sub-retinal implants activate V1 at the appropriate position, scalable to a wide range of visual luminance, but with an aspect-ratio and an extent much larger than expected (Fig. 2). Such distorted activation profile can be accounted for by the existence of passive electrical diffusion (identified in a previous work thanks to numerical simulation and impedance spectroscopy [2]) and undesired activation of ganglion cells' axons en passant. Reverse engineered electrical pulses based on impedance spectroscopy (patented by Leti) is the only tested solution that decreases the extent and aspect-ratio (Fig. 2), providing a promising solution for clinical applications.
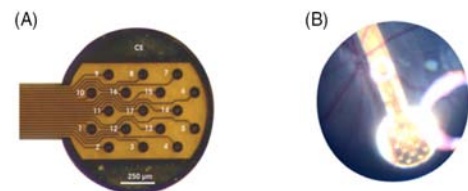

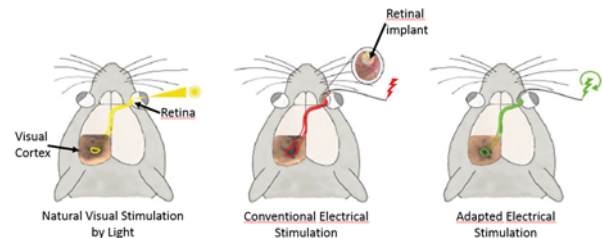Fig. 1: Flexible sub-retinal prosthesis (Leti) (A), eye fundus with an implanted sub-retinal prosthesis (B).

Fig. 2: INT rat primary visual cortex (V1) experimentation: the activation (the colored circles on the visual cortex) of the visual system by sub-retinal electrical stimulation (middle, red, the insert shows the eye fundus image where the retinal implant is visible) is bigger than the activation obtained with natural/light stimulation (left, in yellow). With Adapted Electrical Stimulation (right, green), the size and the form of the activation is smaller than the conventional stimulation (middle, red) and closer to the natural visual activation (left, yellow).

Perspectives

This pre-clinical long-term parametric study shows that prosthetic activation focality can be increased using a more intelligent electrical stimulation strategy. It opens the way of promising improvements of the retinal prostheses for humans.

RELATED PUBLICATIONS:
[1] S. Roux, F. Matonti, F. Dupont, L. Hoffart, S. Takerkart, Picaud S., P. Pham, F. Chavane, "Probing the functional impact of sub-retinal prosthesis", F. eLIFE, 23 août 2016.DOI: http://dx.doi.org/10.7554/eLife.12687.
[2] P. Pham, S. Roux, F. Matonti, F. Dupont, V. Agache, F. Chavane, "Post-implantation impedance spectroscopy of subretinal micro-electrode arrays, OCT imaging and numerical simulation: towards a more precise neuroprosthesis monitoring tool", J Neural Eng 10, 2013. doi: 10.1088/1741-2560/10/4/046002.

MULTISPECTRAL ELECTRICAL IMPEDANCE TOMOGRAPHY USING OPTIMIZATION OVER MANIFOLDS

RESEARCH TOPIC:
Electrical impedance tomography, Medical imaging

AUTHORS:
S. Bonnet, A. Fouchard, [1]O. David

ABSTRACT:
Electrical impedance tomography (EIT) under spectral constraints uses a material basis decomposition to combine the different information embedded in the tissue spectra. In this work, a computational framework is presented to deal with the extra frequency dimensions and the constraints during reconstruction. A fraction volume approach is demonstrated on synthetic data with explicit Euclidean gradient, usage of a finite volume element solver and minimization over the oblique manifold.

SCIENTIFIC COLLABORATIONS: [1]Université Grenoble Alpes (Grenoble Institute of Neuroscience)

Context and Challenges

Medical electrical impedance tomography (EIT) is a soft-field, non-invasive imaging technique. The setting of EIT consists in reconstructing internal electrical property distributions from current and voltage boundary measurements at specific frequencies. The ill-posed EIT inverse problem limits the applications of static imaging, i.e. the deduction of quantitative conductivity maps. This work describes an alternative by using a spectrally constrained approach. It could open application areas in acute stroke, brain injury and cancer screening.

Main Results

scEIT makes use of a material basis decomposition: the conductivity of a control volume is a linear combination of known tissue conductivities. The reconstruction then focuses on the proportion values of individual tissues within the imaging domain

In order to optimize the scEIT imaging method, we propose here a numerical framework based on the joint use of a finite volume element (FVE) forward solver and an optimization on a manifold for the quantitative parameter estimation. FVE brings a model reduction capability compared to finite element methods (FEM) without loss of information [1-3].

Further, the minimization of the objective function is performed over the oblique manifold to efficiently handle the constraints over proportions.
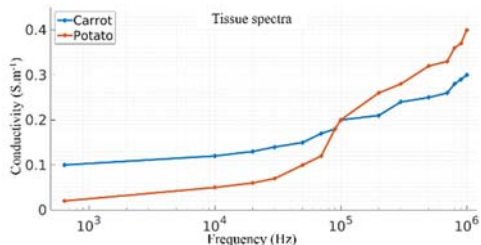

Figure 1: Conductivity spectra assumed known in the reconstruction - background: saline mixed with hashed carrots in blue, inclusion: potato in blue.

This approach standardizes the integration of spectral constraints. It offers a way to reduce the computational complexity of multi-frequency imaging. The ill-posed inverse problem is handled by a Markov random field (MRF), to constrain neighboring control volumes to have close proportion values.

As expected, compared with a mono-frequency implementation, the quality of the multispectral reconstruction was enhanced. The minimization under equality constraints allowed ensuring valid proportions of materials at each control volume, as can be seen in Fig. 2.

The proposed scEIT framework also enabled to enhance the efficiency of the inversion process, through the use of a FVE forward solver. With the 14 electrode phantom and the 15 frequencies used in this work, the size of the multi-frequency Jacobian that was computed at each iteration (20 iterations on average) was halved, translating into effective computation and assembly time savings.
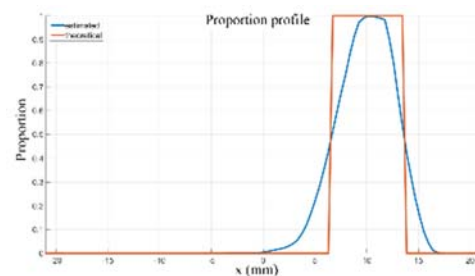

Figure 2: Proportion profiles deduced by multispectral inversion (scEIT).

Perspectives

Further studies will focus on applying these numerical developments to in vitro and in vivo data, with a straightforward extension to 3D and numerous tissues. They will also consider the robustness to the noise in the data and to the variations of the contact impedance at the medium-electrode junction.
The proposed framework can be easily extended to other modality like in DOT imaging, or X-ray imaging.

RELATED PUBLICATIONS:
[1] A. Fouchard, S. Bonnet and O. David, "Multispectral Electrical Impedance Tomography using Optimization over Manifolds", 6th International Workshop on New Computational Methods for Inverse Problems. Journal of Physics: Conference Series 756 (2016).
[2] A. Fouchard, S. Bonnet, L. Hervé and O. David, "Méthodes numériques pour le problème direct et l'analyse de sensibilité en tomographie d'impédance électrique ", GRETSI 2015 (Lyon, France).
[3] A Fouchard, S. Bonnet, L. Hervé and O. David, "A current-density conservative nodal framework for EIT", International Conference on Biomedical Applications of Electrical Impedance Tomography, Neuchatel, Suisse, 2015.

ROBUST AND ACCURATE NEURAL SIGNAL DECODING WITH MINIMAL LATENCY FOR UPPER LIMB TRAJECTORIES RECONSTRUCTION: TOWARD A BRAIN COMPUTER INTERFACE CLINICAL TRIAL

RESEARCH TOPIC:

Brain Computer Interface, Neural decoding, Machine learning, Real-time, Robustness, Smoothness, Minimal latency

AUTHORS:

A. Yelisyeyev, M. C. Schaeffer, G. Charvet, C. Mestais, T. Aksenova

ABSTRACT:

CLINATEC® Brain Computer Interface (BCI) project will 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. Real time neural signal decoding robustness and accuracy are challenges for BCI clinical applications. A new penalization approach was proposed to improve neural decoding smoothness of the predicted limb trajectory without supplementary latency. Proposed algorithms combine data tensor representation with the predicted trajectories smoothness control.

