Project No: 201792
Project Acronym: FMTXCT
Project Full Name: Hybrid Fluorescence Molecular Tomography and X-ray Computed Tomography system and method.

Periodic Report

Period covered: from 01/03/2010 to 28/02/2011
Start date of project: 01/03/2008

Project coordinator name:
Prof. Vasilis Ntziachristos

Project coordinator organisation name:
HELMHOLTZ ZENTRUM MUENCHEN
DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH

Date of preparation: 19/04/2011
Date of submission (SESAM):
19/04/2011 16:21:16 CET

Version: 1
### PROJECT PERIODIC REPORT

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<td>Hybrid Fluorescence Molecular Tomography and X-ray Computed Tomography system and method.</td>
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<td><strong>Name of the scientific representative of the project's coordinator and organisation:</strong></td>
<td>Prof. Vasilis Ntziachristos HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH</td>
</tr>
<tr>
<td><strong>Tel:</strong></td>
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<tr>
<td><strong>E-mail:</strong></td>
<td><a href="mailto:v.ntziachristos@helmholtz-muenchen.de">v.ntziachristos@helmholtz-muenchen.de</a></td>
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<td><strong>Project website address:</strong></td>
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Declaration by the scientific representative of the project coordinator (1)

I, Prof. Vasilis Ntziachristos HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH, as scientific representative of the coordinator of the project FMTXCT and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

The project has fully achieved its objectives and technical goals for the period.

The attached periodic report represents an accurate description of the work carried out in this project for this reporting period.

The public website is up to date.

To my best knowledge, the financial statements which are being submitted as part of this report are in line with the actual work carried out and are consistent with the report on the resources used for the project (section 6) and if applicable with the certificate on financial statement.

All beneficiaries, in particular non-profit public bodies, secondary and higher education establishments, research organisations and SMEs, have declared to have verified their legal status. Any changes have been reported under section 5 (Project Management) in accordance with Article II.3.f of the Grant Agreement.

<table>
<thead>
<tr>
<th>Name</th>
<th>Prof. Vasilis Ntziachristos HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH</th>
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This declaration was visaed electronically by Vasilis NTZIACHRISTOS (ECAS user name nntziava) on 19/04/2011 at 19/04/2011 16:21:16 CET.
1. Publishable summary

Summary description of project context and objectives

The overall goal of this project, is to develop a truly unique imaging system, the likes of which exists nowhere by combining Fluorescence Molecular Tomography (FMT) and X-ray CT (XCT) into a hybrid, quantitative system and method, engineer the optimal theory and inversion approaches for achieving a highly performing and synergistic system and perform pre-clinical imaging with a view towards clinical translation and therapeutic intervention. The work of the project is split into 9 work packages including two work packages (WP1 and WP9) exclusively dealing with management, co-ordination, training and dissemination activities.

Description of work performed and main results

Year 1 and 2

Ahead from schedule within WP5 a fully functional FMT-XCT prototype, based on a commercial XCT system, has been developed. First in-vivo studies have been already performed, using proprietary, user-friendly software to control the acquisition. The self-developed gantry (CT Imaging, WP2) which could offer faster acquisition times through minimization of FMT/XCT interference was developed. As intended within WP2 CEA Leti has designed and implemented a dual energy micro CT imaging system. Both the functional prototype and a calibrated, dual energy processing software have been demonstrated during the training session in July 2009 and are available to the consortium.

WP 3 and WP4 engineer the optimal theory and inversion approaches to achieve the best performing synergistic system. FORTH in strong collaboration with UZH, UCL, CEA-LIME and FIHGM has developed an ultra-fast inversion algorithm which was already extensively tested with phantom and experimental data. The multispectral algorithm and a user friendly software, dramatically improve usability, efficiency and attractiveness to the end user have been completed (WP3). Furthermore a fast image reconstruction algorithm using data compression and a combined reconstruction-classification method for diffuse optical tomography were developed and published. (WP4).

As intended in the proposal WP6 and 7 successfully developed and evaluated various cancer animal models as well as different targeted fluorescent probes which are available to all partners. Furthermore essential aspects of hypoxia induced signaling were already investigated. To evaluate the imaging performance of the FMT-XCT scanner and compare with PET/CT, different optical phantoms were customized and distributed amongst the partner within WP8. First measurements have been performed.

Two training session (WP9) were carried out in 2009. A workshop on "Advanced X-Rays imaging techniques" was organized by CEA Leti, a second one on "Reconstruction Methods" was organized by UCL.

Year 3

One of the main tasks of the third year was to install the final FMTXCT prototype (Work package WP5) at HMGU, which was finalized in cooperation with Ct-imaging. Additionally within WP5 training data sets have been acquired and uploaded to common data server, algorithms for the assignment of optical properties were integrated in the reconstruction code, optimal acquisition parameters have been defined and a user friendly software has been installed and is operational on acquisition.

The CT acquisition software tool is designed such that it supports all free parameter as defined by CEA-LETI (WP2). The selection of an appropriate XCT technology for FMT-XCT system was the main objective of WP2 for Year 3. Therefore WP2 can be considered as completed. In addition - independent of the final prototype developed within WP5 - FIHGM (beneficiary 5) also developed an FMT-XCT prototype and made it available to the consortium members (WP2, WP4) for the acquisition of the dual-energy and contrast agent enhanced XCT datasets.

The work performed in WP3 has been focused on finalizing the multispectral algorithm, implementing the multispectral capacity into the fast inversion approach and quantitatively evaluating the direct inversion with hybrid data. Also WP3 can be considered as "successfully completed". Within WP4 "FMT Inversion with Priors" the theory behind FMT reconstructions using XCT image priors has been explained and the inversion algorithms were tested on both simulations...
and real data, provided by partners 1 (HMGU) and 5 (FIHGM). The algorithm implementation is based on the software package "Time-resolved Optical Absorption and Scattering Tomography" (TOAST) developed at UCL. Finally the user-friendly software that incorporates the new inversion algorithm was developed within WP4.

The use of animal models is essential in order to evaluate FMT-XCT for its intended application, i.e in-vivo imaging. In WP 6 mouse models of breast cancer as well as other cancer types were developed and first FMTXCT in vivo experiments have been performed. Additionally within WP6 commercial and custom made probes targeting tumours or tumor related processes were developed and evaluated. The task of WP7 is to research methods for monitoring treatment response, first experiments were performed. Additional a FMT-MRI system (funded by another source) was developed and essential aspects of hypoxia induced signaling were investigated. Since the success of the project depends to large extend on the exchange of knowledge a third training session on animal imaging (WP9) was carried out in January 2011 in Zürich (UCL). The communication between the different partners is excellent and special ties have been developed virtually between all work-packages. The collaborative working infrastructure is coupled with effective communication. There are no known problems within the network. A regular exchange of information between the members of the consortium and the project co-originator have taken place. A webpage (http://www.fmt-xct.eu), presenting the FMT-XCT European funded project to the public, was developed and is up-dated regularly.

