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Multispectral photoacoustic imaging of tumors in vivo

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Introduction: Photoacoustic imaging (PAI) is an emerging technology that combines the most compelling features of optical and ultrasound imaging, providing both optical high sensitivity and ultrasound high resolution in depth in living organisms. PAI provides unique opportunities to measure noninvasively, endogenous compounds (such as hemoglobin, melanin and fat) together with exogenous compounds such as specific markers.
In this study we evaluated the input of PAI for the biological characterization of cancer in preclinical animal models.

Methods: We set up 2 animal models of cancer (colon cancer liver metastases and orthotopic glioblastoma) and we combined several preclinical imaging modalities i.e. bioluminescence (BLI), 3D fluorescence/microCT and echography/PAI to monitor tumor development at two stages. Angiostamp®800 were used as a tumor specific contrast agent for both fluorescence and PA. Nano6000 was used as an X-rays liver contrast agent.

Results: BLI offered a very sensitive and rapid longitudinal monitoring of liver metastases and glioblastoma development that allows to choose relevant time points for in depth exploration with other imaging modalities.

Angiostamp®800 was found to be a very good fluorescent contrast agent for both liver metastases and glioblastoma. Although microCT was not appropriate to visualize brain structures, it offered a useful morphological visualization of liver metastasis with the use of Nano6000. Thus the combination of 3D Fluorescence and microCT provided relevant molecular information localized in an anatomical context.

Angiostamp®800 also displayed a spectral signature that was well suited for PAI. Thereby, liver metastases were well visualized by PAI and the PA signals were shown to increase along tumor development. Moreover, the multispectral PAI approach provided additional information on the hemoglobin content and the oxy and deoxyhemoglobin proportions in the same tumor.

In the glioblastoma model, despite a strong labeling of the tumor by Angiostamp®800, PAI failed to provide noninvasive visualization of the tumor. Brain exploration by PAI needs some technological improvements that are currently in progress.

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Conclusion: Multispectral PAI is a very innovative technology that valuably extends the panel of imaging modalities by providing a non-invasive and real time molecular characterization of tumors by reporting both endogenous and exogenous markers distribution at a microscopic scale in vivo.

Mots-Clés: Photoacoustic, Oncology, Multimodal

An optimized method enables in vivo microscopy of the murine lung without stabilization

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Lung tissue motion arising from breathing and heart beating has been described as the largest annoyance of in vivo imaging. Consequently, infected lung tissue has been only scarcely imaged in vivo thus far, and little is known concerning the kinetics of the mucosal immune system at the cellular level. We have developed an optimized post-processing strategy to overcome tissue motion, based upon two-photon and second harmonic generation (SHG) microscopy.
In contrast to previously published data, we have freed the lung parenchyma from any strain and depression in order to maintain the lungs under optimal physiological parameters. Excitation beams swept the sample throughout normal breathing and heart movements, allowing the collection of many images. Given that tissue motion is unpredictably, it was essential to sort images of interest. This step was enhanced by using SHG signal from collagen as a reference for sampling and realignment phases. A normalized cross-correlation criterion was used between a manually chosen reference image and rigid transformations of all others. Using CX3CR1+/gfp mice this process allowed the collection of high resolution images of pulmonary dendritic cells (DCs) interacting with Bacillus anthracis spores, a Gram-positive bacteria responsible for anthrax disease. We imaged lung tissue for up to one hour, without interrupting normal lung physiology. Interestingly, our data revealed unexpected interactions between DCs and macrophages, two specialized phagocytes. These contacts may participate in a better coordinate immune response.
This technique allowing microscopic imaging of the living lung without involving any mechanical stabilization of the lung may prove to be of the highest interest for scientists investigating the subtle behaviour of any cell population which would suffer from an invasive stabilization of the lung.