Context and Challenges

The goal of CLINATEC® Brain Computer Interface (BCI) project is to provide the proof of concept that it is possible to control complex effectors, such as a 4-limb exoskeleton, thanks to brain activity monitoring and decoding. This will open new opportunities to motor disabled subjects. The wireless 64-channel ElectroCorticoGram (ECoG) recording implant for chronic use WIMAGINE® [1] and an innovative signal processing, should allow subjects controlling a 4-limb exoskeleton EMY (Enhancing MobilitY) [2] (Fig.1).
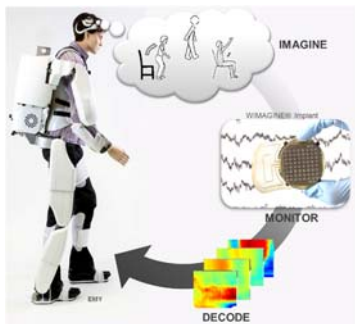

Figure 1. BCI project at CLINATEC.

Real-time neural signal decoding robustness and accuracy for effector control with a large number of degrees of freedom are challenges for BCI clinical applications. The smoothness of the predicted trajectory is an important property of motor-related BCI systems. In this context, improvement of the prediction smoothness for upper limb trajectories reconstruction from ECoG data is one of the goals of the BCI team research. At the same time, minimal system latency is an essential requirement for comfortable use of real-time BCI systems.

Main Results

One approach to improve smoothness consists in smoothing post-processing of the predicted trajectories. However, post-processing increases the system latency. Another solution consists in identifying the model for which smoothness is an intrinsic property. A Kalman Filter (KF) is generally used in BCI systems to generate smooth trajectories. However, this method is not adjusted for high-dimensional data. Moreover, the KF brings a latency to decoding. We propose a new penalization approach to improve neural decoding smoothness, in particular the Polynomial Penalized Multi-Way PLS (PNPLS) [3].
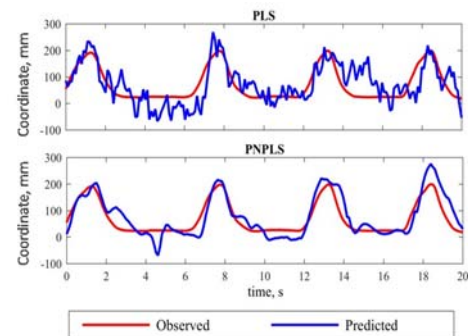

Figure 2. Reconstruction of upper limb continuous trajectory from ECoG data of non-human primate using traditional (PLS) and invented (PNPLS) methods.

New methods combine data tensor representation with the predicted trajectories smoothness level control. No supplementary latency is introduced. The decoders were evaluated and compared in preclinical studies (Fig.2).

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 will allow testing and refining BCI paradigms and decoding algorithms to evaluate the 4-limb exoskeleton ECoG based BCI control. In particular, hybrid discrete/continuous decoding algorithms [4] 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 upcoming clinical trial.

RELATED PUBLICATIONS:

[1] C. Mestais, G. Charvet, F. Sauter-Starace, M. Foerster, D. Ratel, and AL. Benabid, (2015). WIMAGINE: Wireless 64-Channel ECoG Recording Implant for Long Term Clinical Applications, EEE Transactions on Neural Systems and Rehabilitation Engineering, 23(1), 10-21.
[2] Y. Perrot, A. Verney, B. Morinière, and P. Garrec, (2013) EMY: Full-body Exoskeleton, in ACM SIGGRAPH Emerging Technologies, Anaheim, USA.
[3] A. Eliseyev & T. Aksenova, (2016). Penalized Multi-Way Partial Least Squares for Smooth Trajectory Decoding from Electrocorticographic (ECoG) Recording. PloS one, 11(5), e0154878.
[4] MC. Schaeffer, T. Aksenova (2016) Hybrid Trajectory Decoding from ECoG Signals for Asynchronous BCIs. ICANN 2016. LNCS, 9886, 288-296. Springer.

CHARACTERIZATION OF INTRACEREBRAL NEAR INFRARED ILLUMINATION NEUROPROTECTIVE EFFECTS IN PARKINSON'S DISEASE MODELS

RESEARCH TOPIC:

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

AUTHORS:

AL. Benabid, C. Moro, C. Chabrol, J. Molet, D. Agay, F. Darlot, V. Auboiroux, N. Torres, F. Reinhart, C. Cretallaz, T. Costecalde, [1]J. Mitrofanis, [1]N. El Massri, [1]D. Johnstone, [1]J. Stone

ABSTRACT:

There is no curative therapeutic strategy for Parkinson disease (PD) nowadays, but only symptomatic ones. On preclinical model of PD, we already demonstrated a neuroprotective effect of intracerebral near infra-red illumination, In 2016, we evaluated various illumination modalities (wavelength, dose…) and their contribution on neuroprotective therapeutic potential on rodent and non-human primates parkinsonian model. These results are supporting a new therapeutic strategy for patients. Our preclinical results contribute to clinical trial set up aiming to evaluate NIR therapeutic potential.

SCIENTIFIC COLLABORATIONS: [1]University of Sydney, Australia

Context and Challenges

Parkinson's disease (PD) is a progressive disorder with distinct cardinal signs of resting tremor, lead-pipe rigidity, akinesia, bradykinesia, or all of these. The current treatment options of drug therapy and surgery treat the motor signs of the disease very well, at least initially, but they do not stop the progression of the disease. The dopaminergic cells in the substantia nigra pars compacta (SNc) continue to die throughout the treatment period. In essence, these treatments do not offer neuroprotection, it means that they do not slow or stop pathology's progression.

We have reported previously that intracranial application of near-infrared light (NIR) reduces clinical signs and offers neuroprotection in subacute MPTP animal model of Parkinson's disease [1]. Here we continue preclinical experiments to increase evidences of NIR neuroprotective therapeutic effect, and support a clinical trial.

Main Results

To evaluate the universality of NIR neuroprotective potential, we used a newly-developed intracranial optical fiber device to deliver NIR light to the midbrain of 6-hydroxydopamine lesioned rats, another well-known model of Parkinson's disease. We showed that there was no evidence of tissue toxicity by NIR in the midbrain [2]. Regardless of mode of delivery or total dose, NIR reduced apomorphine-induced rotations at the stronger, but not at the weaker power. Neuroprotection, as assessed by tyrosine hydroxylase expression in midbrain dopaminergic cells, could account for some, but not all, of the observed behavioral improvements. There may have been other "symptomatic" elements contributing to behavioral improvements in these rats.

Also, we demonstrated that NIR treatment influenced the cellular response to parkinsonian insult [3]. Our results (Figure1) showed that NIR reduced dramatically (~75 %) MPTP-induced astrogliosis in both SNc and striatum. Among microglia, however, NIR had a more limited impact in both nuclei. Although a reduction in overall cell size was observed, there were no changes in the number of microglia after NIR treatment.

Figure 1: NIR reduces astroglyosis (GFAP+ cells) in SNc and striatum

We also explored potential beneficial outcomes when 670nm and 810nm wavelengths were applied together, either concurrently (at the same time) or sequentially (one after the other) [4]. Our results showed that applied in a specific combination, there was a greater overall beneficial outcome (increased locomotor activity, and higher number of tyrosine hydroxylase immunoreactive cells in the SNc).

In comparison with previous studies using lower NIR dose, we explored whether a higher NIr dose (5 times more) generated beneficial effects [5] Our results showed that the higher NIR dose did not have any toxic impact on cells at the midbrain implant site. Furthermore, this NIR dose resulted in a higher number of nigral tyrosine hydroxylase immunoreactive cells when compared to the untreated group. However, the higher NIR dose animals showed little evidence for an increase in mean clinical score, number of nigral Nissl-stained cells and density of striatal tyrosine hydroxylase terminations, meaning that higher dose may not be as efficient as lower ones.

Perspectives

Within the limitations of preclinical models of PD, our results provide useful insights into the effectiveness of NIR therapy (regarding a broad therapeutic time and dose window), laying groundwork for future endeavours on humans, and support to upcoming clinical trials.

RELATED PUBLICATIONS:

[1] F. Darlot et al, "Near-infrared light is neuroprotective in a monkey model of Parkinson disease", Annals Neuro. 2016 vol 79 (1): 59-75;
[2] F. Reinhart et al, "Intracranial application of near-infrared light in a hemi-parkinsonian rat model: the impact on behavior and cell survival".. J Neurosurg. 2016 Jun;124(6):1829-41.
[3] N. El Massri et al, "Near-infrared light treatment reduces astrogliosis in MPTP-treated monkeys", Exp Brain Res. 2016 Nov;234(11):3225-3232.
[4] F. Reinhart et al, "The behavioural and neuroprotective outcomes when 670nm and 810nm near infrared light are applied together in MPTP-treated mice", Neurosci Res. 2016 No18.
[5]. C. Moro et al, "Effects of a higher dose of near-infrared light on clinical signs and neuroprotection in a monkey model of Parkinson's disease". Brain Res. 2016 Oct 1;1648(Pt A):19-26.
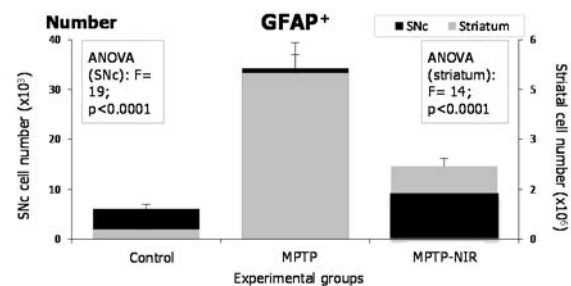
5.