### Expected final results and potential impacts

This system advances multi-modality imaging by offering the first FMT-XCT system worldwide and thereby increases the competitiveness of European health care biotechnology and medical technology sectors. The system can enable new levels of therapeutic discovery, by yielding highly accurate information on animal models of disease and quantitatively resolving effects of treatment in-vivo and longitudinally on the same animal. In this role it can facilitate time-efficient and accurate observations of a large number of possible treatment combinations and optimize dose of drugs and radiation as a function of a particular cancer. The technology can also be used in key clinical applications as well, i.e, in breast cancer imaging or arthritic joint imaging. In this role this technology can be used as an efficient imaging tool for personalized medicine, especially since the system can be used for frequent observations of treatment progression and help in patient specific decision making.

**Project public website address:** http://www.fmt-xct.eu

### 2. Core of the report

**Project objectives, Work progress and achievements, and project management during the period**

The Project Summary Pdf document contains the core of the report.
### 3. Deliverables and milestones tables

**Deliverables (excluding the periodic and final reports)**

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4. Explanation of the use of the resources

**HELMHOLTZ ZENTRUM MUENCHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH**

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<th>Explanations</th>
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**COMMISSARIAT A L ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES**

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### FOUNDATION FOR RESEARCH AND TECHNOLOGY HELLAS

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### UNIVERSITY COLLEGE LONDON

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### FUNDACION PARA LA INVESTIGACION BIOMEDICA DEL HOSPITAL GREGORIO MARANON

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**Total:** 79172.839999999998

### UNIVERSITAET ZUERICH

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**CT Imaging GmbH**

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### Grant Agreement number:
201792

### Project acronym:
FMTXCT

### Project title:
Hybrid Fluorescence Molecular Tomography and X-ray Computed Tomography system and method.

### Funding Scheme:
FP7-CP-FP

### Project starting date:
01/03/2008

### Project end date:

### Name of the scientific representative of the project's coordinator and organisation:
Prof. Vasilis Ntziachristos HELMHOLTZ ZENTRUM MUECHEN DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH

### Period covered - start date:
01/03/2010

### Period covered - end date:
28/02/2011

### Name

### Date
19/04/2011

This declaration was visaed electronically by Vasilis NTZIACHRISTOS (ECAS user name nntziava) on 19/04/2011 at 19/04/2011 16:21:16 CET.
**Publishable Summary**

**Summary description of project context and objectives**

The overall goal of this project is to develop a truly unique imaging system, the likes of which exists nowhere by combining Fluorescence Molecular Tomography (FMT) and X-ray CT (XCT) into a hybrid, quantitative system and method, engineer the optimal theory and inversion approaches for achieving a highly performing and synergistic system and perform pre-clinical imaging with a view towards clinical translation and therapeutic intervention. The work of the project is split into 9 work packages as shown in the figure below, including two work packages (WP1 and WP9) exclusively dealing with management, co-ordination, training and dissemination activities.

**Description of the work performed since the beginning of the project and the main results achieved so far.**

**Year 1 and 2**

Ahead from schedule within WP5 a fully functional FMT-XCT prototype, based on a commercial XCT system, has been developed. First in-vivo studies have been already performed, using proprietary, user-friendly software to control the acquisition. The self-developed gantry (CT Imaging, WP2) which could offer faster acquisition times through minimization of FMT/XCT interference was developed. As intended within WP2 CEA Leti has designed and implemented a dual energy micro CT imaging system. Both the
functional prototype and a calibrated, dual energy processing software have been demonstrated during the training session in July 2009 and are available to the consortium.

**WP 3** and **WP4** engineer the optimal theory and inversion approaches to achieve the best performing synergistic system. FORTH in strong collaboration with UZH, UCL, CEA-LIME and FIHGM has developed an ultra-fast inversion algorithm which was already extensively tested with phantom and experimental data. The multispectral algorithm and a user friendly software, dramatically improving usability, efficiency and attractiveness to the end user have been completed (WP3). Furthermore a fast image reconstruction algorithm using data compression and a combined reconstruction-classification method for diffuse optical tomography were developed and published. (WP4).

As intended in the proposal **WP6 and 7** successfully developed and evaluated various cancer animal models as well as different targeted fluorescent probes which are available to all partners. Furthermore essential aspects of hypoxia induced signaling were already investigated. To evaluate the imaging performance of the FMT-XCT scanner and compare with PET/CT, different optical phantoms were customized and distributed amongst the partner within **WP8**. First measurements have been performed.

Two training session (WP9) were carried out in 2009. A workshop on “Advanced X-Rays imaging techniques” was organized by CEA Leti, a second one on “Reconstruction Methods” was organized by UCL.

**Year 3**

One of the main tasks of the third year was to install the final FMTXCT prototype (**Work package (WP) 5**) at HMGU, which was finalized in cooperation with Ct-imaging. Additionally within **WP5** training data sets have been acquired and uploaded to common data server, algorithms for the assignment of optical properties were integrated in the reconstruction code, optimal acquisition parameters have been defined and a user friendly software has been installed and is operational on acquisition.
The CT acquisition software tool is designed such that it supports all free parameter as defined by CEA-LETI (WP2). The selection of an appropriate XCT technology for FMT-XCT system was the main objective of WP2 for Year 3. Therefore WP2 can be considered as completed. In addition - independent of the final prototype developed within WP5 - FIHGM (beneficiary 5) also developed an FMT-XCT prototype and made it available to the consortium members (WP2, WP4) for the acquisition of the dual-energy and contrast agent enhanced XCT datasets.

The work performed in WP3 has been focused on finalizing the multispectral algorithm, implementing the multispectral capacity into the fast inversion approach and quantitatively evaluating the direct inversion with hybrid data. Also WP3 can be considered as "successfully completed". Within WP4 "FMT Inversion with Priors" the theory behind FMT reconstructions using XCT image priors has been explained and the inversion algorithms were tested on both simulations and real data, provided by partners 1 (HMGU) and 5 (FIHGM). The algorithm implementation is based on the software package “Time-resolved Optical Absorption and Scattering Tomography” (TOAST) developed at UCL. Finally the user-friendly software that incorporates the new inversion algorithm was developed within WP4.

The use of animal models is essential in order to evaluate FMT-XCT for its intended application, i.e in-vivo imaging. In WP 6 mouse models of breast cancer as well as other cancer types were developed and first FMTXCT in vivo experiments have been
performed. Additionally within WP6 commercial and custom made probes targeting tumours or tumor related processes were developed and evaluated. The task of WP7 is to research methods for monitoring treatment response, first experiments were performed. Additional a FMT-MRI system (funded by another source) was developed and essential aspects of hypoxia induced signaling were investigated.

Since the success of the project depends to large extend on the exchange of knowledge a third training session on animal imaging (WP9) was carried out in January 2011 in Zürich (UCL). The communication between the different partners is excellent and special ties have been developed virtually between all work-packages. The collaborative working infrastructure is coupled with effective communication. There are no known problems within the network. A regular exchange of information between the members of the consortium and the project co-originator have taken place. A webpage (http://www.fmt-xct.eu), presenting the FMT-XCT European funded project to the public, was developed and is up-dated regularly.

Expected final results and their potential impacts and use (including socio-economic impact and the wider societal implications of the project so far)

This system advances multi-modality imaging by offering the first FMT-XCT system worldwide and thereby increases the competitiveness of European health care biotechnology and medical technology sectors.

The system can enable new levels of therapeutic discovery, by yielding highly accurate information on animal models of disease and quantitatively resolving effects of treatment in-vivo and longitudinally on the same animal. In this role it can facilitate time-efficient and accurate observations of a large number of possible treatment combinations and optimize dose of drugs and radiation as a function of a particular cancer.