Mots-Clés: lung in vivo microscopy, tissue motion, two, photon

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Autofluorescence spectrale pour le diagnostic précoce et minimal invasif des nodules suspects

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L'analyse spectrale de l'autofluorescence peut fournir des informations en temps réel sur les propriétés morpho-fonctionnelles des tissus biologiques. Cependant la méthode est très peu développée dans le domaine médical, en particulier à l'attention des radiologues dans leur exercice quotidien. Probea® est un dispositif médical original développé par Nodea Medical dédié à l'analyse spectrale de l'autofluorescence de lésions suspectes. Il se différencie des dispositifs d'endoscopie classiques, par l'utilisation d'une aiguille fibrée de 25G connectée à une diode laser émettant à 405 nm (laser de classe 2), permettant l'exploration en profondeur des nodules au sein d'organes pleins de manière minimale invasive et en toute sécurité pour des mesures in vivo. Probea® se positionne comme un dispositif d'aide au diagnostic là où les méthodes d'imagerie (échographie, scanner, élastographie, etc..) peuvent s'avérer peu sensibles ou peu spécifiques, nécessitant la réalisation d'examens supplémentaires comme la biopsie ou le prélèvement extemporané au cours d'une chirurgie.

Trois études précliniques dans l'aide au diagnostic des cancers du sein, bronchopulmonaires et hépatiques ont été menées sur pièces opératoires en partenariat avec différents centres hospitaliers experts français. L'analyse des données de l'autofluorescence spectrale des nodules malins vs bénins démontre que le dispositif Probea® permet d'atteindre une sensibilité et une spécificité

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de 100 % et 85% dans le cancer du sein, 77% et 92% dans les cancers bronchopulmonaires et 81-100% et 75-80% dans le cancer du foie.
Sur la base de ces résultats très prometteurs, une première étude clinique multicentrique nationale a été initiée chez la femme pour des lésions mammaires suspectes à l'échographie. Dans le cas des cancers pulmonaires et hépatiques, une validation de la méthode est toujours en cours afin d'affiner et d'optimiser les critères de jugement déjà existants. Par ailleurs, une ANR en technologies de la santé vient d'être obtenue dans l'indication visant les pathologies hépatiques (projet ALIVE).

Mots-Clés: autofluorescence, diagnostic, aiguille fibrée, minimal invasif, oncologie

Improving lung cancer patients stratification using a Radiomics approach applied to routine FDG PET/CT images

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Non-small cell lung cancer (NSCLC) patients without distant metastases (stage IV) are usually managed in clinical practice based on their clinical staging, including for therapeutic decisions (combinations of surgery, radiochemotherapy or palliative chemotherapy). Staging is based on visual assessment of diagnostic FDG PET/CT images and clinical exams. The Radiomics approach aims at automatically extracting as much quantitative information as possible from available diagnostic images in order to characterize the tumours, including their volume, shape and level of tissue and metabolic heterogeneity. In this work, we investigated how such a Radiomics approach can improve the stratification of patients at diagnosis over the standard clinical staging. We carried out a monocentric, retrospective analysis of routine diagnostic FDG PET/CT images of 116 patients with stage I-III NSCLC. Primary lung tumours were automatically segmented and then characterized using shape and textural features analysis in both the FDG PET and CT images, in order to obtain a multimodal PET/CT vector of quantitative metrics describing the tumours. Using machine learning, a prognostic model was built by selecting the most reliable, least redundant and most clinically relevant (in terms of overall survival) tumour quantitative features. Our results show that such a model can identify with a much higher specificity patients with worse prognosis among stage II and III patients, compared to the conventional approach (clinical staging). Patients with large, nonspherical tumours combining high FDG PET uptake heterogeneity and high CT tissue density homogeneity had a very short median survival of less than 7 months and a 18-months survival rate of 0%, compared to patients with more favorable PET/CT image tumours characteristics (median survival of 21 months, 3-year survival rate of 30%). Compared to clinical staging that provided a hazard ratio of only 1.4 between stage II and III patients and 4.7 and 6.6 with respect to stage I patients, the proposed Radiomics-based model provided a stratification resulting in hazard ratios of 4.9 and 17.4 for medium and high risk groups compared to stage I patients, and of 3.6 between medium and high risk groups. This model now needs validation in prospectively recruited patients. When validated, it could be very useful in the context of clinical trials, and for identifying patients that are unlikely to benefit from standard treatment and should receive intensified and/or targeted therapies.