MICRO & NANO-TECHNOLOGIES

- **Lipid nanoparticules**

- **SiOCH Thin Films**

- **Surface chemistry**

- **MEMS sensor**

NANOSTRUCTURED LIPID CARRIERS AS DELIVERY SYSTEMS OF CHEMICALLY GRAFTED PROTEIN ANTIGENS

RESEARCH TOPIC:
Vaccine, Protein antigen delivery, HIV, Nanostructured lipid carriers, Nanomedicine, Biotechnology, Immune responses

AUTHORS:
E. Bayon, J. Morlieras, T. Courant, A. Gonon[1], F.N. Marche[1], F.P. Navarro

ABSTRACT:
We have designed a novel vaccine formulation based on lipid nanoemulsions (Lipidots®) carrying protein antigens onto their surface. Ovalbumin, a model antigen, and P24, a protein from Human Immunodeficiency Virus capside, have been chemically modified to be efficiently grafted onto thiol or maleimide-functionalized lipid nanoparticles. The resulted nanoformulations are stable several months at 4°C with no protein release over this period. They are very well tolerated by dendritic cells *in vitro* and allow the enhancement of immune responses. The production of antigen-specific antibodies and activation of cellular immunity have been assessed on mice immunized with ovalbumin and P24-grafted nanoparticles.

SCIENTIFIC COLLABORATIONS: [1]Institut Albert Bonniot – INSERM U1209, Grenoble, France.

Context and Challenges
Despite the great progress in the field of vaccines during the last century, there are still some deadly diseases which are out of control. One of them is caused by Human Immunodeficiency Virus (HIV) which is responsible for more than 30 million deaths until now and keeps infecting new people. HIV is constituted of circulating virions which have the ability to infect cells and hide inside them. Furthermore HIV has the ability to irremediably mutate, which makes it extremely complicated to develop efficient defenses. One of the most serious options to address all these challenges is to develop a new generation of vaccines able to induce both humoral and cell-mediated immune responses of high quality. For this purpose, we have designed a novel biocompatible lipid-based nanocarrier [1] for the delivery of antigens and the induction of potent immune responses [2].

Main Results
Lipid nanoemulsions (Lipidots®) are made of a lipid core surrounded by a shell of phospholipids and pegylated surfactants. All ingredients are bioabsorbable, biocompatible, and FDA approved for human-use. Thiol or maleimide-functionalized PEGylated surfactants have been synthetized and incorporated in the formulation to allow the chemical grafting of proteins on the nanoparticle surface via thiol-maleimide chemistry. Two antigen proteins were successfully grafted: ovalbumin (OVA) as a model antigen and, the protein from HIV capside named P24 which has the huge advantage to be indifferent to the virus mutations.The mean diameters of grafted Lipidots® ranged from 80 nm to 90 nm with no significant changes and no release of protein upon 9 weeks of storage at 4°C. The cytotoxicity of naked and antigen-grafted Lipidots® was assessed by exposition to JAWSII murine dendritic cells. Independantly to the payload, lipid nanoparticles induce no cell death after a 24-hour exposure at different significant concentrations thus demonstrating that such engineering of nanoparticles does not affect their safety.
At last, the ability of the ovalbumin and P24-grafted nanoparticles to induce immune responses in combination with immunostimulants (MPLA or CpG) is evaluated in vivo by immunization of mice. For both ovalbumin and P24 the antibody results show that the antigen vectorized by the nanoparticle is much more immunogenic than in a soluble state (Figure 1). It is the fact to graft the antigen onto the nanoparticle which improves the immunogenicity, by protecting the antigen from environment and delivering it directly to the immune cells located in the lymph nodes. In addition to that, the vectorization of the P24 on the nanoparticle gives a chance to activate the cell-mediated immunity, as it was shown that 3 mice over 9 displayed a potent activation of their T lymphocytes in response to exposition to P24.
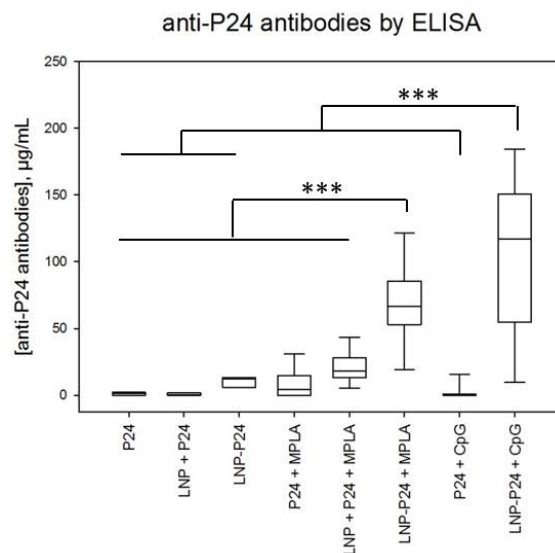

Figure 1: P24-specific antibody titers.

Perspectives
These results confirm that our lipid-based nanocarrier potentializes the induction of mixte immune responses. Current work is focused on the delivery of relevant antigens e.g. influenza antigens or HIV envelop proteins.

RELATED PUBLICATIONS:
[1] T. Delmas, H. Piraux, A.C. Couffin, I. Texier, F. Vinet, P. Poulin, M.E. Cates, J. Bibette, "How To Prepare and Stabilize Very Small Nanoemulsions", Langmuir 27, 1683–1692, 2011.
[2] E. Bayon, J. Morlieras, T. Courant, A. Gonon, P.N. Marche, F.P. Navarro, "Nanostructured lipid carriers as delivery systems of chemically grafted protein antigens", Advanced Materials - TechConnect Briefs 2016, 71-74, 2016.

PHASE II CLINICAL STUDY OF INTRA-OPERATIVE FLUORESCENCE IMAGING GUIDED SURGERY IN CANCER-BEARING DOGS WITH LIPIMAGE™ 815

RESEARCH TOPIC:

Nanomedicine, Lipid nanoparticles, (Lipidots®), Fluorescence Imaging

AUTHORS:

I. Texier, F. Navarro, R. Boisgard, Q. Cabon[1], D. Sayag[1], F. Ponce[1], C. Carozzo[1], D. Watrelot[1]

ABSTRACT:

LipImage™ 815, lipid nanoparticles loaded with infrared fluorescent dye, were used as contrast agent for intra-operative fluorescence imaging guided surgery for the excision of soft tissue carcinoma in dogs. First, the safety of the particles was demonstrated. Second a clinical phase II study was performed on 9 proprietary dogs. LipImage™ 815 appeared as a suitable contrast agent to allow for good discrimination between tumoral and healthy tissues and the fast screening of the infiltration of lymph nodes by metastatic cells.

SCIENTIFIC COLLABORATIONS: [1]VAS: VetAgroSup Campus vétérinaire de Lyon

Context and Challenges

The goal of tumor surgery is to achieve complete excision to prevent tumor recurrence while being minimally deleterious to preserve patient quality of life and promote healing. The assessment of intraoperative surgical margins can be difficult in case of infiltrative tumors. Fluorescence-guided surgery has emerged in the last 10 years as an innovative easy-to-implement technique to improve clean margin resection and help fast identification of sentinel lymph node where metastatic cells can be infiltrated [1]. However, only IndoCyanine Green fluorophore is presently approved for human use as near-infrared contrast agent. Development of contrast agents with improved affinity for tumor cells is therefore greatly needed to expand the potentialities of the technique. In this study, we demonstrated the potential of dye-loaded lipid nanoparticles, LipImage™ 815, for fluorescence guidance of soft tissue carcinoma excision during surgery in dogs.

Main Results

First, the safety of LipImage™ 815 was demonstrated in a biodistribution and toxicology study conducted in both rats and dogs [2]. The contrast agent was cleared in 48 h from the circulation and metabolized by the hepatobiliary pathway. No evidence of acute or delayed general, hepatic, renal or hematologic toxicity was observed, even after injection of 10-fold the injected dose for imaging.