The technology can also be used in key clinical applications as well, i,e, in breast cancer imaging or arthritic joint imaging. In this role this technology can be used as an efficient imaging tool for personalized medicine, especially since the system can be used for frequent observations of treatment progression and help in patient specific decision making.
PROJECT PERIODIC REPORT 3

CORE OF THE PROJECT

PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PROJECT ACRONYM: FMT-XCT

GRANT AGREEMENT NUMBER: 201792

FUNDING SCHEME: Collaborative project (small or medium-scale focused research projects)

DATE OF LATEST VERSION OF ANNEX I AGAINST WHICH THE ASSESSMENT WILL BE MADE:
March 6th, 2008

PERIODIC REPORT: 3rd

PERIOD COVERED: from March 01, 2010 to February 28, 2011

NAME, TITLE AND ORGANISATION OF THE SCIENTIFIC REPRESENTATIVE OF THE PROJECT’S COORDINATOR:
Prof. Dr. Vasilis Ntziachristos
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Ingolstaedter Landstr. 1
85764 Neuherberg
TEL: +49-89-3187-4180
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E-MAIL: v.ntziachristos@helmholtz-muenchen.de
PROJECT WEBSITE ADDRESS: http://www.fmt-xct.eu
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1) Project objectives for the period

No recommendations from previous report (report 2). The project objectives of
reporting period 3 (March 01, 2010 – February 28, 2011) are, as included in Annex I of
the Grant Agreement, as follows:

Objectives for the Work Package 1- Management

WP leader: HMGU

1.1. Management of the interaction of the co-ordinator, the Executive Committee and
the Advisory Committee in order to design, monitor and optimise the experiments.

1.2. Management of the pre-existing and new intellectual property (IP) and know-how.

1.3. Maintenance of the Consortium Agreement.

1.4. Regular meetings and reports on the scientific and financial progress, ethical and
welfare issues.

Objectives Work Package 2 - XCT Development

WP leader: CEA-LETI

2.4 To research and minimize possible interference of X-ray with optical components.

2.5 To research the contrast between organs and tissues achieved by the dual energy
method.

2.6 To research the contrast between organs and tissues achieved by contrast agents

2.7 To provide an optimal XCT design to be incorporated with the free- space FMT
system in WP5

Objectives Work Package 3 -Theory for 360-degree FMT

WP leader: FORTH

3.2 To research optimal direct inversion approach with simulations and experimental
data

3.6. To invert training data acquired from FMT-XCT system for algorithmic finalization.
**Objectives Work Package 4 - FMT inversions with image priors**  
*WP leader: UCL*

4.5 To quantitatively examine optimal inversion methods based on experimental data.
4.6 To develop user-friendly software for inversion of FMT-XCT data based on a-priory inversion.

**Objectives Work Package 5 - FMT-XCT integration**  
*WP leader: HMGU*

5.1 To develop a fully functional multi-spectral XCT-FMT prototype and minimize XCT and FMT interference
5.2 To integrate algorithmic developments from WP2, WP3 and WP4 and control software operating the hardware components.
5.3 Acquire training data sets and optimize image settings.
5.4 To provide optical attenuation maps corresponding to the collected XCT images.

**Objectives Work Package 6 - Cancer imaging with focus on breast cancer**  
*WP leader: CEA-Lime*

6.3 To develop animal models of other cancer for studying FMT-XCT performance.
6.4 To perform in-vivo imaging of key animal models of cancer and correlate the findings with standard laboratory tests and growth measures.

**Objectives Work Package 7 - Imaging Cancer Therapy for enabling intervention**  
*WP leader: UZH*

7.1 To determine the accuracy of the FMT-XCT system in imaging breast cancer response to standard chemotherapeutic protocols as compared to histological gold standards.
7.2 To measure the quantification accuracy of the FMT-XCT method to assess standard chemotherapy effects vs. combinations of chemotherapy with targeted therapy.
7.3 To phenotypically characterize an animal model developed for HIF-related pathways in vivo using conventional FMT and compare imaging findings with FMT-XCT.

**Objectives Work Package 8- FMT-XCT imaging accuracy vs. PET-XCT**  
*WP leader: FIHGM*

8.1. To develop hybrid FMT-PET-XCT phantoms.
8.2 To develop aptamers labelled with fluorescence and 18F for PET to image cancer of the same animal model and offer exact co-registration for validation purposes.

Objectives Work Package 9 - Training and Dissemination

WP leader: HMGU

9.1 Training of scientists on FMT-XCT technology and underlying technologies.
9.2 Dissemination of the results and progress within the partners and to scientific, industrial and public sectors.
9.3 Technology transfer activity.
Work plan of FMT-XCT: Objectives for the reporting period 3 are highlight in green.
2) Work progress and achievements during reporting period 3
March 01, 2010 – February 28, 2011

Work Package 2 - XCT Development

WP leader: CEA-LETI

Written by: Veronique Rebuffel, CEA-LETI (Partner 2), Marco Brambilla, CEA-LETI (Partner 2), with contribution from: Markus Mronz, CT-Imaging (Partner 7) and Juan José Vaquero, FIHGM (Partner 5)

Summary

The different tasks and the corresponding planning are given by the following table: initial planning in grey, revised one in green (from revisions accepted at Year 2 meeting). The milestone corresponding to WP2 is M5; it consists in the "selection of an appropriate XCT technology for FMT-XCT system", and should be achieved in two steps: preliminary recommendation on month 18, and official decision by the executive committee on (initially) Month 24, postponed to Month 29 at Year 2 meeting.

The first year of the project was mainly concerned by tasks 2.1 and 2.2. A complete requirements specification was produced in Nov. 2008 (deliverable D2.1 - XCT design) and approved by HMGU. The second year was devoted to completion of tasks 2.2, and 2.3, summarized in the following deliverables, distributed in March 2010: D2.2 – A functional prototype for dual energy cone beam XCT, and D2.3 – Calibrated, dual energy processing software, D2.4 – Preliminary technical specification for XCT design to be implemented with the hybrid system.

Task 2.7 was also completed in Year 2, leading to a deliverable common with WP5: D2.7 – Final Technical Specifications for XCT system and D5.3 – Gantry development.

Achievement of tasks 2.4 was postponed, and only a draft version of the corresponding deliverable was provided. Tasks 2.5 and 2.6 (comparison of contrast strategies) were delayed. The reasons of the required delay were:

- CEA-LETI bench is different from final prototype,
- CEA-LETI bench is not convenient for extensive tests using living mice,
- Final prototype was not available for experiments at end of Year 2.

During Year 3, task 2.5 and 2.6 were completed, even if additional experiments using the final prototype would clearly consolidate the conclusions. Task 2.4 is still not completed because of the lack of availability of the final system. These points are discussed hereafter.

Progress towards objectives

Task 2.4 “Minimization of interference of X-ray with optical components”

The objective of this task is to provide an estimation of the radiation field which the optical components of the hybrid prototype will be subject to in order to allow the
design and the optimization of a suitable screen, in first instance for the optical sensor, which, being a very sensitive device, could be damaged if reached by high energy radiation.

