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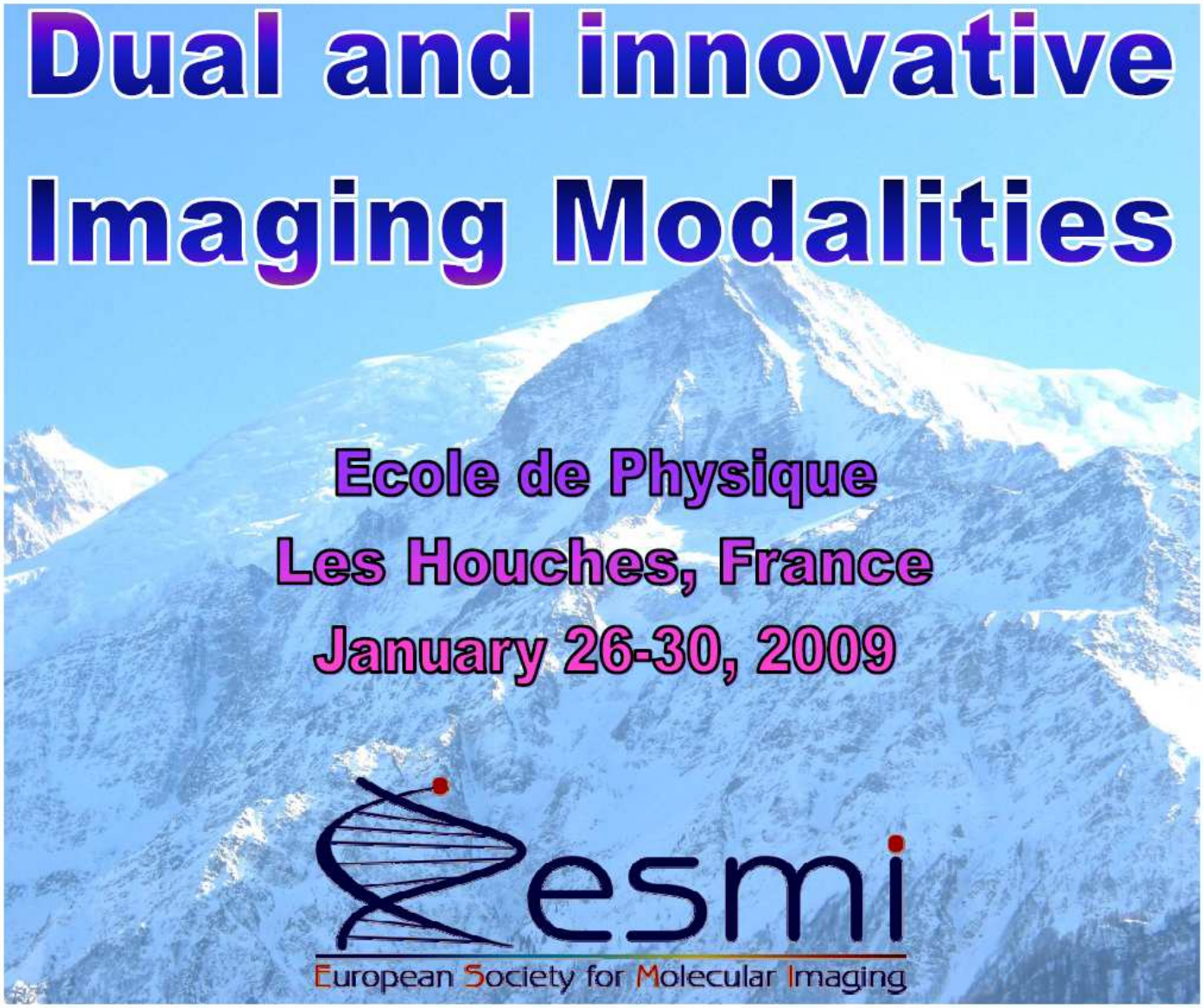
European Society for Molecular Imaging

TOPIM'09

Hot Topics in Molecular Imaging 2009

Dual and innovative Imaging Modalities



Ecole de Physique
Les Houches, France
January 26-30, 2009

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Programme committee

Bertrand Tavitian, Andreas Jacobs, Vasilis Ntziachristos, Silvio Aime, Clemens Lowik, John Clark

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European Molecular Imaging Laboratories

DiMI Diagnostic Molecular Imaging

Centre de Physique des Houches

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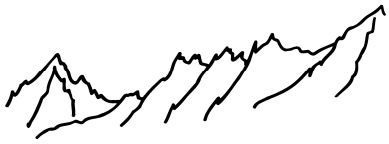
ECOLE DE PHYSIQUE DES HOUCHES	
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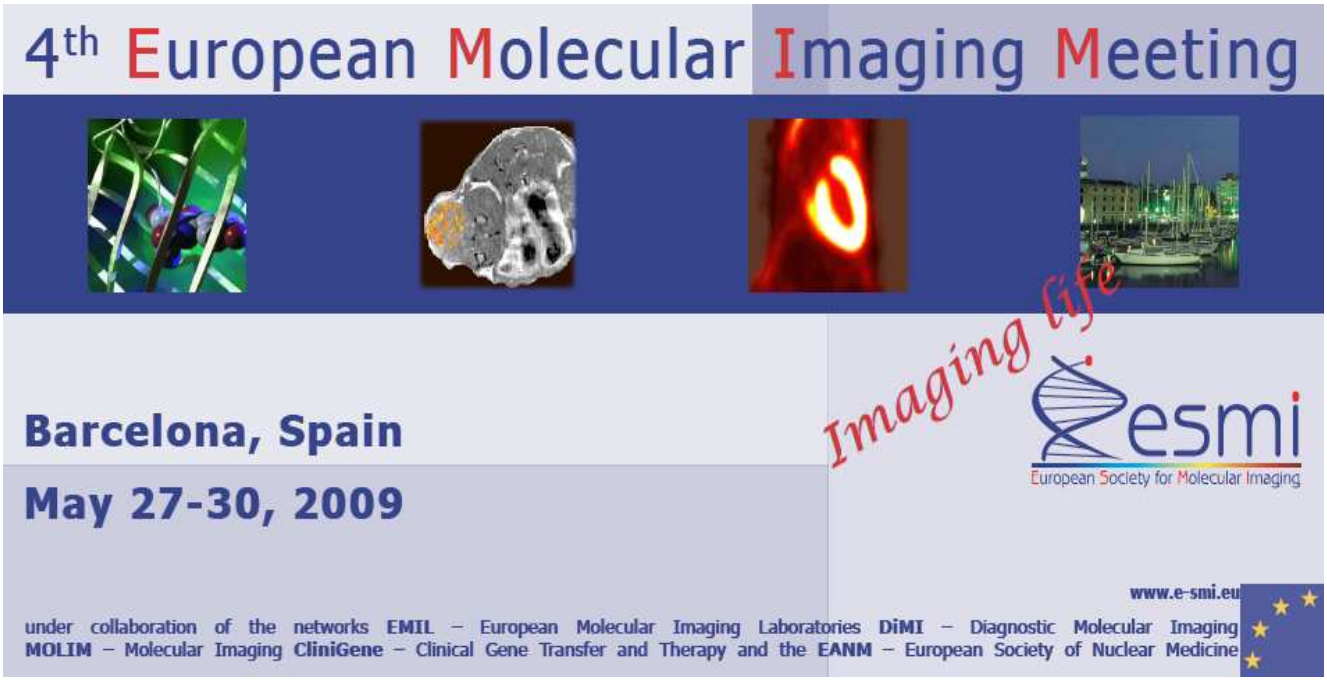
The **European Society for Molecular Imaging** (**ESMI**) is a non-profit and apolitical society, which promotes the development and practical application of Molecular Imaging within Europe. ESMI extends actions initiated in the scope of the 6th framework with the EMIL (European Molecular Imaging Laboratories) & DiMI (Diagnostic Molecular Imaging) Networks of Excellence and the Molecular Imaging Integrated Project, aspiring to be the premier body in its field within Europe and to promote research and practice of Molecular Imaging for benefits in healthcare, science and technology.
Membership in the organization is open to all persons who share the vision of the organization and have educational, research, or practical experience in some aspect of molecular imaging. For additional information on our Society, you can reach the ESMI website at: www.e-smi.eu.

ESMI is pleased to announce the venue of the **4th European Molecular Imaging Meeting** that will take place from 27th to 30th May 2009 in Barcelona, Spain. The conference is organized in close collaboration with the networks **EMIL** - European Molecular Imaging Laboratories, **DiMI** - Diagnostic Molecular Imaging, **CliniGene** – Clinical Gene Transfer and Therapy and **MOLIM** - Integrated Technologies for *in-vivo* Molecular Imaging and the **EANM** - European Association of Nuclear Medicine. The meeting is reflecting our ambition to create and ensure a sustainable and effective European Molecular Imaging community beyond the boundaries of disciplines!
Additional information is available on the ESMI web-site.



FOREWORD

Imaging is a new science with already a strong influence on medicine and biology, leading the way in exploiting molecular, biological and genetic information to develop precise, precocious and predictive diagnostic methods. These methods are increasingly precious for the follow-up and the evaluation of new treatments of many pathological states.

Two of the features of imaging – the bridge that it creates between biology, chemistry, physics and mathematics, and its growing importance in medicine – suffice to justify the creation of the European Society for Molecular Imaging (ESMI). But even more crucial is the fact that imaging is asserting itself as an original means of discovery, and opens new avenues to address unexplored questions. This is what TOPIM, *Hot Topics in Imaging*, is all about: capture these assertions by providing an instant picture of the field, and foster new ones through the discussions between participants. By combining expert descriptions of the most up-to-date imaging technologies and/or applications, TOPIM contributes in collectively describing the imaging approaches, categorizing them and drawing the landscape of *in vivo* imaging applied to a specific scientific issue, chosen each year according to its pertinence and timeliness.

The fundamental principles of interaction and image formation differ significantly between the imaging modalities used for *in vivo* imaging. Combined with elaborate methods of inducing biological contrast, the plurality of technologies and the diverse performance characteristics may at times appear daunting not only for the biologist or even for the medical imaging specialist, not to mention other scientists who lack the knowledge of what the imaging technology could bring to their own line of research and to its applications. Gathering all this knowledge is of particular importance today, as interest in newer tools, integrating two or more imaging modalities, is exponentially increasing.
With the occasion of the third TOPIM edition, it appeared timely to engage the discussion on multimodal imaging approaches and on newly emerging imaging modalities.

When thinking of multimodal imaging, this old Buddhist tale comes to mind: six blind men were gathered together by the raja to examine an elephant. When reporting to the raja their individual experience, each blind man described his version of the truth: the blind man who felt the head said the elephant is like a pot; the one who felt the ear said the elephant is like a fan; the one who felt the trunk said the elephant is like a snake; the one who felt the body said the elephant is like a granary; the one who felt the foot said the elephant is like a pillar; and the one who felt the tail said the elephant is like a rope.
The men came to blows which delighted the raja, who concluded:

"O how they cling and wrangle, some who claim
For preacher and monk the honored name!
For, quarreling, each to his view they cling.
Such folk see only one side of a thing."

When trying to see the invisible with our imaging systems, we are much like those blind men. Each imaging modality sheds some light on a different aspect of the general physiological picture, and combining several techniques provides the complementary information that should bring us to eventually obtain a correct and consistent picture of the elephant.

The programme committee (Bertrand Tavitian, Andreas Jacobs, Vasilis Ntziachristos, Clemens Lowik, Silvio Aime, John Clark) has been most fortunate to attract a panel of prestigious speakers, all at the fore point of research in their discipline. We would like to thank them heartily, especially those who have travelled a long way to the French Alps, for having accepted to share their knowledge with us. We are also indebted to the Ecole de Physique des Houches and to its committee for supporting the TOPIM project since the first *Hot Topics* event in February 2007. We would like as well to warmly thank our sponsors, many of them being with us from the very beginning of the ESMI, for their generous support.