In a second step, 9 proprietary dogs presenting spontaneous soft-tissue sarcoma or subcutaneous tumors located on limbs, maxilliary cheek, or mandibular angle, were enrolled in a verterinary clinical study [3]. All the dogs received an intravenous injection of dye-loaded lipid nanoparticles, LipImage™ 815, at a dose of 2.0 µg/kg, 24 hours before surgery. Wide (6 dogs) or radical (limb amputation, 3 dogs) resection was realized after Computed Tomography examination. Real-time intra-operative fluorescence imaging was performed before and after skin incision, and after tumor excision. In cases of radical resection necessitated by the wide spreading of the tumor, the lymph nodes were imaged.

Important fluorescence signal was observed in all tumors in comparison to healthy tissues (Tumor/Healthy fluorescence ratio >2.0). The margins were clean in 5 of 6 dogs after wide surgical resection, and the margin/healthy tissues fluorescence ratio was close to 1.0 in all of these dogs (Fig. 1). Infiltrated margins were observed in case 1, with a margin/healthy tissue fluorescence ratio of 3.0.
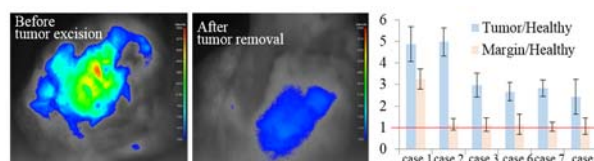

Fig.1. Example of images acquired before and after tumor resection (fluorescence superimposed to visible image). Quantification of the Tumor/Healthy tissue and of the Margin (after resection)/Healthy tissue fluorescence.

In cases of radical resection, metastasis was confirmed in 2 of 3 analyzed lymph nodes, associated with a Lymph Node/Healthy tissues fluorescence ratio of 2.1 and 4.2, while non-metastatic lymph node was associated with a ratio of 1.0 (case 9) (Fig. 2).
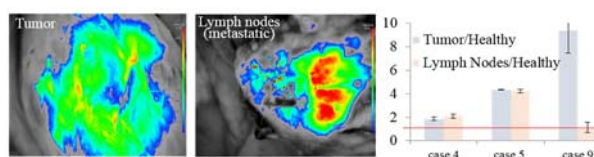

Fig. 2. Example of images of tumor and lymph node (fluorescence superimposed to visible image). Quantification of the Tumor/Healthy tissue and of the Lymph Node/Healthy tissue fluorescence.

Perspectives

This first veterinary clinical study paved the way for a larger study (Phase II / Phase III) in veterinary oncology, as well as for a clinical study in humans by strengthening confidence in the LipImage™ 815 technology.

RELATED PUBLICATIONS:

[1] S. Gioux, H.S. Choi, J.V. Frangioni, "Image-guided surgery using near-infrared light: fundamentals of clinical translation", Mol. Imaging, vol. 9, pp.237-255 (2010).
[2] D. Sayag, Q. Cabon, I. Texier, F. P. Navarro, R. Boisgard, D. Virieux-Watrelot, C. Carozzo and F. Ponce, "Phase-0/phase-I study of dye-loaded lipid nanoparticles for near-infrared fluorescence imaging in healthy dogs", Eur. J. Pharm. Biopharm. vol. 100, pp. 85-93 (2016).
[3] Q. Cabon, D. Sayag, I. Texier, F. P. Navarro, R. Boisgard, D. Virieux-Watrelot, F. Ponce and C. Carozzo, "Evaluation Of Intra-Operative 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).

BENEFIT OF ENCAPSULATION OF PHOTOSENSITIZER USING LIPIDOT® NANOCARRIER PLATFORM FOR PHOTODYNAMIC-MEDIATED CANCER TREATMENT. IN VITRO AND IN VIVO EVALUATION

RESEARCH TOPIC:

Photodynamic therapy, Lipid nanoparticles, Photosensitizer, Encapsulation, Cancer treatment

AUTHORS:

A-C. Couffin, F. Navarro, J-S Thomann, F. Mittler, D. Hinger[1], C. Maake[1], S. Gräfe, A. Wiehe[2]

ABSTRACT:

Demonstration of photosensitizer encapsulation into lipid nanoparticles as nanocarrier platform. Investigation of suitable payload for in vitro PDT efficiency while avoiding intrinsic weaknesses of free PSs such as poor water solubility or skin photosensitivity of patients. In vivo study involving these designed nanoformulations reveals its promising potential for further PDT clinical use.

SCIENTIFIC COLLABORATIONS: [1]University of Zurick (Institute of Anatomy), [2]Biolitec Pharma

Context and Challenges

PhotoDynamic Therapy (PDT) is a clinically approved modality based on the combined action of molecular oxygen, visible light and a photoactive drug (photosensitizer: PS). By irradiation of PS by light, reactive oxygen species and singlet oxygen ($^1O_2$) are created, amenable to rapidly cause significant toxicity leading to cell death and damage tumors. However, the limited solubility and specificity of PSs hampers routine use in clinical practice. Encapsulation into nanoparticle carriers provides a great potential to overcome the intrinsic weakness of PS drugs. Our Lipidot® platform may bring an innovative alternative as versatile nanomedicine for delivering phototherapeutic agents.

Main Results

In a first study, the encapsulation of mTHPC into Lipidot® was investigated by engineering our lipid nanoparticles with different particle sizes and various PS contents [1]. After their extensive characterizations including drug loading efficiency, physico-chemical and photophysical properties, its PDT efficiency was successfully evidenced while highlighting the key parameters driven its nanodelivery system for PDT use.

In the course of our research, we have set out to investigate PDT efficacy of these nanoparticles under *in vitro* and *in vivo* conditions to estimate their potential for PDT clinical use.
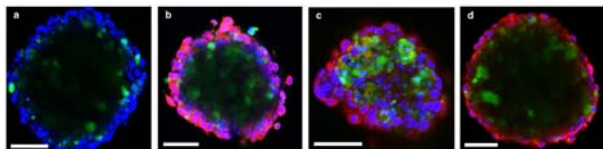

Figure 1: Confocal laser scanning microscopy images of the fluorescent labeled inhibitor of caspases (FLICA) apoptosis assay. mTHPC (red). Untreated control (a) and incubations with mTHPC (b), 50 nm Lipidots (c) or 120 nm Lipidots (d). Incubation time 24 h. With permission of D. Hinger [2]

Using an advanced in vitro 3D multicellular cancer spheroids, the photodynamic effects of mTHPC encapsulated were evaluated [2]. Compared to free drug, higher apoptosis activity of 50 nm nanoparticles was detected after PDT treatment (Fig 1), confirmed by an investigation about modes of cell death at the ultrastructural level with electron microscopy.

The tumor regression mediated by m-THPC nanoformulations has been studied in CAL-33 tumor bearing nude mice. Fourteen days after mTHPC-Lipidot® injection were visually observed a tumor reduction in size and a healing of skin with only slight burns compared to free m-THPC. However, an outer rim surrounding the tumor remains with a crust from destroyed tissue in the middle without complete tumor regression (Fig 2).

Figure 2: Images of *in vivo* tumors after PDT treatment with low light dose (10 J/cm²). Irradiation areas are delimited by 1.5 cm-diameter circles

In addition, regard with treatment tolerance, an outstanding biocompatibility is evidenced by painless injection, no change in behavior of mice and no body weight loss [3]. For further experiments, these fostering findings let us suggesting an optimization of the PDT protocol by improving the drug-light intervals and the injection dose to reach effective tumor eradication.

Perspectives

The development of a nanoformulation is of major importance because the free PS is related to several issues such as poor bioavailability and increased photosensibility of patients. Lipidot® show great potential to revolutionize PDT in the future.

RELATED PUBLICATIONS:

[1] F. Navarro, et al., "Preparation and characterization of mTHPC-loaded solid lipid nanoparticles for photodynamic therapy", Journal of Photochemistry and Photobiology B: Biology, 130, 161–169 (2014).
[2] D. Hinger, F. Navarro, A. Käch, J.S. Thomann, F. Mittler, A.C. Couffin, C. Maake, "Photoinduced effects of m-tetra-hydroxyphenylchlorin loaded lipid nanoemulsions on multicellular tumor spheroids", Journal of Nanobiotechnology 14:68 (2016).
[3] D. Hinger, S. Gräfe, F. Navarro, B. Spingler, D. Pandiarajan, H. Walt, A.C. Couffin, C. Maake,"Lipid nanoemulsions and liposomes improve photodynamic treatment efficacy and tolerance in CAL33 tumor bearing nude mice", Journal of Nanobiotechnology 14:71, (2016).