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Mots-Clés: NSCLC, lung cancer, PET/CT imaging, biomarkers, quantitative imaging, radiomics, texture, shape

Local Image Feature Extraction (LIFEx): a new freeware for tumor characterization in multimodality imaging.

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Objectives: Detailed characterization of tumors is gaining considerable interest in medical imaging, in particular to identify parameters that might characterize tumor heterogeneity. We developed an easy-to-use freeware enabling calculation of a broad range of conventional, textural and shape indices from PET, MR and CT raw or parametric images.
Methods: The LIFEx software does not rely on any commercial libraries. LIFEx is dedicated to researchers, radiologists, nuclear medicine physicians and oncologists and includes a user interface giving access to 37 conventional or advanced histogram, textural and shape indices. Default settings are proposed in the two simplest operation modes but users can change calculation options in the "advanced" operation mode. LIFEx reads DICOM images locally or over a network using a DICOM browser, is compatible with Osirix and includes a powerful 3D slice viewer. Volume of interest can be either loaded from external files or drawn and manipulated using LIFEx. Results are exported in Excel format files. LIFEx runs on Windows, MacOs and Linux platforms. It is distributed with examples and includes a tutorial. User support is offered for the "validated" operation mode. Users can optionally contribute to the gathering of index values in different tissue types and different images and a public data bank of reference values is currently being built and integrated in the software for assisting the users with the interpretation of results.

Results: LIFEx has already been distributed to research labs, nuclear medicine and radiology departments for investigating different tumor types (gliomas, cervix, lung, breast, and colorectal tumors) and has been very positively received. New features are continuously added based on users feedback. The intuitive interface made it fast to master for staff, and allowed us to start building databases of normal index values in brain (white and grey matter), breast, liver, lung, fat, and muscles for various imaging equipment and protocols in PET and CT, while MR data are being collected and processed. Such data make it possible to precisely characterize the variability of different indices in a given imaging modality as a function of the scanner and imaging protocol.
Conclusions: Initial feedback suggests that offering such a free software to the community considerably speeds up the collection of data by independent centers. The on-going creation of a data bank should contribute to a better understanding of the potential and limitation of advanced parameters in PET, CT and MR.

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Mots-Clés: texture, histogram, SUV, conventional indices, PET, MRI, CT, characterization, tumors, software

Potential of textural indices derived from images for characterizing the spatial organization of tumor cells

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Objectives: Characterizing tumor heterogeneity using texture analysis has shown promise to predict treatment response in oncology. Yet, the relationship between texture indices (TI) and biological heterogeneity needs to be clarified.
Methods: Thirty days after the implantation of mammary tumor derived from transgenic MMTV-PyMT mouse and after the injection of 7.4 MBq of F18-Fluorodeoxyglucose, a mouse was sacrificed. Tumor was sliced, autoradiography (AR) was performed and tumor slices were stained using haematoxylin and eosin. Using one tumor slice, we defined 80 subregions of 30 x 30 pixels on the AR images (pixel: 50x50 μm2) and copied them on the image of the hematoxylin component in which nuclei are colored in blue-purple. We selected 30/80 subregions displaying three cell patterns: A – a large continuous map of cells with no more than three small islands ($<$ 15% of the surface) of extracellular matrix, or the opposite, B – two distinct tissue types (cells and extracellular matrix) clearly separated in terms of locations and in comparable proportions, and C – two mixed tissue types in comparable proportions. For the corresponding subregions in AR images, we resampled pixels intensities into 64 gray-levels between 0 and 0.1 and we computed 6 TI and the maximum intensity (Max). To characterize the relationship between texture analysis and cell distribution, we performed Wilcoxon tests between the A, B and C groups.