We would like to address our warmest welcome to all of you, and to encourage you to participate without restriction in the scientific debates as well as to enjoy the beauties of the mountains surrounding us.

Irina Carpusca, *EMIL Manager*
Bertrand Tavitian, *Past President of the European Society for Molecular Imaging*

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09:45 - 10:30	**Radiochemistry at the Service of the Nuclear Imaging Modalities PET and SPECT : Selected Examples within Macromolecules and Nanoobjects** Frederic DOLLE , Orsay	**Nanoplatform-based Multimodality Imaging Probes** Xiaoyuan CHEN, Stanford	**Bringing the best out of Light: multi-modality photonic Imaging** Vasilis NTZIACHRISTOS, Münich	**Dual Bioluminescence and Fluorescence Imaging combined with Intraoperative Imaging** Hans DE JONG, Groningen	**The Challenge of multi-modal Imaging of Acute Ischemic Stroke – a clinical Perspective** Jan SOBESKY, Koeln
10:30 - 10:50					
10:50 - 11:35	**Hyperpolarization and Multi-modality Imaging: the new Challenges in the Design of MRI-based Agents** Silvio AIME, Torino	**MRI and Optical Imaging** Thomas MUEGGLER, Zürich	**Multi-modality Imaging of Breast and Prostate Cancer Bone Metastasis using 3D optical Imaging and CT** Clemens LOWIK, Leiden	**MRI guided focused Ultrasound** Chrit MOONEN, Bordeaux	**PET/MRI: the next Step in Multimodality Imaging** Bernd PICHLER, Tübingen
11:35 - 11:50	Presentation submitted abstract **Tri-modal SPECT-CT-OT Imaging System** Liji CAO, Heidelberg	Presentation submitted abstract **Longitudinal and multi-modal *in vivo* Imaging of Tumor Hypoxia and its downstream molecular Events** Michael HONER, Zürich	Presentation submitted abstract **A multi-spectral Reconstruction Algorithm for Multimodality tomographic Imaging** Giannis ZACHARAKIS, Heraklion	Presentation submitted abstract **Optical Imaging in Breast Cancer: from Bench to Bedside** Rick PLEIJHUIS, Groningen	Presentation submitted abstract **Imaging of Inflammation in experimental Stroke** Jan KLOHS, Berlin
11:50 - 12:05	Presentation submitted abstract **An Image Analysis Pipeline for quantitative Analysis of multi-temporal and multi-modal *in vivo* small Animal Images** Janaki Raman RANGARAJAN, Leuven	Presentation submitted abstract **GPI anchored Avidin - a novel Protein for multimodal, *in vivo* Imaging** Steffi LEHMAN, Zürich	Presentation submitted abstract **Combining Light and Sound – multispectral optoacoustic Tomography for high Resolution molecular Imaging** Daniel RAZANSKI, Neuherberg	Presentation submitted abstract **Co-registration of Fluorescence and Oxymetry using multi-modal optical Tomography** Rosy FAVICCHIO, Heraklion	Presentation submitted abstract **Multimodality imaging: Prostate Cancer Diagnosis and Follow up by TOF-PET & MRI/MRS** Franco GARIBALDI, Roma
12:05 - 12:20	Presentation submitted abstract **Combined Micro X-ray CT and optical Tomography yields structural and molecular Information during Bone Growth** Florian STUKER, Zürich	Presentation submitted abstract **ClearPET-XPAD : simultaneous PET/CT for small Animal** Stan NICOL, Marseille	Presentation submitted abstract **Peering into the Future: Multimodal Imaging of Cellular Therapy in Clinical Trials** Mangala SRINIVAS, Nijmegen	**Cardiac Imaging in Mice using multi-isotopes gated SPECT and gated CT** Philippe CHOQUET, Strasbourg	Presentation submitted abstract **An NF-κB inducible bidirectional Promoter** Anders KIELLAND, Oslo
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PROGRAM

	Monday, 26th January
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10:30 - 10:50	**BREAK**
10:50 - 11:35	**Hyperpolarization and Multi-modality Imaging: the new Challenges in the Design of MRI-based Agents** Silvio AIME, University of Turin, Turin, Italy
11:35 -11:50	Presentation submitted abstract **Tri-modal SPECT-CT-OT Imaging System** Liji CAO, German Cancer Research Center, Heidelberg, Germany
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09:45 - 10:30	**Nanoplatform-based Multimodality Imaging Probes** Xiaoyuan CHEN, Stanford University School of Medicine, Stanford, CA, USA
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12:05 -12:20	Presentation submitted abstract **ClearPET-XPAD : simultaneous PET/CT for small Animal** Stan NICOL, CPPM, IN2P3 /CNRS / Université de la Méditerranée, Marseille, France
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17:45 - 18:30	**Multi-modality Imaging of Brain Tumors using Fluorescence Molecular Tomography, MRI and CT** Frederic LEBLOND, Dartmouth College, New Hampshire, USA
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10:50 - 11:35	**MRI guided focused Ultrasound** Chrit MOONEN, CNRS, Functional and Molecular Imaging, Bordeaux, France
11:35 - 11:50	Presentation submitted abstract **Optical Imaging in Breast Cancer: from Bench to Bedside** Rick PLEIJHUIS, University Medical Center Groningen, Groningen, The Netherlands
11:50 -12:05	Presentation submitted abstract **Co-registration of Fluorescence and Oxymetry using multi-modal optical Tomography** Rosy FAVICCHIO, Foundation for Research and Technology – Hellas, Heraklion, Greece
12:05 - 12:20	**Cardiac Imaging in Mice using multi-isotopes gated SPECT and gated CT** Philippe CHOQUET, University Hospital Hautepierre, Strasbourg, France
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17:45 - 18:30	**Multimodal Nanoparticle for Tumor Characterization** Jinzi ZHENG, University of Toronto / Princess Margaret Hospital, Toronto, Canada
18:30 - 18:45	**BREAK**
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11:35 - 11:50	Presentation submitted abstract **Imaging of Inflammation in experimental Stroke** Jan KLOHS, Charité Hospital, Berlin, Germany
11:50 -12:05	Presentation submitted abstract **Multimodality imaging: Prostate Cancer Diagnosis and Follow up by TOF-PET & MRI/MRS** Franco GARIBALDI, ISS and INFN, Roma, Italy
12:05 - 12:20	Presentation submitted abstract **An NF-kB inducible bidirectional Promoter** Anders KIELLAND, University of Oslo, Oslo, Norway
12:20 - 13:00	**CONCLUSION** Bertrand TAVITIAN, CEA, Orsay, France

ABSTRACTS OF PRESENTATIONS

DUAL AND INNOVATIVE IMAGING MODALITIES

RADIOCHEMISTRY AT THE SERVICE OF THE NUCLEAR IMAGING MODALITIES PET AND SPECT: SELECTED EXAMPLES WITHIN MACROMOLECULES AND NANO-OBJECTS

Frédéric Dollé

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Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are nuclear molecular imaging modalities used in daily clinical practice and, especially where it concerns PET, in biomedical research. Both techniques provide images of the *in vivo* distribution of radioactive molecules, often called radiotracer, administered to a patient or to a laboratory animal and give information on biological function rather than on anatomical structure. Whereas PET uses positron-emitting radioisotopes, often of short half-life like carbon-11 ($T_{1/2}$ = 20.4 minutes) and fluorine-18 ($T_{1/2}$ = 109.6 minutes), SPECT employs single-photon emitters like technetium-99m ($T_{1/2}$ = 6.0 hours) and iodine-123 ($T_{1/2}$ = 13.2 hours). A feature common to both techniques is the preparation of radiolabelled probes (or molecules), involving state-of-the-art methodologies for incorporating the radioisotope into a defined appropriate chemical structure, a discipline termed radiochemistry.
During the thirty years or so that these imaging modalities are around, radiochemistry has served the preparation of essentially small (low molecular weight) labelled molecules for tracing metabolism, blood flow and receptor concentration. More recently, the labelling of macromolecules, *i.e.* peptides, proteins and nucleic acids, has been the subject of increasing attention, especially in cancer research.
The ideal way of radiolabelling a molecule would be replacing one of its atoms by a radioactive isotope of itself. The most prominent example of this in the field of molecular imaging is carbon-11 for carbon-12 substitution for PET as most bioactive molecules are organic molecules that contain carbon. However, this so called true labelling is not practical for macromolecules. Leaving aside some exceptions, macromolecules do not contain carbon atoms that are easily accessible for the fast chemistry required for the short-lived carbon-11 and reaction conditions in much of carbon-11 chemistry may be too harsh for the macromolecule to survive. The strategy that has been followed for the labelling of macromolecules is conjugation chemistry. In this approach the macromolecule to be labelled is provided with a relatively small molecular tag, called prosthetic group, for carrying the radioisotope. The radioisotope can be introduced into the prosthetic entity before the latter is attached to the macromolecule. This is notably the case with fluorine-18. This order has the advantage that the macromolecule does not need to be exposed to the rather

harsh reaction conditions associated with fluorine-18 introduction as the linking of the prosthetic group to the macromolecule must be a mild process. However, when the prosthetic group is a radiometal-cation chelating entity (as for technetium-99m) the radioactivity is usually introduced in the final step.

Radiochemistry approaches applied to the labelling with positron- and single-photon emitters of nucleic acids in particular will be exemplified to illustrate the general use of conjugation chemistry in the radiolabelling of macromolecules. In-house examples of radiolabelling of peptides, proteins and nano-objects with fluorine-18 (the preferred positron-emitter for PET-radiopharmaceutical chemistry) will also be presented as well as a selection of macromolecules tagged with dyes, bridging nuclear imaging and another imaging modality: optical fluorescence.

HYPERPOLARIZATION AND MULTI-MODALITY IMAGING: THE NEW CHALLENGES IN THE DESIGN OF MRI-BASED AGENTS

Silvio Aime

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The impressive technological advances in the field of in vivo diagnostic instruments prompt the chemist to tackle new avenues in the design of imaging agents that match at best the characteristics of the imaging technology and the peculiar properties of the imaging reporter.
In MRI, the most innovative achievement is probably represented by the set-up of Hyperpolarization-based procedures. These methodologies aim at overcoming the low sensitivity of the NMR/MRI experiment. Much interest is currently focussed on the search of ^{13}C- and ^{15}N-labelled small molecules in which the heteronuclear resonance has to be characterized by a very long T_1. Thus a detailed knowledge of the relaxation processes of the hyperpolarized molecule is necessary in order to control at best the relaxation time of the heteronuclear resonance under different experimental conditions.
Next, Molecular Imaging has prompted new technological developments in the direction of dual modality approaches, i.e. MRI/PET, MRI/SPECT, MRI/OI, MRI/US. Whereas the latter approach may allow to tackle important tasks in the field of imaging guided drug delivery, the other dual imaging systems involving MRI can be exploited to make applicable in vivo the peculiar characteristic of properly designed MRI responsive agents. Examples will be shown in which the responsiveness of a MRI agent toward a given physicochemical parameter (i.e. pH) can be fully exploited thanks to the possibility of assessing the actual concentration of the agent through the quantitative detection of the PET or SPECT response.