POROUS SIOCH THIN FILMS OBTAINED BY FOAMING

RESEARCH TOPIC:
SiOCH, Foaming, Nanoporosity, Thin films

AUTHORS:
J. El Sabahy, G. Castellan, F. Ricoul, V. Jousseaume

ABSTRACT:
Porous organosilicate SiOCH thin films have been prepared using a simple and innovative process, foaming. Contrary to classical strategies, this approach uses a SiOCH thin layer deposited by PECVD, without any porogens, and intentionally covered by a dense crust. The porosity generation is obtained through an ultraviolet (UV)-assisted thermal annealing of the stack. The highest porosities ever demonstrated for SiOCH PECVD thin films were obtained (porosity close to 65%). The impact of different process parameters (choice of precursor, deposition, and annealing conditions) on the creation of porosity was studied. Finally, this new approach could be extended to other type of materials and open the way to the development of new nanoporous thin films

Context and Challenges

Highly porous organosilicate thin films (SiOCH) deposited by plasma-enhanced chemical vapor deposition (PECVD) were first used as ultra-low-k (ULK) dielectrics in integrated circuit interconnects. More, recently, these porous SiOCH have revealed interesting properties when they were considered for others applications like moisture sensors, biological fluid analyses, or gas sensing. For all these applications, porosity (with pores in the nanometer range) is a key parameter. It allows tailoring specific material properties like dielectric constant or specific surface area. Generally, porous SiOCH are deposited using a subtractive strategy: the porogen approach. But unfortunately, this technique is limited and a porosity upper limit is reached, close to 50%. In analogy to polymer foaming processes we propose an innovative and simple strategy to perform highly nanoporous SiOCH thin films (even for thickness lower than tens of nanometers) without the use of any templates or external blowing agents [1].

Main Results

This approach uses a SiOCH thin layer deposited by PECVD (without any porogens) intentionally covered by a thin oxide layer which acts as a crust. The porosity generation is obtained through an ultraviolet (UV)-assisted thermal annealing of the stack (cf. Fig. 1).

Figure 1: Schematic representation of the process proposed to obtain the foaming of SiOCH thin films.

Porosity close to 65% (the highest values demonstrated for this type of films) was obtained on very thin films (thickness lower than 500 nm). The porosity introduction with this original method is believed to be related to a foaming mechanism

Two critical factors were identified for the success of the approach: the matrix reorganization and the existence of gas releasing. The first one requires low-temperature deposition of the SiOCH thin films. The film ability to deform and to reorganize itself during UV curing is pointed as a major factor.

The second key factor is the presence of Si−(CH3)x bonds which decrease during the UV curing. This methyl depletion is expected to form a gas which is at the origin of the thin film foaming. This can be tailored through UV curing conditions and SiOCH methyl loading. Fig. 2 summarizes the different open porosities measured in function of the Si−CH3 depletion obtained depending on the chosen precursor (trimethylsilane (3MS) or octamethylcyclotetrasiloxane (OMCTS)) or the curing conditions.
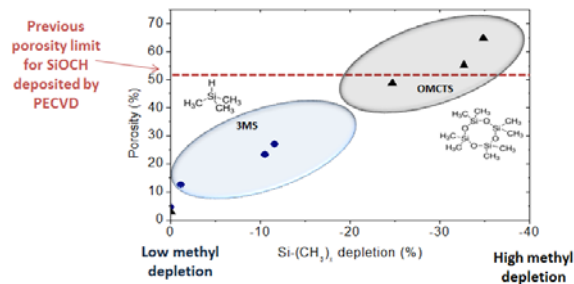

Figure 2: Open porosity (obtained by Ellipsometric Porosimetry EP) vs Si−CH3 depletion for SiOCH 3MS (circles) and SiOCH OMCTS, (triangles) deposited at 150 °C. The SiO2 capping layer was removed before the EP measurements.

Perspectives

In addition to the use of these new highly porous SiOCH thin layers for various applications with higher performances, the simple approach presented here could be extended to other type of materials and open the way to the development of new nanoporous thin films.

RELATED PUBLICATIONS:
[1] J. El Sabahy, G. Castellan, F. Ricoul, and V. Jousseaume, "Porous SiOCH Thin Films Obtained by Foaming," Journal of Physical Chemistry C, vol. 120, pp. 9184-9191, May 5 2016.

USING A « CLICK » SURFACE CHEMISTRY APPROACH TO BUILT A DNA ARRAY FOR DETECTION OF BASE EXCISION REPAIR ACTIVITIES

RESEARCH TOPIC:
Click Chemistry, Functionalized Surface, Surface Analysis, DNA Array, Enzymatic Activities

AUTHORS:
G. Nonglaton, G. Costa, M. Flaender[1], G. Delapierre, C. Saint-Pierre[1], D. Gasparutto[1]

ABSTRACT:
A surface chemistry process was developed to immobilize organic molecules *via* the alkyne/azide Huisgen cycloaddition. The functionalized surface was fully characterized by infrared (FTIR), water contact angle measurements and X-ray photoelectron spectrometry (XPS). In this study, this surface chemistry based on a "click" approach was used to immobilize DNA on a glass slide to build a surface-DNA biosensor to measure base excision repair activities of different enzymes. The results showed that this new fluorescent DNA microarray platform proved an easy, rapid and robust method for detecting DNA *N*-glycosylase (UNG) and AP-endonuclease (APE1) activities.

SCIENTIFIC COLLABORATIONS: [1]Université Grenoble Alpes, INAC - SyMMES/CEA

Context and Challenges

DNA is exposed to many exogenous and endogenous agents, such as chemicals, reactive oxygen species and radiation which can alter its structure and functions. Among the different DNA repair processes, the base excision repair (BER) pathway is the major mechanism. The present work deals with the conception and application of a new, miniaturized and parallelized on surface-DNA biosensor to measure BER activities [1].

The alkyne/azide Huisgen cycloaddition is a well-known 1,3-dipolar cycloaddition between an azide and a terminal alkyne. Due to its numerous advantages such as modularity, high chemical yields, stereospecificity, biocompatibility, stability and atom economy, this reaction is considered as a "click" chemistry reaction. Recently we have adapted this reaction for surface modification and we have demonstrated that the Cu(I)-catalyzed variant of Huisgen cycloaddition can efficiently graft redox active molecules such as ferrocene for memory applications [2].

Main Results

The functionalization procedure is an indirect grafting method and consists in a silanization with an alkylsilane terminated with chlorine atom, which was then substituted by an azide function. The modified surface was thus ready to be coupled to the propagyl modified DNA probes *via* the cycloaddition (Fig.1).
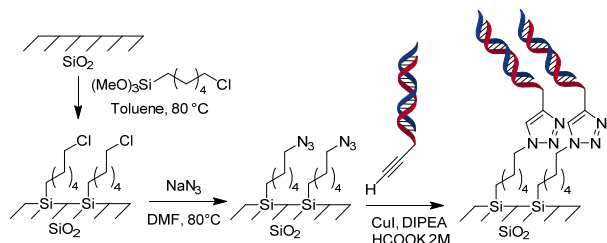

Fig.1. Scheme of DNA probes grafting protocol using Huisgen cycloaddition

The water contact angle of the glass surface significantly increased after the first step from 25° to 85°. The presence of the azide was confirmed by XPS spectrometry (2 peaks centered at 400 eV and 402 eV with a 1:2 ratio) and FTIR spectroscopy using a multiple internal reflection setup (strong band at 2097 cm^{-1}).

Fluorescent hairpin-shaped oligonucleotides were immobilized by spotting using a non-contact micro-arraying system. Each oligonucleotides contained a DNA-damaged site. The excision repair assays were conducted such that the enzymatic cleavages at the DNA-damaged site are detected *via* decreases, after a washing step, in the fluorescence intensity, proportionally to the targeted enzyme concentration (Fig.2).
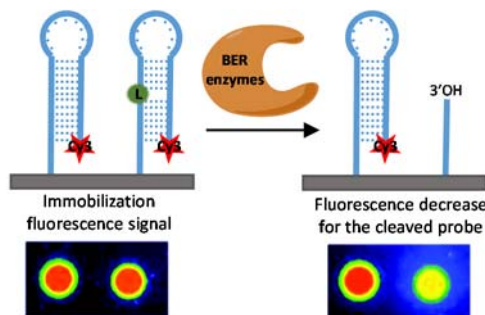

Fig.2. Principle of the on-support BER assay using hairpin-shaped lesion-containing DNA probes.

The present functional test was first applied to detect both UNG and APE1 activities from purified enzymes and was then extended to the detection of these repair activities in Hela cell nuclear extracts. To validate our assay we also tested the effects of two known selective inhibitors of UNG and APE1 enzymes, namely the Uracil DNA glycosylase inhibitor and the methoxyamine respectively.