Results: Homogeneity, Entropy, SRE and LRE were significantly different between the three types of pattern ($p < 0.05$): the A subregions were more homogeneous than C subregions that were in turn more homogeneous than B subregions. Among the A subregions, HGZE, LGZE and Max could separate subregions with a majority of cells ($>$ 85% of cells, called A+) from subregions composed of a majority of extracellular matrix ($<$ 15% of cells, called A-), while other indices could not. HGZE, which measures the distribution of high gray-level zones, and Max were higher in A+ than in A-. LGZE varied in the opposite direction. These results suggest that TI could be classified into two categories: those sensitive to the spatial distribution of cells (Homogeneity, Entropy, SRE and LRE) and those sensitive to the density of cells (HGZE and LGZE), and can thus reflect different types of heterogeneity.

Conclusions: TI measured on AR images can distinguish between various tumor cell density

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and spatial distribution measured on pathology slides, paving the way for image-based in vivo histopathology using appropriate tracers or contrast agents.

Mots-Clés: Texture analysis, oncology, radiomics

Prediction of cervical cancer recurrence using textural features calculated from 18F-FDG PET images

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Objectives: We assessed the ability of textural features to predict recurrence of cervix cancer using pre-treatment 18F-FDG PET images.
Methods: 118 patients with cervical cancer treated in Gustave Roussy between 2005 and 2014 were included. All patients underwent a 18F-FDG PET-CT scan before radiotherapy: 77 on a Siemens Biograph scanner (G1) and 41 on a GE Discovery scanner (G2). PET images were acquired 60.1±4.9 min post-injection. Treatment consisted of a concomitant radio-chemotherapy followed by uterovaginal brachytherapy.

The primary tumor was delineated using a threshold of 40% SUVmax (VOI). Five conventional indices (SUVmean, SUVmax, SUVpeak, metabolic volume, TLG) and 6 textural features were calculated in 3D after resampling the VOI SUV between 0 and 20 using 64 gray levels: Homogeneity and Entropy from the Gray-Level Co-occurrence Matrix, Short-Run Emphasis (SRE), Long-Run Emphasis (LRE), Low Gray-level Zone Emphasis (LGZE) and High Gray-level Zone Emphasis (HGZE).

ROC analyses were performed in G1 and G2 to study whether the measured parameters were significantly different between relapsing and non-relapsing patients. To determine whether the same threshold values could be used for the two scanners for patient classification, we performed a ROC analysis when merging G1 and G2. Binomial logistic regression including all indices was performed to investigate whether combining all indices could improve classification.

Results: 27 patients from G1 and 13 from G2 relapsed during the follow-up time (3.3±2.1 years, minimum 1 year). Only Entropy (AUC_G1 = 0.709, AUC_G2 = 0.698), LGZE (AUC_G1 = 0.664, AUC_G2 = 0.706), SUVmean (AUC_G1 = 0.661, AUC_G2 = 0.701) and SUVmax (AUC_G1 = 0.664, AUC_G2 = 0.695) identified patients who showed tumor recurrence ($p < 0.05$) in G1 and G2. When merging the 2 groups, these indices were still predictive of recurrence with AUC systematically lower (Entropy: 0.670, LGZE: 0.671, SUVmean: 0.662, SUVmax:

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0.667) than the best performance achieved by each index in G1 or G2, due to changes in index values between the scanners. With the binomial logistic regression, AUC were significantly higher than using individual indices (AUC_G1 = 0.82, AUC_G2 = 0.81, AUC_G1+G2 = 0.75).
Conclusion: Some tumor textural indices measured in baseline FDG-PET were as good as SUVs to predict tumor recurrence in 2 patient cohorts. Although the optimal performance (not achievable in practice) obtained by combining all indices was significantly higher than using a single index, more biomarkers are required to accurately predict tumor recurrence. Textural and conventional values being different between scanners, the use of a machine-independent threshold for tumor classification is suboptimal.