During year 2, a preliminary study has been carried out based on CEA-LETI bench and presented in Deliverable D2.5 – Measurement of scattered X-rays. This document is only a draft, because it is based on measurements performed on the CEA-LETI bench: since the scattered field is highly dependent on the geometry of the bench, on the X-ray generator and on the objects and the materials physically present around the X-ray chain, diffuse radiation measurements should be repeated on the final prototype in order to have an accurate estimation of the radiation that can reach the sensitive components. Task 2.4 is partially completed and could be easily completed as soon as measurements on the final prototype would be possible.

Task 2.5 “Contrast evaluation between organs and tissues achieved by the dual energy method” and

Task 2.6 “Contrast evaluation between organs and tissues achieved by contrast agents”

Notice that tasks 2.5 and 2.6 are related within a common objective, which is the comparison of contrast enhancement strategies in order to choose the best one to be provided as high resolution prior to FMT reconstruction algorithms. The corresponding deliverable is D2.6.

Experiments at FIGHM

LETI bench is not convenient for extensive tests using living mice. Because of vertical position, fixation by forelegs, successive CT scans (Dual energy + with contrast agent) are not possible on a living mouse. Due to the fact that final prototype was not yet available when the measures were performed, FIGHM proposed to CEA-LETI to perform experiments using an easily usable bench at their site. The objective was to directly compare the two contrast enhancement strategies on the same machine and on the same mice instead of, as it was done until now, on different machines and different mice, thus avoiding inter-machine and inter-animal variability. The FIGHM system is different from the final FMT-XCT hybrid prototype; nevertheless the characteristics of the two scanners are sufficiently close to consider the obtained results valid also on FMT-XCT machine.

The FIGHM FMT-XCT system

During the present year, the FMT-XCT prototype developed in FIGHM has been finished and it has been made available to the consortium members for the acquisition of combined FMT-XCT data and for the acquisition of the dual-energy and contrast agent enhanced XCT datasets to be compared looking for the maximal achievable contrast between organs. The CT subsystem integrated in the FMT-XCT prototype is a cone-beam CT system based on a flat-panel X-ray detector and a micro-focus X-ray source. The micro-focus X-ray source is a modified Hamamatsu L9631 in which the control unit has been split from the high voltage source and the X-ray tube, thus significantly reducing the size and the weight of the device. The size and weight reduction obtained simplifies to a great extent the integration of the source in a moving gantry. The X-ray source has a power of 50W with maximum peak energy of 110keV and maximum anode current of 0.8mA. The flat-panel X-ray detector included in the prototype is a Hamamatsu C7940DK-02 which consists of a needle shaped CsI:Tl scintillator directly deposited on top of the CMOS detector surface, with a 1m thick carbon input window placed on top of the scintillator. The detector has a pixel pitch of 0.05mm that can be expanded by means of pixel binning (up to 4x4 binning). The minimum integration time for the acquisition of an image is 125ms when 4x4 binning is used. The detector is placed on a motorized linear stage which allows its radial
movement to set the desired Field Of View (FOV) size and magnification factor (that fixes the achievable resolution in the reconstructed data). Besides the possible modification of the FOV size and magnification factor, the motorized stage allows the data acquisition performing non-circular trajectories. Thanks to the selectable FOV size and magnification factor it is possible to acquire high-resolution truncated datasets that can be joined with a low quality, low dose dataset to obtain truncation artefact free, high resolution reconstructed datasets of small areas of the sample. The system mounts also a motorized linear translation stage for the positioning of the animal and the axial extension of the FOV size. To stop the X-ray flux outside the acquisition system, the prototype is equipped with a shielding box integrated in the rotating gantry that blocks also the scattered radiation that can be harmful for the FMT acquisition components and the light reaching the FMT FOV.

FMT-XCT system built at FIHGM.

FIHGM welcomed Marco Brambilla from CEA-LETI on 16-17 December 2010 at Madrid. Four different experiments were carried out on 2 mice, changing at each experiment the contrast agent or the way it was administered to the animal (intravenous iopamiro, intravenous Fenestra, intraperitoneal iopamiro, oral ingestion iopamiro, at different time after injection/ingestion). Experimental system and protocols are detailed in deliverable D2.6 as well as the resulting CT images and contrast evaluation.

Results and conclusion

An example based on Intravenous iopamiro is given hereafter. In this protocol, contrast agent rapidly diffuses from the blood flow to the kidneys, that are readily put in evidence (figure 1 b & c) in the image with an absorption coefficient closer to bones rather than to soft tissues. Also the low energy image (figure 1 a) allows to clearly distinguish kidneys, but the achievable contrast in not comparable at all with the one obtained in contrast agent images. Quantification of the contrast is performed by tracing a profile plot of the reconstructed x-ray linear attenuation coefficient as reported in figure 2.
Figure 1. Comparison of the images obtained with the low energy configuration of the dual energy imaging protocol (a) and with the contrast agent protocol (b and c). Image b was acquired 5’ after CA injection, image c 15’ after CA injection.

Figure 2. Contrast comparison between low energy image and intravenous Iopamiro contrast agent protocol. On the right the profile plot of the reconstructed signal and on the left the images and the corresponding profile.

Other protocols are presented and the corresponding obtained contrasts are discussed in D2.6.

To summarize ours conclusion, our sensation is that when a very specific organ is of interest for a given study and if a suitable contrast agent and/or a related administration protocol are available to target it, the attainable contrast enhancement is by far superior compared to what can be obtained without a contrast enhancing product. On this point we also recall that other more complex protocols are cited in literature and used for small animal imaging (but also on human patients) that combine the use of a contrast agent with a dual energy technique to further improve contrast when a contrast enhancing drug is to be used. On the contrary, when this is not possible, the low energy configuration is a good technique to push to the maximum the imaging performances of the machine in use.

**Investigation of other contrast enhancement strategies**

The dual-energy protocol initially planned in Annex1 (decomposition upon a material basis) was found to be not convenient for FMT-XCT context, and the dual-energy
protocol finally chosen required less development efforts. CEA-LETI proposed to use the corresponding saved man-months to investigate an alternative technique for soft tissues contrast enhancement: X-ray phase contrast imaging (XPCI). This novel technique, exploiting the wave nature of X-Rays, allows putting in evidence very low contrast features at material boundaries, especially when dealing with low Z material and low energy. The challenge is to implement this technique using a laboratory X-Ray source rather than a synchrotron one.

This study has been carried out at no additional man-months. A short report has been issued (attached to deliverable D2.6) presenting a review of the different XPCI techniques and preliminary evaluation. No experiment has been performed, neither the FMT-XCT generator nor the detector being suitable. Our conclusion is that XPCI could be investigated for mice (2cm of tissue is a maximum for the technique) but at acquisition time not acceptable for FMT-XCT need. A complete system should be specified, outside of scope of this study.

**Deviation from Annex1**

The main deviation from Annex1 or more precisely from the modified tasks and planning approved at Year 2 meeting is due to the delay of availability of the prototype. The prototype development got partially delayed because it was unclear what would be the best way to finalize the FMTXCT prototype and how to operate it. Thus to accelerate the system integration of both the FMT-chain and the CT-chain HMGU and CT-Imaging worked out a concept to provide a fully functional and operational prototype. CT Imaging was commissioned by HMGU to realize the concept. The concept comprised of basically four steps:

1) Providing a mechanical interface for the FMT components,
2) Upgrading the machine control to support some FMT components,
3) Providing a software tool enabling the user to start a standard CT acquisition
4) Providing an interface between the machine control and the existent FMT-application used by HMGU.