Perspectives

Altogether, the results demonstrate the power of such fluorescent DNA arrays to develop miniaturized, robust, selective and parallelized functional assays for diagnostic, prognostic and therapeutic applications relating to DNA repair activities.

RELATED PUBLICATIONS:
[1] M. Flaender, G. Costa, G. Nonglaton, C. Saint-Pierre, and D. Gasparutto, "A DNA array based on clickable lesion-containing hairpin probes for multiplexed detection of base excision repair activities," *Analyst*, vol. 141, pp. 6208-6216, 2016.
[2] V. Aiello, N. Joo, J. Buckley, G. Nonglaton, F. Duclairoir, L. Dubois, J. C. Marchon, M. Gély, N. Chevalier, and B. De Salvo, "Redox behavior of a ferrocene monolayer on SiO2 obtained after click-coupling," *Surface Science,* vol. 612, pp. 57-62, 2013.

MEMS WITH AN EMBEDDED FLUIDIC MICROCHANNEL FOR SENSITIVE WEIGHING OF LIQUID SAMPLES

RESEARCH TOPIC:

MEMS, Mass sensors, µfluidics, Liquid density measurement

AUTHORS:

C. Hadji, M. Cochet, F. Baléras, V. Agache

ABSTRACT:

This paper reports hollow MEMS plate oscillators for the characterization of liquid samples, with a one-fold improvement in both Q-factor and Allan deviation compared to previous alike structures, and fluidic constriction larger than 1µm. These new characteristics make the devices amenable for the first time to liquid weighing with a 100 Hz.(g.L-1)-1 sensitivity and a few g.L-1 detection floor

Context and Challenges

With the benefits of miniaturization, MEMS and NEMS (Micro- and NanoElectroMechanical Systems) oscillators allow precise mass measurement, as small as fg down to the zg consistent with analysis of various micro- and nanoscale objects. These systems are promising for various applications, biomedical research and particle metrology among others, and can be easily integrated in miniaturized multifunctional systems. However, when the characterization needs to be achieved in real-time in liquid, their sensitivity is often degraded because of the viscous damping arising from the oscillator motion in the aqueous medium. In order to overcome this technological lock, this work introduces a new class of MEMS Resonators, with a microfluidic channel inserted into the core of the micromechanical part. The devices are based on hollow thin plates with integrated capacitive readout, operating in contour mode for reduced viscous damping with the surrounding air medium, so that no vacuum packaging is required (contrary to SMR cantilevers [1]). With this paradigm, the mass and density of fluid circulating inside the buried channel can be precisely monitored by measuring the device resonant frequency.
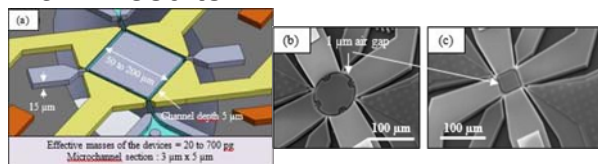
Main Results

Figure1: Schematic views and SEM pictures of the sensor. (a) Typical square plate sensor with four transduction electrodes. (b) SEM picture of a 100µm-wide disk plate, and (c) a 50µm-wide square plate

to previous generation of hollow MEMS plates [2], these new devices feature specific improvements allowing faster operation and better sensitivity. A microchannel is etched inside the silicon plate to flow the liquid; this channel is in bypass configuration with two main channels in order to ease and accelerate the filling procedure. The chip interfacing is performed by a customized

plug and play platform, which is hosting pogo pins for electrical contact and o-rings for hermetic fluidic connection
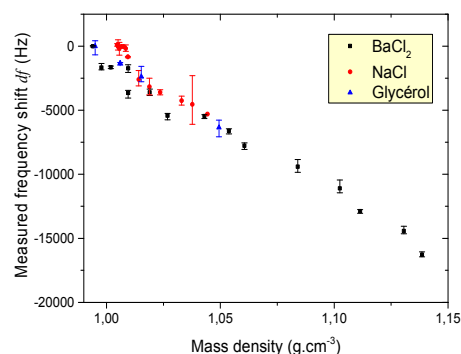

Figure2: Graph with all the measurements (NaCl, BaCl2 and glycerol solutions) gathered. The sensitivity (100 Hz.(g.L1)-1) is unaffected although the samples have different viscosities

Degassed and filtered aqueous samples with different concentrations were prepared: NaCl solutions (with lower density) were used to extract the mass detection floor of our systems, while BaCl2-water mixings samples (with broader range of mass density values) were used to evaluate their sensitivity. Figure 2 shows the frequency shift measured for each solution flown in a 200µm-wide disk plate: a sensitivity of 100 Hz.(g.L-1)-1 was extracted for a detection floor of 3 g.L-1 mass precision. The DMA500 densimeter commercialized by Anton Paar® shows a 1g.L-1 precision but requires at least 1mL samples.

Perspectives

This approach is currently being further investigated in order to apply it to the counting of nanoparticles, their weighing and extraction of mechanical parameters. Other microfluidic-based devices are being developed to connect these sensors with upstream sample preparation modules and enable complex sample analysis.

RELATED PUBLICATIONS:

[1] T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock, and S. R. Manalis, "Weighing of biomolecules, single cells and single nanoparticles in fluid.," Nature, vol. 446, no. 7139, pp. 1066–9, Apr. 2007.

[2] G. Blanco-Gomez and V. Agache, "Experimental Study of Energy Dissipation in High Quality Factor Hollow Square Plate MEMS Resonators for Liquid Mass Sensing," J. Microelectromechanical Syst., vol. 21, no. 1, pp. 224–234, Feb. 2012.

6.

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- Damien Barbes

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- Maxime Huet

- Fanny Marticke

- Sophie Morel

- Julie Oziat

- Lisa Racine

- Gaetan Scheiblin

- Veronica Sorgato

- Artur Sossin

- Judy Zouaoui

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MICROFLUIDIC SYSTEM FOR CELL ENCAPSULATION CONTROL AND OPTIMIZATION FOR CELL THERAPY

Transplantation of microcapsules containing pancreatic islets, cell clusters regulating blood sugar, show promising results for type 1 diabetes therapy. However, many challenges remain to improve long-term graft functionality. The lack of standardization of current encapsulation technologies has aroused interest in microfluidic systems that enable more precision and automation.

This thesis focuses on two of the current encapsulation technologies stakes: improving system productivity and microcapsules surface.

In the first part of this thesis, we characterized a pressure-driven microfluidic flow focusing device (MFFD) droplet generation system. Analytical and numerical models were developed in order to determine and predict flow rates. Droplet formation was characterized as a function of the system input parameters. This study led to scaling laws enabling to predict these system input parameters in order to optimize alginate microcapsules production frequency. In the second part of this thesis, a microfluidic system enabling the production of core-shell microcapsules was developed. First experiments of pancreatic islets encapsulation have shown the ability of this system to minimize the immune reaction towards these capsules.

This work is a first step towards encapsulation system optimization, which eventually, may provide capsules that meet all the capsule requirements for transplantation.

DAMIEN BARBES

Doctoral School of Physics, Université Grenoble Alpes

NEW MEDICAL IMAGING SYSTEM MEASURING THE COHERENT SCATTERING OF X-RAYS WITH ENERGY RESOLVED CDZNTE-BASED DETECTORS

This thesis studies the interest of measuring the coherent scattering of X-rays for breast diagnosis imaging. Nowadays, most of X-ray-based medical imaging techniques use the information of X-rays attenuation through the tissues. It is the case for mammography, the most common breast imaging modality. The recent emergence of energy resolved detectors (based on semiconductors in particular) allows to consider using another phenomenon: the coherent X-ray scattering. Measurement of diffracted spectra can provide new information related to the molecular structure of the examined tissues, in order to improve their characterization and therefore improve the final diagnosis. Two modalities are considered: the breast cancer detection in vivo, following a suspicious mammography result, or biopsy analysis.