Mots-Clés: radiomics, texture, PET, cervix cancer

Mapping myocardial fiber orientation using ultrasound Backscatter tensor imaging

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Introduction

The orientation of myocardial fibers is linked to the mechanical and electrical properties of the heart and its assessment is of great interest to better understand the progression of myocardial disease. Yet there is no clinical imaging modality to map the myocardial fiber orientation routinely. In this study, we present Backscatter Tensor Imaging (BTI), a novel 3D ultrasound-based technique that can map the myocardial fibers by analyzing the spatial coherence of backscattered echoes. Application in phantoms, ex-vivo pig hearts and in-vivo human hearts will be shown.

Methods

Acquisitions were performed using a 2D matrix array (3 MHz, 32x32 elements, 0.3-mm pitch) driven by a customized, programmable, 1024-channel ultrasound system in phantoms, and in ex-vivo and in-vivo hearts tissue. Up to 81 tilted 3D plane waves were emitted and coherently compounded to focus in each voxel of the volume. The coherence of the signals recorded by each element of the probe was calculated as described in Figure 1 for each focal zone and an elliptic fit was used to identify the local fiber orientation.

Results

The isotropic phantom was used to verify the Van Cittert Zernike theorem, which predicts that if the medium is isotropic, the coherence function will only be impacted by the geometry of the probe. 3D BTI was then applied in ex-vivo pig hearts (Figure 2) and in-vivo human hearts (Figure 3), which were found to be approximately orthotropic.

Conclusion(s)

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3D BTI was successfully applied to the ex-vivo pig heart and in-vivo human heart. These results suggest that 3D BTI could be used for in vivo applications, as the technique can be applied with high frame rates. Its application in the human heart in-vivo is still the subject of ongoing work.

Acknowledgement/References

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Mots-Clés: ultrafast, ultrasound, heart, fiber

Effects of healthy aging and parkinson's disease on the superior colliculus response to luminance contrast.

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Luminance contrast is a fundamental visual cue. Testing contrast response is of interest with respect to alterations in aging or in the progression of diseases, such as Parkinson's disease (PD). Indeed, some visuo-motor impairments observed in the early stages of PD might be related to a dysfunction of the Superior Colliculus (SC), a subcortical structure that is part of a distributed network mediating saccadic eye movements, fixations and directed attention, particularly sensitive to low luminance contrasts. Luminance contrast response functions were assessed: i) in healthy control subjects with varying age (Young: n=10, 26±3Yo; Middle- aged: n=10, 47±4Yo; Elderly: n=10, 65±3Yo) and ii) in de novo PD patients before (n=4, 56±9Yo) and after (+2 months: n=3, 59±10Yo; +6 months: n=2, 63±9Yo) the introduction of the first anti-parkinsonian treatment. For these subjects with normal or corrected to normal vision, we used high-resolution 3T-fMRI techniques to measure the luminance contrast response of three regions of interest (ROIs), located along the visual pathway and anatomically defined in each individual: the Superior Colliculus (SC), the Lateral Geniculate Nucleus (LGN) and primary visual cortex, area V1. We used achromatic checkerboards varying in luminance (1-9%), flashing at 4Hz and alternately presented in each visual hemi-field. Additionally, we estimated the perceptual response to contrast by using Maximum Likelihood Difference Scaling (MLDS), a method based on paired-comparisons of stimulus intervals and a signal detection model, allowing estimation of response scales by maximum likelihood. In this psychophysical task, each participant performed 3 sessions, each session consisting of a 5 min random presentation of 120 ordered triads of checkerboards, draw from a set of 10 luminance contrast levels logarithmically spaced. The observer had to choose whether the middle contrast was more similar to the lower or higher one. First, our results demonstrated for control subjects, that, in all explored ROIs, BOLD responses increased progressively with luminance contrast ($p< 10\text{-}5$). This confirms and extends results to the low contrast range of previously published data. Moreover, a progressive decrease of the BOLD amplitude with age was observed in these ROIs (V1 and LGN: $p< .05$). These data are