All tasks have been successfully accomplished by CT-Imaging and the system was delivered to HMGU (Klinikum Rechts der Isar, Munich) on March 09, 2011. The CT acquisition software tool is designed such that it supports all free parameter as defined by CEA-LETI. That means the CT acquisition can be run with the acquisition protocols suggested by CEA-LETI. This tool can be run independent of the FMT application. A detailed description of the current system design can be found in deliverable 5.4 (HMGU). For WP2, this delay of prototype has resulted in delays of deliverables D 2.5, D 2.6 and M5 (see revised planning hereafter).

**Revised planning**

The final prototype was delivered to HMGU (Munich) on March 09, 2011. As soon as it is available for X-ray measurements, these tasks will be possible:

**Finalization of Deliverable D2.5**

Measurements of scattered energy depend strongly on the tube and the environment. They have to be performed on the final prototype. They will allow the optimization of the CCD camera shielding. The pre-deliverable D2.5 could then be finalized.

**Eventual update of Deliverables D2.4 and D2.7**

The optimized dual-energy protocol has been initially defined for LETI bench. Thanks to simulation, a final protocol was specified, that is slightly different due to the X-Ray tube, but it should be validated using the final prototype. An update of Deliverable D2.4 could be issued. Notice that an update of D2.7 could also be issued after experiments at HMGU if modifications have been done.
**Eventual addition to Deliverable D2.6**

The evaluation of both dual energy and contrast agent techniques performed at FIHGM on living mice could be performed on the final prototype. In that case D2.6 would be completed.

**Milestone M5**

Milestone M5 - Selection of an appropriate XCT technology for FMT-XCT system was the main objective of Year 3 and can be considered as completed. The only missing deliverable for completion of WP2 is finalization of D2.5. Nevertheless, the related activity (minimization of X-ray interference with optical components) does not have significant impact on choice of contrast enhancement strategy thus on M5 conclusions.

**Statement of the use of resources**

WP2 is almost completed and the allocated resources spent.

**Publications**


**Work Package 3 - Theory for 360-degree FMT**

*WP leader: FORTH*

*Written by Giannis Zacharakis, FORTH, 14th March 2011*

**Summary**

The work performed during the 3rd reporting period has been focused on:

- Finalizing the multispectral algorithm
- Implementing the multispectral capacity into the fast inversion approach
- Quantitatively evaluating the direct inversion with hybrid data

Main and very strong collaboration with exchange of data and personnel has taken place during this reporting period with UZH, UCL, CEA-LIME, HMGU and FIHGM.

**Progress towards objectives**

*Task 3.1 Direct inversion*

One of the core contributions of FORTH to the project was to provide a very fast algorithm that, retaining the quantitation and resolution accuracy, significantly reduces the computation time and memory requirements. The algorithm was developed in collaboration with UCL and was extensively tested at FORTH with phantom and experimental data. The task was initially completed during the second reporting period and in this 3rd reporting period has been merged with the existing reconstruction software at FORTH.
**Task 3.2 Experimental optimization:**

A large number of experimental data with single and multiple fluorescent targets has been produced by FORTH and used to evaluate algorithmic developments. The final goal of this task and FORTH’s major involvement during the third reporting period was the implementation of the direct inversion method with the data from the FMTXCT prototype. This Task is also directly related to Task 5.2.

In particular our main objective was to train the Matrix-Free Algorithm for Reconstructions on Fluorescence Molecular Tomography (FMT) to work with the data acquired from the FMT-XCT tomographer in Munich. The matrix free method provides a very fast way to reconstruct for FMT images that is extremely cheap in computational resources such as memory and computational time, allowing for a large amount of data, such as the data created when using 360 degrees rotation of the specimen. The main point of focus in this work has been the interfacing between the .xml data format, used as a standard in the project, and the creation of the necessary input for the matrix-free algorithm. This effort requires the extraction and creation of a Tetrahedral mesh from the XCT images provided during the acquisition with the FMT-XCT apparatus and the projection of sources and detector positions, as well as the choice of the data to be used on the surface of the specimen under imaging. A detailed description of the method is given in Deliverable 3.6 while examples of the results can be seen in Figures 1, 2 and 3.

Figure 1: Slices of the XCT image of the specimen 2: mouse with neck tumour.

Figure 2: Surface of the mouse model: The green dots represent the chosen detector position on the surface, red cycles the sources positions and magenta squares the projected on the surface sources. The red line represents the axis of rotation.
Figure 3: Matrix-free reconstruction on a mouse. Blue is the reconstructed fluorochrome concentration

Task 3.3 Direct inversion vs. conventional FMT inversion

Initially FORTH developed a direct inversion method in order to obtain optimal reconstruction speeds. Due to the experimental setup needed to perform this inversion ($2^n$ sources and $2^n$ detectors, with $n > 8$), and its performance when compared with the matrix-free algorithm, it is clear that the best and most versatile approach for reconstructing FMT data is the matrix-free method.

Task 3.4. Multi-spectral imaging

FORTH has been developing multi-spectral approaches to enable imaging of more than one fluorophore simultaneously. This is a very important capacity of in vivo imaging systems and FORTH has extensively evaluated multispectral unmixing algorithms in both in vitro and in vivo data. The method has been also implemented into the Ultra-fast inversion approach using the matrix-free method, which has been evaluated with test data from the FMTXCT prototype. Examples of unmixed images and data can be seen in Figures 4 and 5. Detailed descriptions are given in Deliverables 3.6 and 5.6.

Figure 4: Coronal views of the 3D reconstructions of the CFSE and ATTO590 fluorescence signal overlaid on a schematic outline of the mouse. The inset shows the axial view of the same reconstructions. a) the mixed reconstructions, b) the unmixed ATTO590 reconstruction and c) the unmixed CFSE reconstruction.
Figure 5: *In vitro* quantification of co-localised GFP and DsRed cell culture plates. Increasing concentrations (0.2-1.8x10^5 cells/μl) of GFP and DsRed cells were mixed to a total volume of 10μl and imaged. The intensities recorded with the multispectral instrument were fitted to a linear regression model. a) intensity values before (*mixed*) and b) after unmixing (*unnmixed*) show excellent correlation to the concentration of cells in the specified volume. The values reported in the table indicate the change in linear relationship between intensity and concentration induced by spectral unmixing and demonstrates that GFP and DsRed intensities are, in quantitative terms, directly comparable between each other only following spectral unmixing.

**Task 3.5. Software development**

Important in the technique dissemination and training activities, is the development of user-friendly software that can be accessible by users and not only developers. The software, developed by FORTH, has been completed for inverting FMT data and has easy to handle inputs and correspondingly straightforward visual outputs to simply guide a user through the inversion process and allow for at least some simple visualization tasks in order to easily view and quantify the reconstructed images. This task has been completed during the second reporting period and the software has been regularly used ever since by FORTH and UZH for data reconstruction and visualization.

**Deviation from Annex 1**

There have been no deviations from Annex 1 during the third reporting period.

**Statement of the use of resources**

There have been no major deviations between the planned and actual person-months. Only a small adjustment has been made for months January and February 2010 on personnel cost and related overheads, explained in detail in the “FORTH Explanation of the use of the resources” document.