TRI-MODAL SPECT-CT-OT SMALL ANIMAL IMAGING SYSTEM

Liji Cao, J. Peter

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Objective: Intended for simultaneous detection of fluorescent/bioluminescent molecular markers and radiolabeled pharmaceutical distribution *in vivo* at coinstantaneous registration with the subject's anatomy we have built a tomographic triple-modal SPECT-CT-OT small animal imaging system.
Methods: All imaging components including an x-ray CT tube and detector pair, a gamma-ray detector, a light source and an optical detection unit are mounted on a single rotatable and translatable gantry and share axially superimposed fields-of-view. To optimize sub-system imaging performance a geometric co-calibration method was developed estimating the mechanical misalignments not only within each modality but also between different sub-systems. Different reconstruction strategies are adopted for the sub-modalities.
Results: For the first time, triple-modality imaging of the aforementioned modalities are presented. Imaging is performed simultaneously at a single acquisition step without moving the subject across modalities. After co-calibration procedure, each modality reaches the highest possible geometric performance and, moreover, data from the different sub-systems are intrinsically co-registered without any post-registration step. Various phantom studies were performed to demonstrate the performance and imaging quality of the system. In addition, several *in vivo* mice studies are presented to demonstrate various possible applications.
Discussion: To fully make use of the single-gantry structure, these three sub-modalities are not regarded as separate systems during both calibration and reconstruction procedures. We have incorporated the CT results as *a priori* information for the calibration of SPECT and OT sub-systems to ensure the accuracy of the geometric parameters as well as the intrinsic registration of the reconstructed volumes. Furthermore, the reconstruction procedure of the optical tomographic detection also benefits from the prior information of the subject's anatomy and the coupled radioactivity distribution acquired from the simultaneous CT and SPECT scanning.

AN IMAGE ANALYSIS PIPELINE FOR QUANTITATIVE ANALYSIS OF MULTI-TEMPORAL AND MULTI-MODAL *IN VIVO* SMALL ANIMAL IMAGES

Janaki Raman Rangarajan[1], G. Vande Velde[2], U. Himmelreich[3], T. Dresselaers[3], C. Casteels[4], A. Atre[4], D. Loeckx[1], J. Nuyts[4], F. Maes[1]

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[2] *Neurobiology and Gene therapy, K.U.Leuven, Belgium*
[3] *Biomedical NMR Unit, Faculty of Medicine, K.U.Leuven, Belgium*
[4] *Division of Nuclear Medicine, K.U.Leuven, Belgium*
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We have developed a multi-temporal and multi-modal image analysis pipeline for in vivo molecular imaging applications to obtain quantitative measurements of structure and function, to fuse complementary information from multiple images and to compare images in longitudinal studies. This pipeline contains dedicated modules for image acquisition, pre-processing, image registration, atlas based segmentation and quantification.
Methods: Following image acquisition, the pre-processing module encompasses methods for removing artefacts like animal movement during acquisition, RF bias field inhomogeneity and inter-scan intensity variation. The registration module of the pipeline uses the Maximization of Mutual Information similarity measure for µMR-µMR and µMR-µPET registration. Such a co-registration of multi-modal and time series images to a common reference template/atlas facilitates atlas based segmentation. After appropriate spatial and intensity normalization, the quantification module provides quantitative measures of morphological and functional changes corresponding to the molecular imaging application.
Results: The pipeline was tested on both a multi-temporal study to quantify in vivo vector based MR reporter gene delivery system and a multimodality study to quantify morphological and functional changes in a transgenic Huntington disease rat model in µMR-µPET data**.**
Conclusion: The image analysis pipeline presents structured modules for analyzing images from quantitative small animal molecular imaging studies. The pipeline is generic and can be adapted for different strain, animals, disease models, imaging modalities and applications.

COMBINED MICRO X-RAY CT AND OPTICAL TOMOGRAPHY YIELDS STRUCTURAL AND MOLECULAR INFORMATION DURING BONE GROWTH

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Aims: Bone remodeling is currently assessed using in-vivo using micro X-ray computed tomography (microCT). The resulting morphological readout yields no direct information on osteoblast or osteoclast activity. Such information can be obtained from a near-infrared (NIR) bisphosphonate (pamidronate) imaging agent, OsteoSense®, with a high specificty to hydroxyapatite (HA) a marker for osteoblast activity. Aim of the current study was to combine sequentially microCT and fluorescence molecular tomography (FMT) allowing to correlate structural with molecular information during bone growth in mice.

Materials and Methods: Bone remodeling was engineered in C57BL/6 mice by application of sinusoidal forces to implanted pins of two interleaved tail vertebrae. Amplitude of sinusoidal force was set to 8N for the treatment group (n=2) and to 0N for control group (n=2). Images of vertebrae were acquired by a high resolution CT scan and immediately after by a FMT scan with OsteoSense680 injected 24h prior.
Results: The regions of induced bone formation in the murine tail vertebrae could clearly be localized in both modalities. For the morphological read out a bone volume change analysis for the pre and post loading state showed a bone mass increase where as for the optical measurement different regions within the tail can be well separated and quantified after tomographic reconstruction.

Conclusion: These preliminary data obtained in-vivo show the feasibility to localize molecular information during engineered bone reformation process with a sequential multimodality approach. Longitudinal studies to monitor bone remodelling process using structural and functional information over time are currently performed.

FLUORESCENT REAGENTS FOR LIFETIME-BASED OPTICAL IMAGING

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Fluorescence Lifetime Imaging is an Optical Imaging methodology that is gaining more and more importance due to its great potential. Each fluorescent dye has its own lifetime; by detecting differences in lifetimes it is possible to distinguish even dyes having the same fluorescent color as well as to identify autofluorescence. In this contribution we present the development of a series of Cy5.5® analogues cyanine dyes with the same fluorescent colors but different lifetimes.

Methods. Cyanine dyes were synthesized and purified following standard methodologies and characterized via ^{1}H-NMR spectroscopy, UV-Vis absorption and photoemission spectroscopy. Fluorescence lifetimes were measured using a time-correlated single photon counting (TCSPC) technique (Horiba Jobin Yvon) with excitation source NanoLed at 636 nm (Horiba) and impulse repetition rate of 1 MHz at 90° to a TBX-4 detector. The detector was set to 700 nm with a 5 nm band pass. The instrument was set in the Reverse TAC mode, where the first detected photon represented the start signal by the time-to-amplitude converter (TAC), and the excitation pulse triggered the stop signal. DAS6 decay analysis software was used for lifetime calculation.

Results and Conclusion. A series of Cy5.5® analogues characterized by significantly different fluorescent lifetimes has been prepared by inserting structural modifications that do neither interfere with the absorption/emission wavelength nor with the bioconjugation properties. Interesting results have been achieved by testing these cyanine dyes for bioconjugation and by using the bioconjugates in optical imaging applications.

THE CHALLENGES AND OPPORTUNITIES IN PET/MR

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Opportunities MR is arguably more data rich than CT with many scan sequence modes available permitting multiple contrast opportunities**.**
If it were possible to acquire PET and MR data simultaneously it would open up many novel *in vivo* imaging opportunities e.g. real time motion correction including dynamic processes especially below the neck, MR guided PET partial volume correction for small structures, MR spectroscopy and Pharm fMRI and PET biomarker synergy.
For truly simultaneous PET/MR an instrument will need to achieve uncompromised PET and MR performance. To achieve this “cross talk” between the two instruments must be eliminated or controlled to a manageable level. Heavy MR gradient coils should not interfere with the PET co-incident gamma emissions Electronic “noise” from the PET electronics must be screened from the MR RF coils. Electromagnetic interferences on PET from the Main and gradient magnet coils and RF system must be eliminated or controlled to a manageable level. MR driven PET attenuation correction will need to be developed and validated. Examples of clinical studies that would be likely to benefit from simultaneous PET/MR will be shown together with some solutions to the interferences.

In a later lecture this week Prof Bernt Pitchler will describe his experience with the Siemens PET/MR insert.

MULTIMODALITY IMAGES FUSION

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This talk will present a survey of registration techniques. First, geometry-based techniques, that require the extraction of geometrical landmarks, will be introduced. These methods generally minimize the distance between paired points. Among others the Iterative Closest Point (ICP) approach will be detailed. Second, intensity-based techniques that rely on similarity measures will be presented. Different similarity measures (from the Sum of Squared Differences to the Mutual Information) will be detailed together with their application context. This will allow introducing hybrid methods, such as block matching based methods. Then, some insights in non-linear registration methods will be given.
Examples of application will be presented.

- Reconstruction of a 3D volume from a stack of 2D histological section
- Construction of an “average” image from a database of several images
- Reconstruction of an isotropic volume from several anisotropic volumes.

PET/CT

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PET/CT is a multimodal imaging modality, which provides accurately coregistered fused positron emission tomography (PET) and X-ray computed tomography (CT) images by hardware and software integration of the scanners. After the resolution of initial methodological problems, e.g. breathing mismatches, attenuation correction artifacts, and acquisition protocol issues, the logistical advantage of replacing multiple separate studies by a single PET/CT scan was quickly recognized and led to the success of PET/CT. PET with [^{18}F]fluorodeoxyglucose (FDG), a tracer of glucose metabolism, plays an important role together with morphological imaging modalities for staging and therapy stratification of cancer patients. The fusion of functional PET and anatomical CT images yields improved diagnostic accuracy by identifying lesions which cannot be detected by a single method. In addition, CT provides the anatomical context of functional abnormalities and PET characterizes the functional status of morphological findings, resulting in improved diagnostic accuracy. The assessment of molecular markers of response in addition to morphological criteria improves therapy assessment and the accurate mapping of molecular data to spatial coordinates by PET/CT provides the basis for treatment planning, e.g. in radiation therapy. PET/CT can be used to image quantitatively a multitude of molecular PET tracers. Therefore, it is an important translational link between experimental molecular techniques, small animal imaging, and clinical applications.

INSTRUMENTATION CONCEPTS FOR INTEGRATED OPTICAL / PET IMAGING

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Whereas interpreting functional/molecular data under anatomical cognizance as provided by CT or MRI offers numerous advantages, combining two molecular imaging modalities such as PET and optical imaging (OI) seems, at first sight, not very instinctive. However, there are a number of potential applications specifically in drug research and development that could make use of fully integrated PET- optical imaging instrumentation. As of today, instrumentation development is still in its infant stage and research is focused almost exclusively on preclinical application. In this presentation, we provide a review on the current state of integration concepts and working systems for small animal PET-optical imaging through classification of approaches into three instrumentation categories: i) systems that employ a single photon sensor for detecting both scintillation and optical probe light, ii) systems that use mirrors to deflect optical photons from the multi-energetic photon flux for external detection outside the field-of-view (FOV) of the PET system, and iii) systems utilizing light detectors that are mounted directly in front and within the FOV of PET detectors. The latter concept will be depicted in detail on the example of a novel optical detector concept that was built in our laboratory. An optical detector as proposed consists of a large-area photon sensor for light detection, a microlens array for field-of-view definition, a septum masks for cross-talk suppression, and a transferable filter for wavelength selection. Multiple detectors are allocated on a rotatable gantry forming a hexagonal detector geometry, and laser scanning and large-field light sources are integrated to facilitate fluorescence imaging. In concert with a newly developed post-acquisition mapping algorithm optical projection images are derived at a higher spatial object resolution than that of the detector's intrinsic capacity. Via back-projection focal planes can be calculated within the object space at arbitrary focus plane-to-detector distances yielding, under specifically applied diffuse lightning conditions, the segmented map of the complex anatomical surface of the imaged object which constitutes an essential constraint for non-contact optical tomography. Such light detector module can be placed inside any PET system with a bore opening of greater than 125 mm in diameter. We prove the working principle employing a clinical Siemens EXACT HR+ scanner. Finally, we discuss this dual-modal imaging embodiment and propose a further optimized fully integrated small animal PET-OT imager blueprint employing genuine raw PET detector blocks as used for dedicated small animal systems.

NANOPLATFORM-BASED MULTIMODALITY MOLECULAR IMAGING PROBES

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Nanotechnology, an interdisciplinary research field involving chemistry, engineering, biology, medicine, and more, has great potential for early detection, accurate diagnosis, and personalized treatment of diseases. Nanoparticles can be engineered as nanoplatforms for effective and targeted delivery of drugs and imaging labels by overcoming the many biological, biophysical, and biomedical barriers. For *in vitro* and *ex vivo* applications, the advantages of state-of-the-art nanodevices (nanochips, nanosensors, and so on) over traditional assay methods are obvious. Several barriers exist for *in vivo* applications in preclinical animal models and eventually clinical translation of nanotechnology, among which are the biocompatibility, in vivo kinetics, targeting efficacy, acute and chronic toxicity, and cost-effectiveness. This talk will exemplify the great potentials and challenges of nanoplatform-based molecular imaging agents, covering microbubbles, quantum dots, carbon nanotubes and iron oxide nanoparticles for ultrasound, near-infrared fluorescence, Raman, photoacoutistic, magnetic resonance imaging, as well as radionuclide (PET and SPECT) imaging applications.