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consistent with the response functions obtained with the psychophysical task, showing on the one hand a response that increases with luminance contrast, reaching an asymptote at 20% and, on the other hand, a tendency for this response to decrease with age at low range contrasts and even in the higher range tested (p=.07). Second, in comparison to controls, our results showed an atypical profile of modulation for the SC in untreated PD patients with a tendency toward the normal results 6 months after the introduction of the treatment. Our findings suggest a significant luminance contrast sensitivity loss with age, as tested by our rapid psychophysical technique and also reflected by a BOLD signal decreasing along the visual pathway. Understanding the mechanisms underlying these changes that occur in normal aging is essential both for understanding the normal aging process and for comparisons with patients with age-related visual and/or cortical/subcortical disorders. Moreover, our preliminary results for PD patients indicate a possible functional deficit in SC that might appear early in the disease course in line with experiments we performed on a small animal Parkinson model. L-Dopa treatment seems to restore progressively SC functionality.

Mots-Clés: Human Vision, Parkinson Disease, Neuroimaging, fMRI, Psychophysics.

Vers une cartographie rétinotopique rapide des aires visuelles en IRMf : application à des sujets jeunes et âgés

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L'imagerie par résonnance magnétique fonctionnelle (IRMf) constitue un outil majeur pour l'étude non invasive de l'organisation fonctionnelle du cerveau humain sain et pathologique. Ainsi, elle permet de localiser l'activité neuronale impliquée lors de tâches cognitives ou de stimulations sensorielles. Pour l'étude du traitement de l'information visuelle chez l'homme, un des atouts majeurs offerts par l'IRMf réside enla possibilité de délinéer les aires, ainsi que la projection du champ visuel central et périphérique au sein du cortex visuel. Les bases d'une méthodologie de cartographie des aires visuelles en IRMf ont été posées par les travaux princeps de Sereno (Sereno et al. 1995). Depuis, cette approche, permettant de délinéer les aires visuelles de V1 à hV4, a été couramment utilisée dans de nombreuses études de neurosciences. Néanmoins, la durée des acquisitions permettant une cartographie précise reste importante et rend difficile son application auprès de participants pour qui le temps d'acquisition demeure un facteur important (enfants, personnes âgéesou patients).Face à ce constat, quelques travaux ont proposé des méthodes pour réduire la durée d'acquisition en augmentant le rapport signal sur bruit par l'ajout d'une tâche attentionnelle centrée sur le stimulus (Bresler &Silver, 2010) ou l'intégration d'une grille de fixation (Schira et al. 2009). D'autres études ont proposé des méthodes de cartographie stimulant uniquement les méridiens vertical et horizontal ou bien le centre et la périphérie du champ visuel (Chang et al. 2014). L'objectif de ce travail est d'intégrer et de tester ces différentes approches dans un protocole de cartographie optimisé qui soit à la fois rapide et précis. Pour ce faire, nous avons réalisé deux études en IRMf auprès de participants jeunes et âgés afin d'étudier l'influence de la réduction du temps d'acquisition sur la qualité des cartes obtenues. Nos résultats ont montré que le temps d'acquisition total pouvait être divisé par deux, tout en conservant une bonne qualité des cartes d'activations. La réduction du temps d'acquisition nous permet de proposer un protocole de cartographie des aires visuelles rapide, applicable auprès de participants jeunes ou âgés et d'envisager son application dans un contexte clinique.

Mots-Clés: IRMf, Cortex Visuel, Rétinotopie, Cartographie Cérébrale, Vieillissement normal

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