The coherent scattering measurement system developed during this thesis work uses energy resolved CdZnTe-based detectors, these detectors combining performances (energy resolution, sensitivity, spatial resolution, and compactness) promising for clinical application. This system is also based on the detector pixelation, which allows to provide an imaging modality capable of characterizing analyzed materials or tissues in one direction without any translation or rotation. A complete study of the measurement system is proposed in this thesis, structured in three main parts: modeling and simulation of the system, development of the processing of the data measured by the detector in order to image and characterize the analyzed sample and finally, designing of a new and more complex experimental setup based on a whole detector and multislit collimation system. An experimental validation is proposed for each of these three parts.
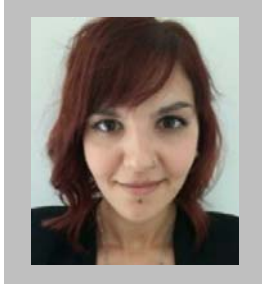
FRANÇOIS BERTHOLON

Doctoral School for Electronics, Power Systems, Automatic Control and Signal Processing, Université Grenoble Alpes

MIXTURES ANALYSIS BASED ON GAS CHROMATOGRAPHY SIGNALS

The chromatography is a chemical technique to separate entities from a mixture. In this thesis, we will focus on the gaseous mixture, and particularly on the signal processing of gas chromatography. To acquire those signal we use different sensors. However whatever the sensor used, we obtain a peaks succession, where each peak corresponds to an entity present in the mixture. Our aim is then to analyze gaseous mixtures from acquired signals, by characterizing each peak.

After a bibliographic survey of the chromatography, we chose the Giddings and Eyring distribution to describe a peak shape. This distribution defines the probability that a molecule walking randomly through the chromatographic column goes out at a given time. Then we propose an analytical model of the chromatographic signal which corresponds to a peak shape mixture model. Also in first approximation, this model will be considered in some case as Gaussian mixture model.

To process those signals, we studied two broad groups of methods, applied upon real data and simulated data. The first family of algorithms consists in a Bayesian estimation of unknown parameters of our model. The order of mixture model can be included in the unknown parameters. It corresponds also to the number of entities in the gaseous mixture. To estimate those parameters, we use a Gibbs sampler, and Monte Carlo Markov Chain (MCMC) sampling, or a variational approach. The second methods consists in a sparse representation of the signal upon a dictionary. This dictionary includes a large set of peak shapes. The sparsity is then characterized by the number of components of the dictionary needed to describe the signal. At last, we propose a sparse Bayesian method.

CELINE HADJI

Doctoral School of Physics, Université Grenoble Alpes

INTEGRATED FLUIDIC MICROCHANNEL IN MEMS FOR CHARACTERISATION AND WEIGHTING OF LIQUID SAMPLES

MEMS and NEMS allow sensitive and precise mass detection consistent with micro- and bio-objects analysis. These systems are promising for biomedical research and particle metrology, and can be easily integrated in miniaturized multifunctional systems. Therefore, characterization in liquid media remains tricky due to viscous dissipation consequent to the movement induced in the fluidic environment.

In order to overcome this technological lock, our laboratory previously designed and fabricated specific MEMS devices for fluidic analysis; these thin plate resonators with an embedded microchannel are actuated in liquid media, with four capacitive electrodes providing both actuation and detection. The circulating fluid mass can be precisely measured by monitoring the device's resonant frequency. The long-term objective is to be able to detect and weigh one single particle transported by the fluid.

Two main objectives were fulfilled during these three years. First, the MEMS behaviour in presence of various liquids was evaluated, providing a fine-grained analysis of their performances as mass sensors. The measured resolution of our sensors is about a few $g.L^{-1}$ with a sensitivity of 100 $Hz.(g.m^{-3})^{-1}$.

Meanwhile, a new generation of NEMS sensors with innovative features was designed; the objective is to decrease the effective mass and reduce the frequency noise for a better mass resolution.

This thesis includes four chapters. The first one consists in a review of the existing techniques for particles characterization in fluid as well as MEMS and NEMS solutions for particles metrology described in the litterature. The second part of the manuscript presents the results of the experimental characterizations carried out on the first generation of sensors. The third chapter gathers the conclusions of these measurements and gives an outlook on possible improvements on both the design and the characterization of the sensors. At last, the fourth part describes the new generation of devices and discusses their characteristics in terms of expected resolution and applications.
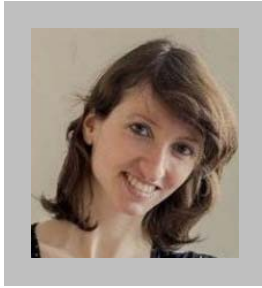
MAXIME HUET

Doctoral School of Physics, Université Grenoble Alpes

AUTOLOGOUS RED BLOOD CELLS AGGLUTINATION BY A BISPECIFIC REAGENT FOR THE QUANTIFICATION OF BIOMARKERS

The detection or quantification of biomarkers in the blood can provide valuable information on human health. An analysis directly performed at the patient bedside is called a Point-Of-Care test (POC). The agglutination of red blood cells by a bispecific reagent combining a biomarker binding part and an erythrocyte binding part is proposed as a basis for an autonomous and quantitative POC test. The integration and automation of the protocol in a microfluidic chip and the optical measurement of the kinetics of agglutination are investigated. The first question concerns the possibility of producing agglutination in passive microfluidic device that is to say without any energy nor any material supply other than the sample. The second and third questions respectively relate to the measurement of the kinetics of aggregation and the existence of a link between this measure and the concentration of the biomarker. The formulation and embedding of the reagents has proved essential to perform a reproducible agglutination reaction in passive microfluidics

and thus answer the first question. Various measurement strategies based on the optical properties of the red blood cells have been proposed. Some of them have been successfully implemented. The kinetic measurement of agglutination has been performed for a blood typing model and allowed the discrimination between positive and negative agglutination reaction in 100 % of the experiments. The effect of biomarker concentration on the agglutination measurement has been demonstrated using a relevant blood target, answering the last question. All this PhD work was done in close collaboration with an industrial partner.

FANNY MARTICKE

Doctoral School for Electronics, Power Systems, Automatic Control and Signal Processing, Université Grenoble Alpes

OPTIMIZATION OF AN X-RAY DIFFRACTION IMAGING SYSTEM FOR MEDICAL AND SECURITY APPLICATIONS

X-ray diffraction imaging is a powerful non-invasive technique to identify or characterize different materials. Compared to traditional techniques using X-ray transmission, it allows to extract more material characteristic information, such as the Bragg peak positions for crystalline materials as well as the molecular form factor for amorphous materials. The potential of this technique has been recognized by many researchers and numerous applications such as luggage inspection, non-destructive testing, drug detection and biological tissue characterization have been proposed.

The method of energy dispersive X-ray diffraction (EDXRD) is particularly suited for this type of applications as it allows the use of a conventional X-ray tube, the acquisition of the whole spectrum at the same time and parallelized architectures to inspect an entire object in a reasonable time. The purpose of the present work is to optimize the whole material characterization chain. Optimization comprises two aspects: optimization of the acquisition system and of data processing. The last one concerns especially the correction of diffraction pattern degraded by acquisition process. Reconstruction methods are proposed and validated on simulated and experimental spectra. System optimization is realized using figures of merit such as detective quantum efficiency (DQE), contrast to noise ratio (CNR) and receiver operating characteristic (ROC) curves.

The first chosen application is XRD based breast imaging which aims to distinguish cancerous tissues from healthy tissues. Two non-multiplexed collimation configurations combining EDXRD and ADXRD are proposed after optimization procedure. A simulation study of the whole system on a breast phantom was realized to determine the required dose to detect a 4 mm carcinoma nodule. The second application concerns detection of illicit materials during security check. The possible benefit of a multiplexed collimation system was examined.

SOPHIE MOREL

Doctoral School of Physics, Université Grenoble Alpes

WIDE-FIELD IMAGING OF TISSUE SLIDES FOR DIGITAL PATHOLOGY APPLICATION

This PhD project aims to develop a simple, fast (35 minutes), wide-field (up to 2.5 cm 2.5 cm) multiscale (m-cm) imaging method for stained and unstained tissue slides for digital pathology application. We present a solution based on lensfree imaging. It is a simple, low-cost technique that enables wide field imaging (10-30 mm2) of sparse objects, like viruses, bacteria or cells. In this project, we adapted lensfree imaging for dense objects observation, like stained or unstained tissue slides. The sample is illuminated under multiple illumination wavelengths, and a new multiwavelength holographic reconstruction algorithm was developed in order to reconstruct the modulus and phase of dense objects. Each image covers 10 mm2 field of view, and is reconstructed in 1.1 second. An image of the whole tissue slide covers 6.25 cm2. It is recorded in 35 minutes by scanning the sample over the sensor. The reconstructed images are multiscale, allowing the user to observe the overall tissue structure and to zoom down to the single cell level (3-4 m). The method was tested on various stained and unstained pathology samples. Besides tissue slides, multiwavelength lensfree imaging shows encouraging results for meningitis diagnosis, bacteria population monitoring for identification and antibiotic susceptibility testing, and cell culture monitoring.