MRI AND OPTICAL IMAGING

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Complementing structural and functional information, 'molecular' imaging methods provide *in-vivo* readouts on cellular and molecular events at the levels of transcription and translation products, individual signaling cascades, and/or protein-protein interactions. In pathological conditions, morphological and physiological aberrations are the result of abnormal molecular processes in tissue and it is a reasonable hypothesis that quantitative mapping of these events in vivo should increase both the sensitivity and the specificity of diagnostic tools.
As molecular events occur at low frequency highly sensitive imaging modalities are required providing high signal-to-background ratios. Therefore molecular imaging has evolved mainly in imaging disciplines other than MRI, in particular in optical and nuclear imaging. Optical techniques benefit from a variety of stable reporter systems available and from many assays that have been developed for imaging of cellular systems: molecular dyes, quantum dots and fluorescent and bioluminescent proteins like red shifted GFP derivatives or luciferases. Spectral properties of fluorophors should be in the red/near infrared spectra domain to allow good tissue penetration of light.
With the exception of monitoring cell dynamics[1] MRI has only recently been used for the visualization of molecular events in vivo. Translation of molecular information into MRI contrast has been achieved through different approaches: by coupling a targeting moiety to a paramagnetic or superparamagnetic reporter, through enzyme-catalyzed chemical modification of metal-based contrast agents or iron-binding/transporter to accumulate iron as a contrast agent (for review see Ref. 2) and recently gene-targeting short nucleic acids (oligodeoxynucleotides, or sODN) cross-linked to superparamagnetic iron oxide nanoparticles (SPION)[3]. A critical issue with exogenous probes is their delivery to the target, in particular when the target is located intracellular. A promising type of imaging agent introduced by Balaban et al.[4] relies on artificial proteins for imaging based on chemical exchange saturation transfer (CEST), proposing MRI-based sensors which can be analogously to fluorescent protein sensors be introduced as reporter gene generating an endogenous contrast without the need of an exogenous imaging agent.
Despite these scientific highly attractive approaches to get access to molecular information, the strength of MRI, at least currently, is that it provides high quality structural and physiological data. This is complementary to optical imaging methods such as fluorescence molecular tomography (FMT), which shows high sensitivity and specificity but poor spatial resolution (on the order of millimeters) due to photon scattering in tissue. A combination of FMT and MRI would benefit from the strength of the two approaches. Combination with MRI as alternative to FMT/CT approaches is attractive, as MRI for many body regions provides superior anatomical data for soft

tissues and in addition can be used to derive functional/physiological and metabolic information. The hybrid approach allows direct allocation of molecular signal to organ structures (images registration). In addition, the structural information derived from MRI (a priori information) can be used to improve reconstruction of optical data[5]. Several realizations of FMT/MRI systems are conceivable. The inherent problem is to handle the hostile environment due to the high magnetic field. The most obvious approach is to achieve coupling of light to the region of interest in the magnet using optical fibers. Such devices provide limited spatial resolution due to the restricted numbers of fibers that can be used and thus the limited number of source detector pairs available. The used of CCD cameras or array detectors similar to PET would reduce this limitation. Currently systems incorporating such features are under development. Although the technical realization of an FMT/MRI system is more complex than that of FMT/CT, which is already fairly advanced, the high potential information content that could be derived from MRI justifies the development of hybrid FMT/MRI.

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LONGITUDINAL AND MULTI-MODAL *IN VIVO* IMAGING OF TUMOR HYPOXIA AND ITS DOWNSTREAM MOLECULAR EVENTS

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The importance of hypoxia in the context of cancer is receiving increasing attention. Not only are hypoxic tumor cells more resistant to radio- and chemotherapy, but hypoxia also is generally associated with more aggressive tumor phenotypes. Intratumoral hypoxia triggers the stabilization and subsequent activation of Hypoxia Inducible Factor (HIF), an oxygen regulated transcription factor. In cancer, HIF has been shown to regulate tumor cell survival, malignancy and metastasis. **AIM:** In order to study the relationship between tumor growth, tumor hypoxia, stabilization and the activation of HIF we have generated an *in vivo* imaging toolset, which allows the longitudinal and non-invasive monitoring of these processes in a mouse allograft tumor model. **METHODS:** We used Positron Emission Tomography (PET) with the hypoxia sensitive tracer [^{18}F]-FMISO to quantitatively assess hypoxia in C51 (murine colon cancer cell line) tumor allografts s.c. implanted into the neck of Balb/c nude mice. In the same tumor model, we monitored the stabilization and the activity of HIF-1α using C51 reporter cells stably expressing either a HIF-1α-luciferase fusion construct or luciferase driven from a HIF sensitive promoter. *In vivo* luciferase activity was measured using the Xenogen IVIS 100 system. **RESULTS:** Longitudinal *in vivo* experiments in tumor allografts allowed semi-quantitative assessment of tumor hypoxia and HIF reporter activity over time (Fig. 1). **CONCLUSION:** Multimodal imaging combining PET and bioluminescence readouts allows studying tumor hypoxia related events in the context of cancer and evaluating effects of therapeutic interventions affecting the HIF pathway.

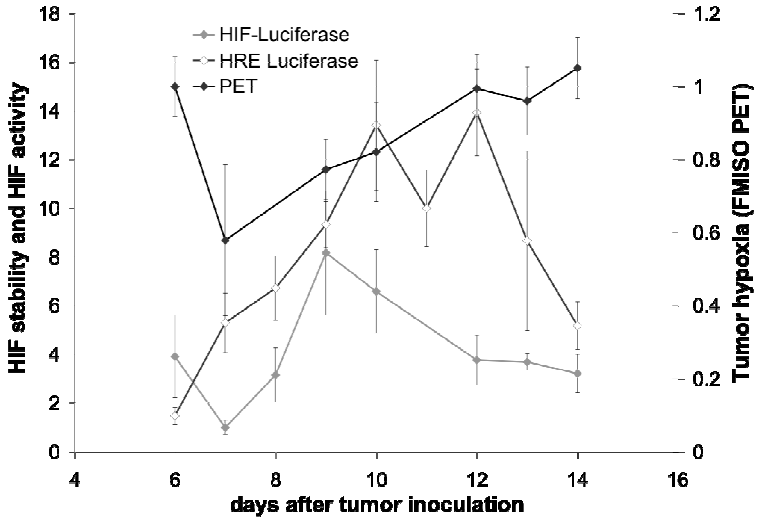

Figure 1 Combination of different imaging readouts. Luciferase activity(HIF stability and activity) was measured using the Xenogen IVIS 100.Tumor hypoxia was determined by quantifying the uptake of FMISO. Voxels were considered hypoxic when the activity displayed a tumor to muscle ratio gerater or equal to 1.4 (Koh et al, 1992). Values for all readouts are shown relative to those on day 6.

GPI ANCHORED AVIDIN- A NOVEL PROTEIN REPORTER FOR MULTIMODAL, *IN VIVO* IMAGING

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The use of reporter systems expressed on the extracellular side of the cell surface as molecular imaging targets has multiple advantages over conventional intracellular protein reporters: 1) endogenous expression levels are generally low - amplification in signal intensity is thus desirable and feasible for surface proteins, and 2) surface reporters can be targeted by multimodal imaging probes.
AIM: In this study, we are investigating the applicability of a novel reporter protein, glycosyl-phosphatidyl (GPI) anchored avidin, for *in vivo* imaging. Via its GPI anchor this protein is linked to the extracellular side of the cell membrane and can be targeted with biotinylated imaging probes. We would like to demonstrate that GPI avidin can be used to study promoter activity in allograft tumor models using *in vivo* imaging approaches.
METHODS: GPI avidin was fused to a hypoxia responsive promoter. The resulting reporter construct was stably expressed in murine C51 colon cancer cells. To evaluate the functionality and regulation of the reporter protein *in vitro* we performed 1) immunofluorescence stainings with biotinylated probes and 2) fluorescence associated cell sorting (FACS) after treating the reporter cells with DMOG, a chemical agent mimicking hypoxia. GPI avidin was subsequently targeted with a biotinylated optical probe in subcutaneous mouse tumors, formed from the reporter cells, using *in vivo* fluorescence reflectance imaging.
RESULTS: *In vitro* experiments confirmed the functionality and the oxygen dependent regulation of the reporter construct in stably transfected C51 cells. First *in vivo* experiments show an accumulation of a fluorescent, biotinylated imaging probe in subcutaneous tumors expressing GPI avidin.
CONCLUSION: Our results show that GPI avidin can be targeted *in vitro* and *in vivo* with biotinylated, fluorescent imaging probes. In a next step, we will study reporter expression with other imaging modalities.

THE ClearPET-/XPAD: DEVELOPMENT OF A SIMULTANEOUS PET-/CT SCANNER FOR MICE

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We are developing a bi-modal PET-CT imaging system for small animal. It consists of a ClearPET prototype associated to a micro CT system made of a RX source and an X-PAD detector.
Methods: The ClearPET prototype, built in Lausanne within the Crystal Clear collaboration, is now in CPPM, Marseilles. It is being modified both to use a better arrangement of the detectors and to allow for the integration of the micro-CT elements on the same rotating gantry. The XPAD, an X-ray photon counting detector developed at CPPM, is based on the silicon hybrid pixel tracker developed for the Atlas collaboration at CERN. It allows for the detection of photons above an adjustable energy threshold by 130 µm square pixels readout every ms without dead time.
Results: Monte Carlo simulations with the GATE software have been used to determine a new arrangement of the PET detectors, leading to a improved sensitivity [1]. Both simulations and measurements have been performed to evaluate the interaction of the diffuse flux from the X-ray source on the object with the PET detector operation (detection of coincidences). An appropriate shielding for the PET detectors has thus been design to allow for the simultaneous use of both modalities. The mechanical modification of the gantry is currently being done. The integration of all components should occur later this year.
Conclusion: The ClearPET-/XPAD has been designed and is being assembled. It will allow for simultaneous operation of a low dose micro-CT and a high resolution PET scanner for mice.

Reference:

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2007 IEEE NUCLEAR SCIENCE SYMPOSIUM CONFERENCE RECORD

A PET/OPTICAL METHOD FOR MOLECULAR IMAGING STUDIES

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In the research field of small animal imaging the combination of two or more imaging modalities can enhance the information content derived from the reconstructed images. In particular quantitative fluorescence Diffuse Optical Tomography (fDOT) can complement the micro-PET technique. The latter is already a highly established modality because of its superior sensitivity and the capacity of quantitatively monitoring molecular events inside small animals. On the other hand fDOT is rising as promising low cost method incorporating a broad palette of fluorescent contrast agents but its quantification performance is still under ongoing research.

In this study, we are using a dual PET/Optical probe to take advantage of the PET quantification accuracy. Images from the PET camera were used for the calibration of the DOT technique. Two series of experiments were performed for the evaluation of the co-registration approach and the calibration of the fDOT respectively. The first series of experiments consisted of capillaries containing a dual PET/Optical probe of known concentration placed under the skin of the mouse. In the second series of experiments, the probe is administrated directly to the animal by IV injection.

To ensure high precision in the co-registration of the reconstructed data a custom-made mouse supporting system was built to fit in several instrumentation geometries (PET/fDOT/CT). The initial results show that our co-registration approach is characterized of sub-millimeter precision. The intensity of the optical reconstructed signal from the kidneys and the liver has been correlated to the PET signal in a series of experiments. Future work involves the use of quantitative DOT along with PET for the simultaneous imaging of both PET and fluorescent agents targeting different biological compounds.