**Revised planning**

There has been no need for corrective actions. We have managed to deliver the final deliverables related to the algorithmic developments, which have been tested with FMTXCT test data and are ready to be fully exploited with the FMTXCT prototype.
Publications


Work Package 4 - FMT inversions with image priors

WP leader: UCL

Written by T Correia (UCL), T Rudge (UCL), V Soloviev (UCL), A Zacharopoulos (UCL) and S Arridge (UCL), José Vaquero, FIHGM (Partner 5), Date: 18th March 2011

Summary

This document reports progress in year three on Workpackage 4 "FMT Inversion with Priors" within the FMTXCT project. The theory behind FMT reconstructions using XCT image priors is explained, and the inversion algorithms are tested on both simulations and real data, provided by partners 1 (HMGU) and 5 (FIHGM). The algorithm implementation is based on the software package “Time-resolved Optical Absorption and Scattering Tomography” (TOAST) developed at UCL. Finally, we present our user-friendly software that incorporates the new inversion algorithm.

Progress towards objectives

Task 4.1 FMT inversion using XCT image priors

Consider the forward problem to be \( y = F(u) \), where \( F : U \rightarrow Y \) is an operator mapping from parameter to data space and \( y \) is the measured data, which is considered to be \( y_{\text{fluo}}/y_{\text{ex}} \), where \( y_{\text{fluo}} \) are the fluorescence measurements and \( y_{\text{ex}} \) the excitation measurements. The image reconstruction involves minimising the following objective function:

\[
\text{Minimise } E(u) = \frac{1}{2} \|y_{\text{meas}} - F(u)\|^2 + \tau \int_\Omega \psi(|\nabla u|) d\Omega
\]

\[
= L(y_{\text{meas}}, F(u)) + \tau \Psi(u),
\]

where \( \tau \) is the regularisation parameter. The term \( \Psi \) is the prior function, which in the case of multimodality imaging, is constructed in terms of an auxiliary reference image. In this particular project, the XCT data is used as the reference image.

Anisotropic diffusion

The minimum of \( E(u) \) is calculated as:

\[
\nabla E(u) = F'(u)^T \Gamma (F(u) - y_{\text{meas}}) + \tau L(u),
\]

(2)

Where \( L \) is the anisotropic diffusion function given by

\[
L(u) = \frac{\partial u}{\partial t} = -\nabla \cdot [g(|\nabla u|) \nabla u],
\]

(3)
where is the diffusivity.

The discrete formulation of the anisotropic diffusion can be written as:

\[
\frac{u_i^{k+1} - u_i^k}{\Delta t} = \sum_{j \in \mathcal{N}(i)} \frac{g_j^k + g_i^k}{2d^2} (u_j^k - u_i^k),
\]

where \( \mathcal{N}(i) \) are the neighbours of pixel \( i \) and \( \Delta t \) is a constant that determines the diffusion rate.

**Edge preserving functions**

The diffusion regularisation must be isotropic in homogeneous regions and in the presence of an edge the diffusion regularisation should only be applied in the tangential direction. There are a few functions with these edge preserving properties:

<table>
<thead>
<tr>
<th>Function</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-order Tikhonov</td>
<td>( g(u) = 1 )</td>
</tr>
<tr>
<td>Perona-Malik (Cauchy or Lorentzian)</td>
<td>( g(u) = \frac{1}{1 + \left( \frac{| \nabla u |}{T} \right)^2} )</td>
</tr>
<tr>
<td>Perona-Malik 2 (Welsh)</td>
<td>( g(u) = \exp \left( - \left( \frac{| \nabla u |}{T} \right)^2 \right) )</td>
</tr>
<tr>
<td>Total variation</td>
<td>( g(u) = \frac{T}{\sqrt{| \nabla u |^2 + T^2}} )</td>
</tr>
<tr>
<td>Bayesian</td>
<td>( g(u) = 1 - P(\text{edge}</td>
</tr>
</tbody>
</table>

\( T \) is the threshold parameter. If this parameter is too large it leads to an oversmoothing of the image. Whereas, if \( T \) is too small, smoothing is not applied and the resulting image is similar to the initial one.

The parameter \( T \) can be selected using a method based on cumulative histogram. The threshold can be calculated from the cumulative histogram by setting the threshold at, for example, 90 percent. Instead of keeping \( T \) constant over the entire image it can vary spatially. It can be kept fixed or it could be updated at every iteration. In the Bayesian formulation, \( P(\text{edge} | \nabla u) \) is the probability of an edge of interest being present.
Incorporation of the anatomical prior

The anatomical information can be used in the prior term to weight the diffusion function. Consider \( u_{\text{ref}} \) to be an anatomical image, which has been filtered previously by a Gaussian filter. Then, in order to obtain the image weighting factor \( \omega(u_{\text{ref}}) \) one can apply one of the diffusion functions in Table 1 to the anatomical image, which will return an image where homogeneous regions are equal to 1 and in the neighbourhood of edges values vary between 0 and 1. The prior term takes the form:

\[
\mathcal{L}(u) = -\nabla \cdot \left[ \omega(|u_{\text{ref}}|) g(|\nabla u|) \nabla u \right],
\]

(5)

Task 4.2 Quantification of algorithmic performance using simulated data

The Digimouse atlas (http://neuroimage.usc.edu/Digimouse.html) was used to generate a mouse mesh. The aim is to simulate a fluorescent target embedded in the liver. Therefore, different optical properties were assigned to the liver \( \mu_a = 0.035 \text{ mm}^{-1} \) and \( \mu s' = 0.68 \text{ mm}^{-1} \) and other tissue \( \mu_a = 0.01 \text{ mm}^{-1} \) and \( \mu s' = 1 \text{ mm}^{-1} \).

Figure 1 a) shows the mesh cross-section. A fluorescent target is simulated in the liver, as shown in figure 1 b). The source and detector, which is placed opposite the source, is rotated around the object in 16 evenly spaced steps.

Figure 1 c) shows the fluorescence image reconstructed using zero-order Tikhonov regularisation. The reconstructed images using the anatomical priors and the exponential Perona-Malik and total variation potential functions are shown in figures 1 d) and 1 e), respectively.

Task 4.5 Quantification of algorithmic performance using experimental data

Phantom

Data were provided by partner 5 (FIHGM)- details see “Summary of FIGH activities for WP4”. The phantom consisted of a resin slab with a small capillary tube filled with a fluorescent dye, which was located close to imaging surface. The optical and CT images were acquired simultaneously. A total of 42 projections were used in the image reconstructions (the CCD camera was static and only the source position varied). Figure 2 a) shows the reconstructed fluorescence using Tikhonov regularisation. Figure 2 b) shows the reconstruction with anatomical prior and Perona-Malik function and figure 2 c) with total variation.
Figure 2. Reconstruction a) with Tikhonov prior. b) with anatomical prior and Perona-Malik function c) and with anatomical prior and Total Variation.

Mouse

Optical data and CT images of an in-vivo mouse with a brain tumour were obtained by partner 1 (HMGU) at 184 different source-camera positions. Their FMX-XCT system has the x-ray tube and detector mounted onto the rotating gantry and, on the perpendicular axis is the laser and CCD camera. The gantry rotates around the mouse placed in the centre. The CCD images consist of 512x512 pixels, where the pixel size is 0.073 mm. Only 29 projections were used in the image reconstruction. Figure 3 a) shows the mesh cross-section. Figure 3 b) shows the reconstruction obtained using Tikhonov regularisation and figure 3 c) shows the reconstruction obtained using the anatomical prior and Perona Malik function.