JULIE OZIAT

Biomaterials and Electrochemistry, Mines St Etienne

3D ELECTRODE OF PEDOT: PSS FOR ELECTROCHEMICAL DETECTION OF METABOLITES OF *PSEUDOMONAS AERUGINOSA*

During infections, microorganisms fast identification is critical to improve patient treatment and to better manage antibiotics use. Electrochemistry exhibits several advantages for rapid diagnostic: it enables easy, cheap and *in situ* analysis in most liquids. Its use for bacterial identification is recent and comes from the discovery of molecules giving strong redox signals in the bacterial supernatant of the *Pseudomonas* genus.

This thesis focuses on the supernatants analysis of the bacterium *Pseudomonas aeruginosa*.This bacteria is the fourth cause of nosocomial infections in Europe. First, the interest of supernatants electrochemical analysis for identification was evaluated. For this, after the study of four redox biomarkers of this bacterium in model solutions, supernatant electrochemical analysis of several strains of *P. aeruginosa* was performed. The results are promising. They highlight a complex strain-dependant electrochemical signature of the supernatant.

Following, we focused in the amplification of the electrochemical detection through the use of the conductive polymer PEDOT: PSS. This polymer was chosen for its good electrochemical properties, its biocompatibility and its easy shaping. It was first used as a thin films to confirm its amplification power through biomarker adsorption. Then, a 3D electrode was made by freeze drying. The use of this type of electrode can further amplify the detection by increasing the exchange surface as well as confining the bacteria in the electrode.

LISA RACINE

Doctoral School of Chemistry and Life Science, Université Grenoble Alpes

DESIGN OF MATERIALS FOR THE CONTROLLED DELIVERY OF LIPOPHILIC ACTIVE INGREDIENTS

Due to their high biocompatibility, macroscale hydrogels have been studied as promising materials for the design of drug delivery systems (DDS). Such systems devoted to the local administration and prolonged drug release can improve the efficacy of pharmaceutical compounds while limiting undesired side-effects. Hydrogels present a high water content and soft consistency with mechanical properties that can match those of biological tissues. Nevertheless, these systems are essentially limited to the delivery of hydrophilic drugs. Our approach for extended release of hydrophobic drugs is to design composite materials composed of lipid nanoparticles (LNPs) entrapped within polysaccharide hydrogels. We selected two polysaccharides which are currently used in pharmaceutical and biomedical applications: carboxymethylcellulose (CMC) and chitosan (CS). We also used poly(ethylene glycol) (PEG) as a plasticizer to tune the matrix mechanical properties. Three types of LNP-loaded hybrid materials were studied; i) bulk CMC/PEG hydrogels, ii) CS/PEG films, and iii) CS/PEG sponges. These materials were chemically crosslinked through attractive click reactions. LNPs were successfully entrapped within the three materials without affecting their properties. A deeper study was conducted with the CMC/PEG composite hydrogel. The LNP release profiles were correlated with the network structure and particles properties. The different materials appear promising systems for the time-controlled delivery of therapeutics.

GAETAN SCHEIBLIN

Science, Engineering and Health, Mines St Etienne

DEVELOPMENT OF LACTATE SENSORS AND TRANSFER TO PRINTED ELECTRONICS

In a context of patient survey at home, the demand in wearable biosensors for continuous body monitoring strongly increases. Thereby, these biosensors have to be minimally invasive and should provide accurate sensing independently of the patient activity. Consequently the need of flexible solid state sensors grows up. Organic electronic is an excellent candidate for such sensing application thanks to the use of low cost flexible materials that could be easily processed using large scale facilities such as screen-printing. More precisely, organic electrochemical transistors (OECT) offer original properties for bio-sensing applications. This work was first focused on the development of fully printed biosensors for lactate and glucose. A fast solid state device working at a voltage lower than 1V, able to detect glucose or lactate (with limits of detection of 30μM and 500μM respectively) and to work in real sweat, was developed.

Conversely to classical electrochemical sensors, OECT can be associated in more complex circuits. Two pH sensitive OECT were integrated in a Wheatstone bridge leading for the first time to the reference-less detection of pH in real sweat samples.

OECTs were also combined for the design of printed logic gates (NOR, NAND…). An advanced biologic NOR gate was fabricated for the first time through the modification of OECT gates with lactate oxidase and glucose oxidase enzymes respectively. This biologic NOR gate shown finally the expected logic response according to the presence in the solution of glucose and lactate biochemical inputs.

VERONICA SORGATO

Doctoral School of The Particle Physics in Condensed Matter

NOVEL MULTISPECTRAL IMAGING TECHNIQUE FOR THE SPATIAL QUANTIFICATION OF OPTICAL PROPERTIES

The Novel 'Dual-Step' Multispectral Imaging Technique that has been developed intends to contribute to the clinical diagnosis of superficial lesions by providing non-invasively quantitative spatial wide field maps of absorption and scattering endogenous optical properties. The approach relies on the combination of a Non-Contact Spatially-resolved Diffuse Reflectance Spectroscopy (DRSsr) technique with a Multispectral Imaging (MSI) technique. Absolute quantification is based on the scattering estimation with Non-Contact DRSsr which is subsequently used by MSI to estimate wide field absorption. The instrumental setups of each technique are built and thoroughly characterized in this work. The optimal quantification of optical properties relies on a newly established calibration algorithm 'ACA-Pro' that achieves minimal estimation errors inferior to 3.3% for scattering and 5.5% for absorption. The developed 'Dual-Step' technique has been validated not only with an extensive intralipid phantom study but also with ex-vivo biological human skin samples and in-vivo inflammation skin models on rats. The results show the potential of the 'Dual-Step' technique as a valid quantitative, wide-field, and non-invasive clinical diagnosis approach.

ARTUR SOSSIN

Doctoral School of Electrical, Electronics and Automation,
INSA Lyon

CORRECTION OF SCATTERED RADIATION IN MULTI-ENERGY RADIOGRAPHY AND TOMOGRAPHY

X-ray imaging coupled with recently emerged energy-resolved photon counting detectors provides the ability to differentiate material components and to estimate their respective thicknesses. However, such techniques require highly accurate images. The presence of scattered radiation leads to a loss of spatial contrast and, more importantly, a bias in radio-graphic material imaging and computed tomography (CT). Additionally, artifacts are also introduced in the case of the latter. The main aim of the present thesis was to develop a scatter correction approach adapted for multi-energy imaging. In order to achieve this task, a secondary objective was also set. Namely, the conception and validation of a simulation tool capable of providing energy-resolved scatter simulations in a reasonable time. Once validated through simulations and experimentally, this tool gave the ability to study the behavior of scattered radiation both in spatial and energy domains. Based on the conducted scatter analysis, a Partial Attenuation Spectral Scatter Separation Approach (PASSSA) adapted for multi-energy imaging was developed. The evaluation of PASSSA in radio-graphic mode through simulations and experiments revealed noteworthy results both in terms of image contrast improvement and scatter induced bias reduction. Additionally, simulation studies examined the performance of the developed approach in CT, where PASSSA also proved to be quite effective at correcting scatter induced distortions. Moreover, the performance improvement in the context of basis material decomposition in radiography after applying the designed method was also analyzed. It was concluded that the application of PASSSA results in a substantial improvement in basis material thickness estimation. Finally, based on the obtained simulated and experimental method evaluation results an analysis of perspective developments was also conducted.

JUDY ZOUAOUI

Doctoral School of Physics, Université Grenoble Alpes

MULTISPECTRAL TIME-DOMAIN DIFFUSE OPTICAL TOMOGRAPHY

In medical imaging, the ability to accurately retrieve and quantify the composition of turbid media is challenging and would enable to diagnose some diseases or to better study physiological processes. Diffuse optical tomography (DOT) is an attractive medical imaging technique which permits to probe in depth using near-infrared light and to reconstruct in three dimensions the composition of biological tissues non-invasively, non-ionizing and with potentially high specificity. To obtain endogenous chromophore (oxy- and desoxy-hemoglobin) features in the depth of a highly scattering medium, a multiwavelength time domain optical setup combined to a three-dimensional reconstruction algorithm was developed. Experimental measurements were conducted in reflectance geometry by illuminating a perturbed medium (with a heterogeneity) with a picosecond laser and by collecting, for several wavelengths and multipositions, the backscattered light via two fibers connected to two dedicated detectors and coupled to a time-correlated single photon counting system. The data processing of these time-resolved measurements and those of a known reference medium was performed by supposing that the propagation of light is governed by the diffusion approximation and using a method based on Mellin-Laplace transform. Numerical and phantom experiments on series of objects similar to biological media demonstrate that this technique has the potential to give quantitative medical images. We have highlighted a correct quantification for the less absorbing objects at 10 mm depth while underestimation results at deeper depths and higher absorptions. Furthermore, the multimodal imaging brings improvements in quantification in depth and thus it can be a good opportunity to DOT for its future clinical applications.

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