MICROSCOPIC AND MESOSCOPIC IMAGING

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The understanding of function of genes and treatment of disease often heavily relies on the ability to optically interrogate molecular and functional changes in an intact living organisms. But while we assist to an increasing focus towards the development of nanometer-resolution optical imaging methods, there have not been too many successful efforts in improving the imaging penetration depth. In fact most existing optical imaging methods are inadequate for imaging at dimensions that lie between the penetration limits of modern optical microscopy and the diffusion-imposed limits of optical macroscopy (>1cm). Pre-chirping for multiphoton microscopy can allow to reach penetration depths of the order of 600 microns, and molecular sensitive optical coherence tomography permits similar penetration depths via optical heterodyning. Therefore many important model organisms, e.g. insects, animal embryos or small animal extremities, remain inaccessible via optical imaging.

One possibility is to optically clear the samples through a chemical procedure and image them with selective plane microscopy or optical projection tomography. Once cleared, the samples present very low scattering and absorption values making their light diffusive contribution almost negligible. Both techniques provide very high resolution tomographic reconstructions and we have recently demonstrated imaging of molecular activity distribution within different organs. Unfortunately the clearing process requires to operate *ex-vivo*.

Alternatively, we can decide to take into account the tissues' diffusive properties. Mesoscopic fluorescence tomography is a method appropriate for non-invasive *in-vivo* imaging at dimensions of 1mm-5mm. The method exchanges resolution for penetration depth, but offers unprecedented tomographic imaging performance and it has been developed to add "time" as a new dimension in developmental biology observations by imparting the ability to image the evolution of fluorescence-tagged responses over time. As such it can accelerate studies of morphological or functional dependencies on gene mutations or external stimuli, and can capture the complete picture of development or tissue function by allowing longitudinal visualization of the same, developing organism.

DEVELOPMENT OF HIGH-RESOLUTION SMALL ANIMAL PET-CT AND FDOT-CT IMAGERS AND THEIR APPLICATION TO *IN-VIVO* BRAIN DEVELOPMENT

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The Laboratorio de Imagen Médica (LIM) at the Hospital Gregorio Marañón, is an interdisciplinary research faculty team of engineers, clinicians, biomedical and physical scientists focused on the development of biomedical imaging technologies and methods, and on their translation to clinical practice. LIM has developed a small animal PET-CT built around a micro-focus X-ray tube and a flat-panel detector assembled in a rotating gantry that is currently in commercial production. A new variation of this design replaces the PET with a non-contact FDOT imaging system, integrated with a high-resolution cone beam CT scanner into the same gantry with co-planar geometry. The FDOT laser is guided to the sample via two galvanometers and the photon density distribution at the surface of the subject is measured with a low-noise, cooled CCD camera. Geometrical projections at two different angles can be performed with the CCD detector parallel to the sample (placed at the FOV center between two antireflective plates), leading to 20000 source-detector pairs (100 sources and 100 detectors at each angle). On-going work consists of expanding the solution for any sample geometry, allowing more angular projections, hence more source-detector pairs. FDOT and XCT images are intrinsically registered by this system. New work with a challenging application for these technologies imaging the development of brain metabolic responses in late-stage chicken embryos in vivo will also be discussed.

ULTRA RESOLUTION SPECT COMBINED WITH OTHER MODALITIES

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Aim: To develop an extremely user friendly and reliable SPECT system with unsurpassed resolution that can be readily combined with other imaging modalities.
Methods: We constructed a new rodent SPECT system (U-SPECT-II [1]) and a CT scanner (U-CT) with dedicated multi-modal animal beds and registration software for localized sub–half-millimeter total-body SPECT. Three optical cameras and a dedicated interface are fully integrated with U-SPECT to allow pre-selection of the field-of-view without adding any dose to the animal. This facilitates focusing the pinholes, thereby maximizing sensitivity for the task at hand. Furthermore, the optical cameras allow for unique registration possibilities, in particular with images from independent optical modalities.
Results: SPECT resolutions better than 0.35mm where obtained for Tc-99m. Resolutions with In-111 and I-125 were barely degraded, compared to Tc-99m. Image registration of SPECT with CT was better than 0.2 mm.
Discussion: U-SPECT-II and combined U-SPECT-II/U-CT can be used for novel applications in the study of dynamic biologic systems and pharmaceuticals at the suborgan level. Discrimination of molecule concentrations between adjacent volumes of about 0.04 μL in mice and 0.5 μL in rats with U-SPECT-II is readily possible. Accurate anatomical localization can be done efficiently and cost-effectively with independent modalities such as CT or MRI. For imaging small animals separate systems offer unique advantages over integrated devices [2].

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DUAL MODALITY MR-SPECT IMAGING

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Aims: We have developed a dual modality imaging system (MR-SPECT) that is capable of acquiring simultaneous MRI and SPECT measurements. A significant challenge in this development was ensuring that the MRI and SPECT systems were compatible with each other.
Methods: The utilization of a cadmium-zinc-telluride (CZT) semiconductor detector that is capable of operating in high magnetic fields, along with proper electronic shielding allows for the MRI and SPECT components to function in the presence of one another. Integration of the two components was achieved through the use of a specialized RF coil that must be calibrated for the presence of additional lead shielding and the collimator.
Results: Artifacts arising from interference between the two components were dependent on the quality of the shielding and RF coil tuning. Such artifacts were minimized to generate simultaneous MR-SPECT images of various resolution phantoms. Methods to address the remaining residual artifacts were also conceived.
Conclusions: Use of CZT detectors and proper instrumentation setup allows for the simultaneous acquisition of MRI and SPECT measurements. The development of this MR-SPECT system will provide a new tool for molecular imaging research.

BRINGING THE BEST OUT OF LIGHT: MULTI-MODALITY PHOTONIC IMAGING

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Optical imaging is unequivocally the most versatile and widely used visualization modality in clinical practice and life sciences research. In recent years, advances in photonic technologies and image formation methods have received particular attention in biological research and the drug discovery process for non-invasively revealing information on the molecular basis of disease and treatment. An increasing availability of endogenous reporters such as fluorescent proteins and probes with physiological and molecular specificity enable insights to cellular and sub-cellular processes through entire small animals, embryos, fish and insects and have revolutionized the role of imaging on the laboratory bench, well beyond the capability of conventional microscopy. This talk describes current progress with instruments and methods for in-vivo photonic tomography of whole intact animals and model biological organisms. We show how new tomographic concepts are necessary for accurate and quantitative molecular investigations in tissues and why it could be potentially a valuable tool for accelerated investigations of therapeutic efficacy and outcome. We further demonstrate that cellular function and bio-chemical changes can be detected in-vivo, through intact tissues at high sensitivity and molecular specificity. Examples of imaging enzyme up-regulation, carcinogenesis and gene-expression are given. The potential for clinical translation is further outlined. Limitations of the method and future directions are also discussed.

NON-INVASIVE 3D MULTI-MODALITY IMAGING OF TUMOR PROGRESSION AND OSTEOLYTIC BONE METASTASES IN MOUSE MODELS OF CANCER.

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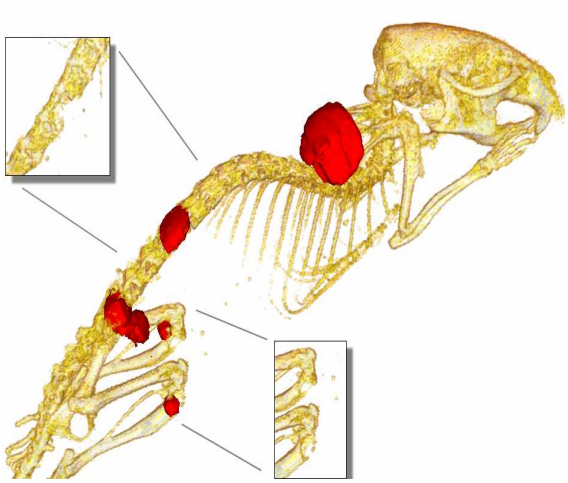
Breast and prostate cancer metastasize preferentially to bone and often leads to osteolytic or osteosclerotic lesions respectively. The development of novel anti-cancer strategies requires more sensitive and less invasive methods to detect and monitor in vivo tumor progression, metastasis and minimal residual disease in cancer models.
Whole body optical imaging, either Bioluminescent (BLI) or Fluorescent (FLI), is the most versatile, sensitive and powerful tool for Molecular Imaging in small animals. We previously have shown that with BLI we can detect small numbers of cells non-invasively and that it enables the quantification of tumor growth and metastasis within an intact animal. Optical imaging, although extremely sensitive, has been based on 2D planar images and, therefore, spatial resolution was poor. New developments have now made it possible to extend FLI and BLI to three-dimensional imaging by 3D optical tomography allowing better quantification of photon emission. In addition, fusing 3D optical images with images obtained from the same animal using MRI or fast CT allows obtaining structural anatomic information and will greatly enhance spatial resolution. Furthermore, structural tissue information obtained by fast CT (and MRI) allows generating a tissue atlas that can be used to correct for tissue-dependent photon scattering and absorption. This will allow to obtain better quantitative data.
In the present work we have used the 3D IVIS BLI system from Xenogen and the 1178 fast CT system from Skyscan to detect osteolytic bone metastases. We also have combined BLI with FLI using Osteosense to detect bone turnover, cRGD-Cy5.5 to detect angiogenesis and EGF-CW800to detect EGFR status.

This mouse was injected intracardiac with MDA-231-luc cells and was scanned 40 days later. Using our in house developed INTEGRIM software 3D reconstruction could be made of the fused images showing a large bone metastases in the scapula, two in the vertebrae of the spinal cord, one in the left femur and two in the metaphyses of both tibia. Osteolytic lesions are also clearly seen from the fast CT scan (inserts). Pictures shown are derived from the actual 3D movies.



A MULTI-SPECTRAL RECONSTRUCTION ALGORITHM FOR MULTIMODALITY TOMOGRAPHIC IMAGING

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Aims: Multi-spectral imaging is emerging as a very powerful tool for in vivo optical imaging. It allows the detection of multiple fluorophores that can be associated with different processes, thus enabling simultaneous multi-targeted imaging.
Methods: In this work we present a volumetric spectral unmixing algorithm capable of separating signals from different probes combined with 3D rendering of tomography data. The algorithm can be used for both fluorescence and absorption modalities, enabling thus the distinction of multiple fluorophores as well as multiple absorbers. The method can be applied for visualizing independent biological processes and pathways, such as cell population variations as well as physiological parameters, such as oxygen saturation and hypoxic burden.
Results: In the first case the method was used to distinguish DsRed- and GFP-labelled T cells in Rag-/- mice and follow in vivo the change in population upon reaction to an antigen-presenting peptide affecting only the DsRed cells. The optical tomographic technique was used to extract information from measurements on four targets for as long as five days after administration of the peptide.
In the second case the method was applied in reconstructing in 3D Oxy- and Deoxy-hemoglobin concentrations and thus visualizing oxygen saturation and blood volume during tumor growth. When both modalities are used, absorption and fluorescence data can be co-registered and directly compared, since measurements and analysis have been performed concurrently and with the same experimental parameters.
Conclusions: We have presented a multi-spectral algorithm that can be used for both fluorescence and absorption data to produce accurate and quantitative 3D reconstructions of fluorophores' and absorbers' concentrations and allow the detection and following of biological pathways and physiological parameters *in vivo*.