Figure 3: Mouse a) Mesh cross-section. B) Reconstruction with Tikhonov prior. b) with anatomical prior and exponential Perona-Malik function.

The images reconstructed using priors clearly show an improvement in the image quality compared to images reconstructed using a simple zero-order Tikhonov regularisation.

Task 4.6 User-friendly software

A Matlab-based software was developed, which reconstructs FMT images using prior anatomical information extracted from CT images. This software requires the software package TOAST and Wavelab850 (or older, http://www-stat.stanford.edu/~wavelab/).
The user can select different edge preserving functions and choose the threshold type. The regularisation parameter value can be inserted manually or alternatively it can be calculated using the L-curve method. There is also an option that does the reconstruction without including any anatomical information. The reconstruction is always performed using data compression, and the compression level can be chosen by the user. A demo is provided that reconstructs FMT images from phantom data provided by WP5.

Summary of FIGHM activities for WP4
To quantitatively examine optimal inversion methods based on experimental data. We have acquired FMT/XCT data to feed the inversion algorithms developed by partner UCL (see above).

FMT/XCT data acquisition
Here we review the current state of the FIHGM’s FMT/XCT system (details see WP2), after all the work developed this year. All the components are attached to a rotating gantry.

Figure 5. Pictures of FIHGM FMT/XCT system, without shielding (left), with shielding (right)
**FMT subsystem**

Regarding the FMT subsystem, the subject to be imaged is placed in a specially designed bed, gently compressed between two antireflective plates ensuring planar boundaries. The bed is inserted in a fixed platform at a known distance from the CCD camera (ORCA II, Hamamatsu, Hamamatsu City, Japan) and objective (Nikon), living the mouse in horizontal position, and the anti-reflective plates parallel to the CCD chip of the camera. The laser is guided to the subject via mirrors into a galvomirror scanning device (Scancube 7, ScanLab AG, Puchheim, Germany) as shown in Figure 1. The laser power delivered to the sample is controlled by means of a TTL signal. Modulating the duty cycle of the signal is equivalent to vary the laser power. The exposure time of the camera is fixed. For every source, the laser power that leads to an optimal maximum number of counts in the CCD camera is obtained, using and automatic algorithm. The fluorescence images are recorded by placing a 10 nm bandwidth filter centred at 700 nm in front of the objective of the camera, while for the transmitted excitation images a 10 nm bandwidth filter centred at 675 nm is used. The filters are placed facing the camera using a motorized filter wheel (Luxiflux V2, Cyberstar, Echirolles, France). All the acquisition processes are controlled via and user friendly software developed in C and IDL code.

**XCT subsystem**

The CT subsystem integrated in the FMT-XCT prototype is a cone-beam CT system based on a flat-panel X-ray detector and a micro-focus X-ray source. The micro-focus X-ray source is a modified Hamamatsu L9631 in which the control unit has been split from the high voltage source and the X-ray tube, thus significantly reducing the size and the weight of the device. The size and weight reduction obtained simplifies to a great extent the integration of the source in a moving gantry. The X-ray source has a power of 50W with maximum peak energy of 110keV and maximum anode current of 0.8mA. The flat-panel X-ray detector included in the prototype is a Hamamatsu C7940DK-02 which consists of a needle shaped CsI:Tl scintillator directly grown on top of the CMOS detector surface, with a carbon input window placed on top of the scintillator. The detector has a pixel pitch of 0.05mm that can be expanded by means of pixel binning (up to 4x4 binning). The minimum integration time for the acquisition of an image is 125ms when 4x4 binning is used. The detector is placed on a motorized linear stage which allows its radial movement to set the desired Field Of View (FOV) size and magnification factor (that fixes the achievable resolution in the reconstructed data). Besides the possible modification of the FOV size and magnification factor, the motorized stage allows the data acquisition performing non-circular trajectories. Thanks to the selectable FOV size and magnification factor it is possible to acquire high-resolution truncated datasets that can be joined with a low quality, low dose dataset to obtain truncation artefact free, high resolution reconstructed datasets of small areas of the sample. The system mounts also a motorized linear translation stage for the positioning of the animal and the axial extension of the FOV size. To stop the X-ray flux outside the acquisition system, the prototype is equipped with a shielding box integrated in the rotating gantry that blocks also the scattered radiation that can be harmful for the FMT acquisition components and the light reaching the FMT FOV.

**Phantom experiments**

We have built a slab phantom, following the same procedure as for the phantoms built for WP8. Its optical properties are \( \mu_a = 0.1 \text{ cm}^{-1} \) and \( \mu_s = 8 \text{ cm}^{-1} \). The slab size is of 5x5x1 cm, and a capillary with 2 µL of Alexa fluor 680 in its tip was inserted on it, close
to the surface. For the FMT the acquisition process 42 sources were selected equally spaced into squared region of 1cmx1cm. The acquisition algorithm is detailed in "FMT subsystem". For the XCT acquisition process the following parameters were used:

- Voltage: 45 Kv
- Current: 120 µA
- Source-detector distance: 215 mm
- Filter: 1mm, Aluminium
- Pixelsize: 0.2 mm
- Integration time: 125 ms
- Number of projections: 360.

Four fiducials points made of pumice were added to the phantom for registration of both modalities. Due to the planar symmetry of the phantom, registration could be made just via rigid transformation. Using for the reconstruction the ART algorithm with 42 iterations and a relaxation parameter of 0.7 gives the reconstruction shown in Fig 6.

![Figure 6. FMT reconstruction (color scale), registered with the CT reconstruction (grey scale). Coronal slice (1.5 mm from the surface).](image)

The raw data were given to UCL for algorithm development.

**Mouse experiments**

A capillary with 2 µL of the fluorophore Alexa Fluor 680 in its tip was inserted on the esophagus of an adult nude mouse. For FMT the acquisition process 48 sources were selected equally spaced into a region of 1cmx1cm situated on the upper torso of the specimen. The acquisition algorithm is detailed in "FMT subsystem". For the XCT acquisition process the following parameters were taken:

- Voltage: 45 Kv
- Current: 120 µA
- Source-detector distance: 215 mm
- Filter: 1mm, Aluminium
- Pixelsize: 0.2 mm
- Integration time: 125 ms
- Number of projections: 360.

Four fiducials points made of pumice were added to the phantom for registration of both modalities. Due to the planar symmetry of the system, registration could be made just via rigid transformation. Using for the reconstruction the ART algorithm with 42 iterations and a relaxation parameter of 0.7 gives the reconstruction shown in Fig 6.
Figure 6. FMT reconstruction (color scale), registered with the CT reconstruction (gray scale). Left, Sagittal slice. Center, axial slice. Right, coronal slice.

The raw data were given to UCL for algorithm development.