COMBINING LIGHT AND SOUND – MULTISPECTRAL OPTOACOUSTIC TOMOGRAPHY FOR HIGH RESOLUTION MOLECULAR IMAGING

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We have developed a multi-spectral optoacoustic tomography (MSOT) method capable of high resolution visualization of molecular bio-markers, including fluorescent proteins and probes, deep within living optically diffusive organisms. **Methods:** The method is based on ultrasonic detection of pressure waves generated by the absorption of pulsed light in elastic media. As such, it offers three-dimensional tomographic ability of optical contrast in conjunction with high spatial resolution resulting from diffraction-limited ultrasonic detection. Additionally, we employ multispectral illumination along with accurate normalization for photon propagation in tissues in order to provide quantified visualization of molecular probe distribution. **Results:** Using MSOT, we were able to achieve high resolution whole-body visualization of flurescent probe distribution in mice as well as tissue-specific expression of eGFP and mCherrry fluorescent proteins in Drosophila melanogaster pupa and the adult zebrafish. So far, those widely used model organisms were not accessible at their particular developmental stages by any of the existing optical microscopy techniques due to an extensive light scattering. For our method, we further demonstrate single cell sensitivity and penetration depths of centimeter and beyond in tissue-mimicking phantom experiments.
Conclusion: This technology fills a significant area in biological imaging that goes way beyond the penetration limit of modern microscopy and can become the method of choice in studying signaling pathways and gene expression, morphogenesis, decease progression and many other targeted mechanisms through whole bodies of opaque living organisms and animals with high spatial resolution and sensitivity.

MULTIMODAL IMAGING OF CELLULAR THERAPY IN CLINICAL TRIALS

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Successful dendritic cell (DC) therapy in cancer patients requires the interaction of functional DCs and immune cells in lymph nodes (LNs). Multimodal imaging enables evaluation of both functionality and localization of injected DCs. We labeled DCs with ^{111}In for quantification via scintigraphy. To obtain anatomical data, we labeled DC with SPIO for localization with MRI. This yielded images with sharp anatomical detail and allowed for immunohistochemical validation. However, metal-based contrast agents are not readily amenable to quantification. Thus, we carried out preliminary studies with clinically-applicable ^{19}F MRI.

Methods: Melanoma patients, scheduled for lymph node resection, were injected i.n. with autologous DC labeled with SPIO and ^{111}In. After vaccination patients were monitored with scintigraphy and MRI (3T). Radioactive LNs were resected and analyzed by immunohistochemistry and studied by MRI (7T).

Results: We show by scintigraphy that only a small proportion of injected DCs emigrate from the injection site. MR imaging allowed assessment of both accurate DC delivery and inter-/intranodal migration patterns. Moreover, histology revealed SPIO-labeled DCs in the T cell areas of the injected and proximal LNs presenting tumor antigen and activating $CD8^+$ T cells.

Conclusion and Future prospects: By exploiting different imaging techniques, we are now able to elucidate the fate of *ex vivo* generated DC in clinical studies. As the next step towards monitoring the immune system *in vivo*, ^{19}F MRI is a promising tool for longitudinal cell tracking and quantification. Updated results on clinically-applicable ^{19}F label for *in vivo* DC tracking, allowing for both anatomical data and cell quantification will be presented.

ULTRA-HIGH RESOLUTION SPECT WITH INTEGRATED OPTICAL CAMERAS

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Aims: In focusing pinhole SPECT, to achieve better sensitivity and resolution it is important to choose the field of view (FOV) as tight as possible around the structure of interest. To this end we integrated optical cameras with SPECT to localize organs (e.g., using an atlas). The optical images are also convenient for registering images of other modalities (CT, PET, MR, a second SPECT) with SPECT. Here we determine the accuracy of the correspondence between optical and SPECT images.
Methods: prior to SPECT, optical images of the animal are acquired from left, top and right. Correspondence between optical images and SPECT is determined by calibration. The user designates the desired FOV on the optical images. Organs invisible from the outside can be localized using an atlas, which can be superimposed on the optical images. To measure the accuracy of the correspondence between the optical images and the reconstructed volume, we scanned and reconstructed a physical phantom containing multiple point sources.
Results: Point source measurements show that the correspondence between optical images and SPECT has accuracy below 0.5 mm.
Conclusion: Scan planning based on optical imaging can be sufficiently accurate and helps to achieve significant image improvements in focusing pinhole SPECT. The combined setup can also be applied in scan planning for other modalities such as CT, MRI and PET and for a variety of image registration tasks.

MULTIMODAL IMAGING OF TUMOR ANGIOGENESIS

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Angiogenesis is crucial for growth and spread of tumors and numerous angiogenesis inhibitors have been developed to treat cancer. In preclinical and clinical studies antiangiogenic drugs have proven promising and some have already gained clinical approval. Nevertheless, their effectiveness could be improved by individualising therapy concerning drug selection, combination and dose. In this regards, it is important to comprehensively understand the physiological consequences of antiangiogenic inhibitors and to have non invasive imaging tools that sensitively assess therapy response.
In this context, measures such as microvascular structure, mean vessel diameter, relative blood volume, perfusion, permeability, vessel maturity, as well as several molecular markers are of high interest. Often they have to be used in concert to gain a comprehensive understanding of vascular remodelling. Unfortunately, there is no single imaging modality that can provide all this information. Therefore, in this presentation it is intended to discuss the different parameters of vascular remodelling and function and to define the most suitable imaging modalities to determine the respective parameter in preclinical research. It will introduce high resolution µCT and MRI, DCE MRI, HF-US, intermittent ultrasound, and molecular imaging with ultrasound, MRI and optical methods.

MULTI-MODALITY IMAGING OF BRAIN TUMORS USING FLUORESCENCE MOLECULAR TOMOGRAPHY, MRI AND CT

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Metabolic pathways for certain types of brain tumors such as gliomas lead to preferential accumulation of the fluorescent molecule Protoporphyrin IX resulting from over-exposure to the precursor molecule 5-aminolevulinic acid (ALA). For example, clinical studies have shown that following the oral administration of ALA, glioblastoma multi-forme (GBM) brain tumors typically lead to large fluorescence contrast-to-background ratios making it possible to discriminate normal and malignant tissue. We are presenting instruments and methods developed to image certain types of brain tumors using PpIX fluorescence in conjunction with more conventional imaging modalities such as CT and MRI. On the clinical side, we are describing a surgical method used to improve the completeness of tumor resection. Initial results of on-going clinical trials are presented with emphasis on those technical aspects relating to studying the effectiveness of fluorescence-guided resection coupled to standard MR-guided neuronavigation techniques. We are also presenting results of brain tumor studies conducted using two small animal fluorescence molecular tomography instruments. Both systems are time-resolved with one being based on frequency-domain technology and the other acquiring time-domain data using single-photon counting techniques. These devices were built in conjunction with other modalities providing structural anatomical information used as prior knowledge by the optical tomography algorithms (MRI for the frequency-domain system, CT for the time-domain imager). We are also presenting novel tomography methods using time-domain data to improve the resolution of optical images.

PRECLINICAL AND CLINICAL MULTIMODALITY IMAGING: SELECTED TRANSLATIONAL EXAMPLES

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Multimodality imaging strategies based on the use of electromagnetic waves (SPECT-CT, PET-CT and MRI) are used daily in clinical routine for fusion of morphologic and functional or metabolic data in order to increase non invasive diagnostic accuracy and to improve follow-up of therapy and patient throughput. In the meantime, preclinical multimodality imaging strategies, based on the same techniques but dedicated to small animal (mice and rats), are applied for better understanding molecular mechanisms of diseases or for improving diagnosis and drug development. Preclinical imaging strategies are currently increasing due to technological developments improving spatial and temporal resolutions and sensitivity.
Based on the daily clinical and preclinical experience of our department, we will emphasize the translational interest of multimodality imaging in oncology and cardiology illustrated by selected examples.
With the help of dedicated multimodality imaging chambers (MINERVE, France), attention will be focused on the importance of maintaining homeostasis of the small animal during long acquisition times which are often observed in preclinical imaging.
MicroSPECT-CT (explore SPECZT Vision 120, GE Healthcare, USA) and microSPECT-low-field MRI (developed in our laboratory) dual modalities will be presented and illustrated with several applications.
Regarding the field of preclinical multimodality imaging analysis, we will also emphasize the need not only for quantification but also for the importance of registering databases of normal references values in particular for cardiac applications in Gated-micro-SPECT.

IMAGING OF INFLAMMATION

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Inflammatory processes are the crucially involved in many diseases, which have a high impact on the health in western societies. Techniques that enable non-invasive visualization of specific inflammatory processes are therefore highly desirable. They enable a better understanding of pathophysiology, a more targeted treatment of inflammation, a more reliable monitoring of response to anti-inflammatory treatment, and are thus highly important for the development of new anti-inflammatory therapies. The immune system may attack own body cells or tissues, as in autoimmune diseases such as rheumatoid arthritis. In arteriosclerosis, the immune system responds to the damage to the artery wall caused by oxidized low-density lipoprotein leading to inflitration of inflammatory cells into the lesion and the development of arteriosclerotic plaques. The immune system can also be activated by injury, as for example in stroke, secondarily enhancing lesion growth. Several approaches are described in non-invasive inflammation imaging using unspecific or specific imaging compounds targeting different processes. Examples include the use of unspecific extravasation markers or probes that bind specifically to cells or molecules crucially involved in inflammation. Furthermore, it has been shown that immune cells can me tracked with non-invasive imaging techniques. The presentation provides an overview of our current possibilities in specific visualization of inflammatory processes using different imaging modalities focusing on arthritis, arteriosclerosis, and stroke.

DUAL BIOLUMINESCENCE AND FLUORESCENCE IMAGING COMBINED WITH INTRAOPERATIVE IMAGING

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Since almost a decade, bioluminescence imaging (BLI) has proven its value in various field of scientific research varying from infectious diseases, to cardiovascular diseases and oncology, among others. In oncology it has proven to be one of the most sensitive in vivo imaging modalities, especially when it concerns superficial tumours to be studied. Besides being a very powerful tool in the study of non-invasive tumor pathogenesis, it can also be considered the gold standard in optical imaging to compare other optical modalities with. While bioluminescence involves the introduction of a reporter gene into the genome of a tumor cell, this technique will most certainly not find its way into the clinic and as such other modalities seems more appropriate, like near-infrared fluorescence (NIRF) imaging. Several preclinical studies have been conducted to show its potential in future human applications like ovarian and breast cancer. Currently, NIRF is in its clinical testing phase for intraoperative usage and as such needs a gold standard to compare with the localisation and presence of tumor tissue while visual observation by the naked eye alone is not sufficient. BLI does seem to fulfil the criteria to act as such a gold standard. In the presentation preclinical mouse and rat models of colorectal cancer and breast cancer will be presented in order to point out the simultaneous use of BLI in concordance with NIRF for preclinical testing of NIRF imaging systems and optical contrast agents in an intra-operative setup.

MRI GUIDED FOCUSED ULTRASOUND

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Local temperature elevation may be used for tumor ablation, gene expression, drug activation, gene and/or drug delivery. High Intensity Focused Ultrasound (HIFU) is the only clinically viable technology that can be used to achieve a local temperature increase deep inside the human body in a non-invasive way. MRI guidance of the procedure allows in situ target definition and identification of nearby healthy tissue to be spared. In addition, MRI can be used to provide continuous temperature mapping during HIFU for spatial and temporal control of the heating procedure and prediction of the final lesion based on the received thermal dose. The primary purpose of the development of MR guided HIFU was to achieve safe non-invasive tissue ablation. The technique has been tested extensively in preclinical studies, and is now accepted in the clinic for ablation of uterine fibroids. MR guided HIFU for ablation shows conceptual similarities with radiation therapy. However, thermal damage generally shows threshold like behavior with necrosis above the critical thermal dose, and full recovery below. MR guided HIFU is being clinically evaluated in the cancer field. The technology also shows great promise for a variety of advanced therapeutic methods such as gene therapy. MR guided HIFU, together with the use of a temperature sensitive promoter, provides local, physical, spatio-temporal control of transgene expression. Specially designed contrast agents, together with the combined use of MR and ultrasound, may be used for local gene and drug delivery.

OPTICAL IMAGING IN BREAST CANCER: FROM BENCH TO BEDSIDE

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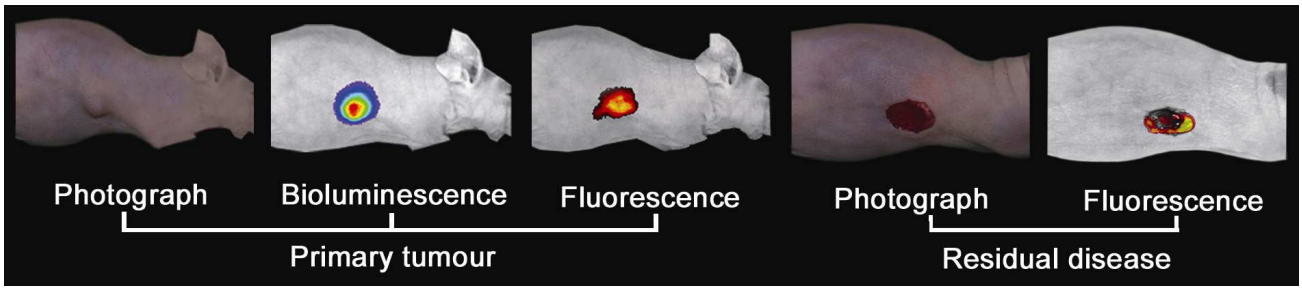
Aim: There is a clear interest and development in near-infrared fluorescence (NIRF) optical imaging in translational research for clinical applications. In this study, the potential of applying NIRF for tumour delineation in the surgical treatment of breast carcinoma was addressed.
Methods: Nude mice were injected with 2x10e6 human MDA-MB-231-luc-D2-H2LN breast cancer cells in the mammary fat pad. Tumour growth was evaluated by bioluminescence imaging. The primary tumour was surgically removed after 4-6 weeks using a NIRF camera system in combination with two optical contrast agents (MMPSense, ProSense) for the intra-operative detection of the primary tumour.
Results: Bioluminescence was found to be an adequate measure for tumour size. NIRF optical imaging was able to detect breast cancer cells in accordance with bioluminescence imaging (gold standard). Furthermore, NIRF imaging detected remnant disease after resection of the primary tumour, confirmed by histology.
Conclusion: NIRF imaging, combined with NIRF optical contrast agents, offers a sensitive method for intra-operative detection of the primary tumour and evaluation of tumour delineation in a breast cancer mouse model. A prototype clinical NIRF camera system together with clinical grade NIRF fluorescent optical contrast agents are in development and testing phase for clinical use.



CO-REGISTRATION OF FLUORESCENCE AND OXYMETRY USING MULTI-MODAL OPTICAL TOMOGRAPHY

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Aims: Oxygen delivery is a fundamental mechanism regulating tumour metabolism. We investigated the relationship between the variability of hypoxia and its effect on tumour growth.
Methods: Fluorescence Molecular Tomography (FMT) is a new technique that detects fluorescence in small animals *in vivo*. We have coupled FMT capabilities with Near Infrared Spectroscopy (NIRS) based oxymetry measurements. The OxyFMT method provides a way to combine a 3D quantitative and volumetric map of fluorophore concentration with one of oxygen saturation and blood volume. HeLa cells labelled with the far-red emitting protein Katushka were implanted in Rag-/- mice and imaged on a daily basis.
Results: We present data from a longitudinal study on tumour xenografts in which the fluctuations in hypoxic burden were followed concurrently with tumour growth. Our results show a correlation between tumour growth and hypoxic burden, consistent with theoretical models of oxygen metabolism.
Conclusions: We suggest fluorescence tomography, combined to tomographic oxymetry, are suitable for modelling the temporal dynamics of hypoxic burden and their consistency and accuracy underline their collective potential to be used for integrated functional and physiological applications.

CARDIAC IMAGING IN MICE USING MULTI-ISOTOPES GATED SPECT AND GATED CT

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We have investigated the feasibility of imaging the mouse heart with single and multiple energy ECG gated SPECT combined with ECG gated CT in healthy and infarcted animals.
Methods: 7 adult mice (Swiss) weighting 35+/-3g were investigated including 2 control mice and 5 mice 4 weeks after a surgical myocardial infarction by ligation of the descending coronary artery. A preclinical SPECT/CT imager (eXplore SPECZT Vision 120, GE Healthcare) was used with a rotating 7 pinholes collimator (1mm hole) for SPECT and 80KV-32mA step and shoot 220 projections for CT. Two radiopharmaceuticals (^{201}Tl and Tetrofosmin-^{99m}Tc) were used for myocardial perfusion and blood pool was imaged with Albumin-^{99m}Tc. Radiopharmaceuticals were administered IP and/or IV. For CT a vascular contrast agent (FenestraVC®) was administered IP. Mice were anesthetized with isoflurane 1.5% and single and multiple isotopes acquisition schemes were tested. ECG Gated SPECT and CT data were analyzed using Microview®, Mirage® and BPGS® softwares.
Results: Isotropic reconstructed SPECT and CT voxel sizes were respectively 330µm and 100µm. Up to 16 time bins per cardiac cycle were obtained for gated SPECT (in a retrospective way) and 10 bins per cardiac cycle were obtained for gated CT (in a prospective way) with a mean total acquisition time of 1h 30 for both modalities. Left (LV) and right (RV) ventricular volumes and corresponding ejection fractions as well as LV necrotic tissue and aneurismal wall sizes were obtained.
Conclusion: *In vivo* ECG gated single and multiple isotopes SPECT schemes combined with ECG gated CT demonstrated powerful capabilities for preclinical SPECT/CT cardiac studies in mice.

MULTIMODALITY TECHNIQUES FOR STEM CELL THERAPY IN CARDIAC MUSCLE REPAIR

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Atherosclerosis is the main cause of myocardial infarction and death in western developed countries. Therapies are not able to reverse the destructive cascade that occurs after acute myocardial infarction. Cell therapy for cardiac repair is one of the most promising treatments of cardiovascular diseases. To develop these very promising techniques, “open”, compact, dedicated detectors with high sensitivity and sub-millimeter spatial resolution are needed for the imaging of atherosclerosis in small animal models. In fact, multimodality imaging techniques are needed for this task. Examples are SPECT/CT, PET/CT combined with optical and MRI imaging techniques modalities. A collaboration was started by the Rome group as a leading partner with the research goal to build a SPECT detector compatible with MRI and/or optical detectors. So far, we have designed and implemented a single photon emission computed tomography (SPECT) system for molecular imaging of cardiovascular diseases with spatial resolution of 300 μm to 500 μm, sensitivity of ~0.3 cps/kBq and an active detector area of 100×100 mm^2. The first prototype is made of a tungsten collimator, a continuous or pixellated scintillator and position-sensitive photomultiplier tubes (PSPMT). Careful choice of the ratio between the dimensions of the scintillator pixel and spacing of the anodes of the PSPMT was needed in order to get high detector intrinsic resolution. For the electronic readout, an individual channel readout with self-triggering capability was designed and built to improve trigger sensitivity, signal quality and flexibility in the data processing phase. A second phase of the system development has been planned and initiated. The photodetector system is being made of silicon photomultipliers (SiPMs) that are insensitive to magnetic fields. This will allow building a prototype of detector to be tested and used in a MRI field of up to at least 7 Tesla for future multi-modality SPECT-MRI system. The PET option has also been also considered. A PET/SPECT/MRI system would allow monitoring of up to three different biological processes. Practical problems of animal handling and routes of delivery of stem cells are being considered. We are starting to study mice with infarction by using SPECT techniques. We will monitor the diffusion of stem cells injected in the mouse’s heart and the effect of therapy. This will be possible by using dual tracer technique, for stem cells labeled with ^{111}In Oxine or

transduced with NIS gene that take up ^{99m}Tc pertechnate.

The radiotracer has to be injected repeatedly, so it would be impossible to use tail vein to deliver the radiotracer. Perfusion measurements have been performed on mice with two routes of delivery, i.e., tail vein and peritoneum injection. Our results indicate that injecting the radiotracer in the peritoneum is possible. However, the uptake in the heart in the later case is lower than for tail vein injections and requires an increase in system's sensitivity. In conclusion, a powerful microSPECT detector system has been designed and partially implemented for molecular imaging of cardiovascular disease in animal models. The performance characteristics of the prototype detector are as theoretically predicted/expected. The evolution of the system for the integration in a multimodality system has been initiated. A photodetector module using SiPMs that are insensitive to magnetic fields is under study. The electronic readout will be similar to that of a detector system based on PSPMT's. To match the dynamic range a different ASIC will be used.

POLARIZATION SENSITIVE SECOND-HARMONIC GENERATION IN NONLINEAR MICROSCOPY

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Aims: The polarisation dependency of second-harmonic generation (SHG) imaging in microscopy can be used to determine the orientation and degree of organization of different SHG elementary source molecular structures (called harmonophores). **Methods:** Fibrilar collagen type I and thick filaments in the anisotropic band (A-band) of the muscle sarcomeres in *C. elegans* body walls were imaged by analysing the SHG intensity variation on the incoming polarization (PSHG) in a microscope (1.4 NA). The obtained images are fitted into a generalised biophysical model that assumes hexagonal symmetry and the nonlinear properties of single-axis organic molecules.

Results: For our PSHG images, we are able to retrieve the effective orientation, θ_e, of the harmonophores and its distribution function. For the body walls muscle the distribution function is centred at $\theta_0 = 63^o$ and has a bandwidth of $\Delta\theta_e = 4^o$ (*FWHM*), and for collagen these values are $\theta_0 = 47^o$ and $\Delta\theta_e = 3^o$.

Conclusion: This methodology allows estimating the effective orientation of harmonophores. Here we have shown that these are different for collagen and for muscle structures. Therefore, this methodology has the potential to be used as discrimination tool for differentiating SHG active structures (information unreachable by conventional SHG microscopy). In addition, this methodology allows estimating the degree of organization of the harmonophores by obtaining the distribution function of the θ_e angles using an optical technique.

MULTIMODAL NANOPARTICLE FOR TUMOR CHARACTERIZATION

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The inherent differences and the complementary nature of existing imaging systems has prompted the quest for multimodality imaging platforms that allow for integration of images acquired at different scales and at various stages of disease detection and treatment. It has become clear that along with hardware and software approaches to multimodality imaging there is a corresponding need to develop contrast agents that can be detected across multiple imaging systems. The successful multimodality contrast agent has the potential to seamlessly bridge wide ranges of spatial, temporal and sensitivity scales and to be employed throughout a variety of clinical scenarios. This talk will focus on the use of a liposome-based multimodality agent for tumor characterization.
Methods: Our research group developed a modular liposome platform that is able to provide prolonged signal enhancement in a number of different imaging modalities (CT, MR, SPECT, PET and/or optical). CT (GE Discovery ST) and MR imaging (GE Signa TwinSpeed 1.5T with head coil) were performed on VX2-sarcoma bearing rabbits over a 14-day period. Micro-CT (GE Locus Ultra), micro-PET (Siemens Focus 220) and optical *in situ* imaging (Leica FCM1000) were performed on mice bearing H-520 NSCLC xenografts.
Results: The multimodal liposome imaging platform has shown to successfully target both primary and metastatic tumor lesions via the enhanced permeation and retention phenomenon as a result of its long vascular half-life (18h in mice and 65h in rabbits). The maximum tumor-to-muscle contrast enhancement ratio was measured to be 13 ± 5 at 7 days post-administration in rabbits. At 48h post-injection, the liposomes distributed throughout $72 \pm 5\%$ of the total tumor volume. *In vivo* and *ex vivo* confocal imaging detected the majority of liposomes to reside in the perivascular regions at 7 days post-administration. The addition of an angiogenic endothelial target resulted in increased tumor accumulation and delayed clearance.
Conclusion: Our proof-of-principle studies demonstrated the feasibility of incorporating combinations of different imaging agents on a single nano-carrier for characterization of tumors over a broad range of sensitivity, as well as spatial and temporal resolutions.

NEW HYBRID OPTICAL TECHNIQUES TO NON-INVASIVELY MEASURE OXYGEN METABOLISM

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We have introduced "diffuse correlation spectroscopy" (DCS) for measurement of blood flow in tissues. We combined it with diffuse optical spectroscopy (DOS/NIRS) to measure oxygen metabolism.
Methods: We concentrate on the application of hybrid DCS/DOS on cerebrovascular diseases such as ischemic stroke and traumatic injury and demonstrate its clinical utility.
Results: DCS has been validated against arterial spin labeled MRI, transcranial Doppler Ultrasound, Laser Doppler flowmetry, fluorescent microspheres and against secondary (non-)invasive measures of physiologiy. DCS/DOS detected distruptions of cerebral autoregulation in ischemic stroke and traumatic brain injury. Clinical feasibility is demonstrated in a range of subjects from premature babies to aging adults.
Conclusion: DCS is a new optical technique that measures microvascular blood flow in deep tissues. It is readily utilized in a multi-modality setting and combined measurements allow access to microvascular oxygen metabolism.

MULTI-MODAL IMAGING OF ACUTE AND SUBACUTE ISCHEMIC STROKE – *EXPERIMENTAL PERSPECTIVES*

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After occlusion of a cerebral artery, onset of focal cerebral ischemia occurs immediately but most severe cerebral blood flow (CBF) suppression may be reached and spontaneous partial or complete recovery of CBF may subsequently develop with some delay. Local CBF alterations coupled to transient depolarisations (cortical spreading depolarisations or peri-infarct depolarisations, CSD/PID) emerge spontaneously at the border of ischemic foci and propagate over cerebral cortex adding to dynamic CBF alterations in acute and subacute stroke. Infarct size seems to be determined by the number of depolarisations, but the basis of this relationship is unclear. - We investigate dynamics of CBF using Laser Speckle Flowmetry (LSF) real-time imaging and repetitive PET imaging in experimental models of focal ischemia. LSF is also used as a surrogate measure to detect CSD/PID. PET allows comparing CBF with other parameters like oxygen or glucose uptake, or expression of peripheral benzodiazepine receptors (PBR) on resident microglia to study inflammatory responses. We show with LSF that waves of CSD/PID cycle repeatedly around the perimeter of ischaemic lesions in the cerebral cortex, resulting in regular periodicity of depolarisation, and enlarging the lesion with each cycle. Our PET results indicate that neuroinflammation occurs in the peri-infarct zone, and that energy demand increases in this zone, possibly resulting from repetitive CSD/PID and inflammatory processes. We argue that CSD/PID cycling mediates upregulation of neurobiological responses (whether beneficial or damaging) to a focal brain lesion, and present evidence from clinical monitoring to suggest that depolarisations may cycle in the injured or ischemic human brain.

THE CHALLENGE OF MULTI-MODAL IMAGING OF ACUTE ISCHEMIC STROKE – CLINICAL PERSPECTIVE

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In modern stroke therapy, neuroimaging plays a key role for the understanding of pathophysiology and for the monitoring of therapy. It is also key tool for translating basic research findings into clinically effective therapy. Current imaging modalities (mainly CT, MRI and PET) focus on different aspects of pathophysiology. Multimodal stroke imaging of stroke pathophysiology" has two major goals:

a) Comparative approach: Positron emission tomography (PET), the gold standard for the *in vivo* imaging of metabolic changes and perfusion after ischemia, introduced imaging markers of penumbra and tissue damage in human stroke. Within the past decade, MRI (magnet resonance imaging) and CT (computed tomography) based surrogates have become available in the clinical setting. Using the mismatch concept, modern neuroimaging is increasingly used clinical decisions. However, there are several open questions concerning perfusion measurement in CT and MRI including the choice of the adequate parameter map, the postprocessing procedure, and the applied thresholds. Due to these methodical constraints, the current use of mismatch imaging by CT and MRI –although used in clinical routine- still suffers from methodical drawbacks. Thus, the ongoing validation of imaging techniques (CT or MRI vs PET) and the clinical validation of innovative imaging protocols are of major importance for stroke therapy.

b) Additive approach: In the future, MRI will be used *in addition* to PET in order to achieve an integrated imaging model of cerebral ischemia. This approach will combine detailed morphological information (DW/PW-MRI=tissue infarction and perfusion) with functional information (PET = receptor imaging, metabolic imaging/molecular level). This approach will yield detailed pathophysiological information about the local impairment and about remote disturbances of widespread receptor systems. Time dependent dynamic changes can be described by longitudinal measurements. PET based markers of cerebral hypoxia, of neuroinflammation and of relevant neurotransmitter networks will be studied in relation to early MR findings in order to specify the mismatch concept.

This approach of "clinical molecular imaging" will generate the rationale for new therapeutic strategies in terms of reperfusion, neuroprotection and early rehabilitation.

PET/MRI: THE NEXT STEP IN MULTIMODALITY IMAGING

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Several dedicated small animal imaging technologies are commercially available, including X-ray computed tomography (CT), magnetic resonance imaging (MRI), optical imaging (OI), ultrasound (US), single photon emission computed tomography (SPECT), and positron emission tomography (PET). These systems provide a wealth of information which is highly complementary. The unrivalled advantage of PET stems from tracking radiolabeled biomarkers with a detection sensitivity reaching concentrations in the pico-molar range, whereas MRI and CT provide high resolution anatomical information. MRI also enables a large variety of tissue contrasts, diffusion imaging, magnetic resonance spectroscopy (MRS), and functional MRI (fMRI). Thus, to combine two or even more imaging modalities providing complementary information, such as morphology and function, is a worthwhile goal. Although the combination of PET and CT has already been realized PET/CT bears many limitations. The major drawback of PET/CT is that the imaging is performed sequentially rather than simultaneously, adding in preclinical studies significant time under anaesthesia for the subjects and eliminates any temporal correlation between the parameters. Furthermore, the CT has limited soft tissue contrast and the radiation dose can be high enough to perturb the animal model under study. Hence, a preferred choice would be to combine PET and MRI. This talk reviews the current activities in combining PET and MRI into one single multimodality imaging system and shows first *in vivo* applications in rodents. Finally, an outlook to first clinical systems will be provided and the problem of MRI based PET attenuation correction will be discussed.

IMAGING OF INFLAMMATION IN EXPERIMENTAL STROKE

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Brain inflammation is a hallmark of stroke, where it has been implicated in tissue damage as well as in repair. Imaging technologies that specifically visualize these processes are highly desirable. They provide powerful tools to further evaluate the contribution of specific processes to the pathophysiology of CNS disease. Moreover, these technologies may be valuable in detecting and assessing disease progression, in stratifying patients for therapy, and in monitoring therapy. The presentation gives an overview on how current imaging technologies and various imaging probes can be used to visualise brain inflammation in rodent models of stroke, with a distinct focus on optical brain imaging. Applications comprise among others the detection of blood-brain barrier impairment with unspecific probes, visualisation of the inflammatory receptor CD40 with a targeted probe and the detection of matrix metalloproteinase activity using activatable probes. The challenges in the use of imaging instrumentation, as well as in the development of imaging probes, including the testing of probe specificity and design of favourable mechanism of contrast generation, are discussed in the context of brain inflammation imaging.

MULTIMODALITY IMAGING: PROSTATE CANCER DIAGNOSIS AND FOLLOW UP BY TOF-PET & MRI/MRS

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Prostate cancer is the most frequently occurring cancer in men and causes a large number of preventable deaths if not diagnosed at an early stage. Nevertheless early detection of prostate cancer is a real challenge both from the cancer biology and detector performance's points of view. Functional and specific imaging techniques are needed. Indeed complementary value can be provided by dedicated nuclear medicine molecular imaging techniques. In fact MRI or ultrasound imaging of prostate are primarily focusing on the structural information, while the functional metabolic or molecular imaging attained with nuclear medicine modalities can offer adjunct information, non only in the diagnosis but also in the staging phase and in the follow-up of the therapy. Moreover, sufficiently specific radiopharmaceuticals are still lacking. Fortunately some progress has been recently and researches in both cancer biology and imaging detector fields realize that they have to work together to succeed in providing the needed imaging for early diagnosis of prostate cancer. This picture makes evident the need of multimodality imaging that enables the combination of anatomical, functional and molecular information by combining images from different modalities taken at the same point, such that biochemical activities can be detected, quantified and registered to a precise location in the organ. In the specific prostate case combination of PET and MRI can have a substantial adjunct value if one could combine it with MRS. In fact the experimental MRI-compatible PET with F18- or C11-choline seems a promising new protocol for prostate cancer. Combining it with MRS allows diagnosis of prostate cancer by detecting the ratio choline/citrate that would greatly enhance the early cancer detection capability. A research program was started in ISS, INFN and in University of Rome, in collaboration with Jefferson lab and West Virginia University (WVU), to look at the possibility of building a TOF – PET detector compatible with a MRI scanner to allow building a prototype for TOF-PET&MRI imaging of prostate cancer. The main technical challenge is related to the interference between the components of the two kinds of detectors. Great progresses have been made lately in this area and prototypes, and even working detectors, exist for small animal imaging. Imagers for brain and whole body imaging will be available soon. Nevertheless there is need (and possibility) for further significant improvements. In fact most of the new detectors are of PET – MR type. They use Avalanche Photo Diodes (APDs) that are insensitive to magnetic fields but are slow

and do not allow to fully benefit from the Time of Flight (TOF) capability of PET detectors. The TOF capability provides significant advantages to the PET imager in terms of Signal To Noise Ratio (SNR), especially in the case of imaging internal organs such as prostate, but it depends strongly on timing resolution. As an example, the advantage in terms of SNR increases from ~1.5 to ~5.2 (for 40 cm diameter objects) with improving time resolution from 1200 ps to 100 ps FWHM. The time resolution of the detector is affected by the scintillator choice and its layout, on the photodetector characteristics and on front-end and digitizing data acquisition readout electronics. Fast scintillators and photodetectors, as well as highly integrated fast ASICs are needed to optimize the timing resolution that presently in working detectors is ~ 600 ps FWHM. Improving this parameter together with the right choice of scintillator and photodetectors is the main part of the collaboration research project. Standard PET is not suited for this task: it is too far away from prostate, so the spatial resolution (6-12mm) and detection efficiency (< 1%) are poor. Moreover it accepts activity outside the organ. A compact endorectal probe (similar to the ultrasound probe or MRI prostate coil for geometry) seems to be the potential choice. An external partial ring detector or panel is sufficient to operate with the probe. The proposed PET probe is made of pixellated scintillator coupled to Silicon Photomultipliers (SiPMs) that so far were shown to be insensitive up to at least 7 Tesla fields in studies performed at WVU. The work continues towards timing resolution ~ 200 ps – 300 ps, needed to get rid of the major overlaying background outside the prostate.

AN NF-κB INDUCIBLE BIDIRECTIONAL PROMOTER

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Co-regulated expression of multiple proteins is a valuable tool in experimental biology and gene therapy. For instance, studies of physiological processes and disease progression may require different types of reporter genes to monitor expression in individual cells (e.g. in tissue section) and in the living animal. In gene therapy, correlated expression of therapeutic transgenes and a marker gene allows identification of the targeted cells. Furthermore, multiple proteins may be necessary to accomplish the desired therapeutic effect.
Bidirectional arrangements of genes are naturally occurring in approximately 10% of the vertebrate genome, and we here describe a simple approach for assembly and optimization of inducible bidirectional promoters. We have used this approach to generate a bidirectional promoter, which is regulated by the transcription factor NF-κB and that express the two reporter genes GFP and luciferase. We have produced a transgenic mouse with this construct and show expression of the two reporter genes.

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