Work Package 5 - FMT-XCT integration

WP leader: HMGU

Written by Angelique Ale (HMGU) and Max Koch (HMGU), March 2011

Summary

The work that has been performed for reporting period 3 is:

- Prototype including proprietary gantry has been installed (deliverable 5.4)
- Common file format has been developed for exchange of data
- Training data sets have been acquired and uploaded to common data server (tasks 5.3, deliverable 5.8)
- Algorithms for the assignment of optical properties were integrated in the reconstruction code (task 5.2, deliverable 5.7)
- Optimal acquisition parameters have been defined (deliverable 5.8)
- User friendly software is installed and operational on acquisition (deliverable 5.9)

Progress towards objectives

The proprietary gantry prototype was finalized in cooperation with Ct-imaging. Thus to accelerate the system integration of both the FMT-chain and the CT-chain HMGU and CT-Imaging worked out a concept to provide a fully functional and operational prototype. CT Imaging was commissioned by HMGU to realize the concept. The concept comprised of basically four steps:

- Providing a mechanical interface for the FMT components
- Upgrading the machine control to support some FMT components
- Providing a software tool enabling the user to start a standard CT acquisition
- Providing an interface between the machine control and the existent FMT-application used by HMGU.
All tasks have been successfully accomplished by CT-Imaging and the system was delivered to HMGU (Klinikum Rechts der Isar, Munich) on March 09, 2011. (for details see deliverable 5.4)

![Final FMTXCT Prototype](image)

**Figure 1: Final FMTXCT Prototype**

User friendly software is installed on the acquisition computer, see deliverable 5.9. The user can choose optimal parameters for acquisition; see task 5.4 and deliverable 5.8.

**Task 5.2 Integration of algorithms from Wp2 and Wp4**

One of the steps in the integration of algorithms was the development of a common file format. The developed common file format for exchange is called fmtxml file format. The format has been agreed upon and used by all cooperation partners. In addition to the file format a matlab reference implementation for importing and reading the dedicated files was developed and was published to the partners. Several datasets that have been uploaded (see also task 5.3) to the transfer drive. All partners were able to download the data from the server and the datasets have been used to evaluate the developed components of the inversion methods. The ultra-fast inversion method has been successfully applied to the training data set, see also results in deliverable 3.7. Also UCL has successfully applied the developed methods to the training data sets. A hybrid reconstruction method was applied at HMGU to the training data sets, see images below.
Task 5.3 To acquire training data sets and optimize imaging settings

Several training data sets have been acquired. Three most diverse training datasets were selected and uploaded to the common data server. The training datasets were converted to a common file format, xml format. Forth and UCL have successfully used the data sets to evaluate the developed algorithms. Optimized imaging settings have been obtained from experience. The optimized imaging settings are summarized in deliverable 5.8. The acquisition software that is installed at the fmt-xct acquisition computers has an interface to the parameters that are important for the acquisition. The user can choose these parameters, following the guidelines of deliverable 5.8.

Task 5.4 Optical property determination

Optimal optical properties were determined and implemented in the calculation of the forward model, in cooperation with UCL. Focus was placed on the thorax region, see deliverable 5.7. The determined optical properties were used to obtain successful reconstruction results.

<table>
<thead>
<tr>
<th></th>
<th>Absorption (cm(^{-1}))</th>
<th>Scattering (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung region</td>
<td>0.25</td>
<td>27.5</td>
</tr>
<tr>
<td>Heart region</td>
<td>0.35</td>
<td>17.5</td>
</tr>
<tr>
<td>Bone region</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>Tissue region</td>
<td>0.3</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1: Determined optimal optical properties

Revised planning

Not applicable
Publications


Work Package 6 - Cancer imaging with focus on breast cancer

WP leader: CEA-Lime

Written by Anikitos Garofalakis (Cea lime), March 21, 2011

Summary

The use of animal models is essential in order to evaluate FMT-XCT for its intended application, i.e in-vivo imaging. In the work package 6 our task is to develop and test mouse models of breast cancer as well as other cancer types. Additionally we have developed and evaluated both commercial and custom made probes targeting tumours or tumor related processes.

Progress towards objectives

Objective 6.3: To develop animal models of other cancer for studying FMT-XCT performance (objective completed)

In the end of the second year of the programme we have reported the successful development of breast cancer tumour models. These included the MDA-MB-231 human breast adenocarcinoma and the MCF-7 tumour cells. In the end of the third year we report the development of a mouse model for brain tumor. We set up an orthotopic animal model for glioblastoma in nude mice, nude rats and Fischer, rats. The model is based on intracranial injection of tumour cells (human U87, human Gli36, human U251 or rat 9L glioma cells) using a stereotactical device (Stoelting Inc.) Injection of 1x10^5 or 2x10^5 cells is performed into the striatum of nude mice or rats, respectively. Tumour growth can be detected by PET imaging from day 8 on (9L in Fischer rats), and is clearly visible in nude mice at day 14 post injection.

![](image1.png)

>Figure 1a: PET image nude mouse 9L intracranially 14 days post injection.
We also generated different cell lines expressing fluorescent proteins or firefly luciferase. U87, Gli36 or 9L cells expressing the firefly luciferase gene could be visualised by bioluminescence imaging early (3 days) after intracranial implantation in nude mice and 6 days post injection in rats.

Objective 6.4 To perform in-vivo imaging of key animal models of cancer and correlate the findings with standard laboratory tests and growth measures.

Development and evaluation of an aptamer for the labelling of the MCF-7 breast cancer model.

In the previous reports we have presented a new aptamer developed (ACE8) in our lab able to bind membrane proteins of cancer cells with high affinity. By looking microscopically, we showed that the ACE8 aptamer binds to the breast cancer cell line MCF-7 first at the membrane and then it gets internalized. The next step was to perform in-vivo small animal measurements. We used the FMT technique to quantify the tumor uptake of ACE8 in the MCF-7 tumor xenografts. Measurements were performed at 3 hours post-injection using the TomoFluo3D prototype (Fig. 3). We
recently calibrated the TOMOFUO 3D using nuclear imaging and demonstrated that it can non-invasively quantify fluorescent probes concentration in small animals (Garofalakis et al, *In vivo calibration of free-space Fluorescence Tomography using Nuclear Imaging*. Optics Letters 35(18) p. 3024-3026 (2010)). The contrast obtained with fDOT was higher than the contrast obtained with epiluminescence fluorescence imaging and revealed ten times more aptamer ACE8 in the tumor compared to a control sequence (0.67 ± 0.16% of injected dose compared to 0.07 ± 0.06%, respectively)(Figure 2).

![Image](image-url)

**Development of a new nano-micelle for the passive targeting of the MDA-MB-231 breast cancer model.**

Nano-particles have the potential of carrying constrainst agents and drugs to tumours. They take advantage of the leaky vasculature surrounding tumours to get trapped based on a phenomenon which is known as Enhanced Permeation and Retention (EPR). We have developed nano-micelles that have very small size in order to improve their diffusion deeper inside tumours. We have three tested polymerized polydiacetylene (PDA)-micelles of different coatings; either nitrilotriacetic acids (NTA) and different poly(ethylene glycol) (PEG) chain lengths.a. PDA-NTA, b. PDA-PEG350 and c. PDA-PEG2000 micelles.

These nano-micelles have been for in-vivo imaging after being labelled with the 730 a infra-red (NIR) fluorescent dye FluoProbes® 730 (FP730). We used Epi luminescence fluorescent imaging which is a fast screening method for the selection of the best of the three nano-micelles. The results showed that the micelle PDA-PEG2000 micelles had the best tumor to target ratio and thus it was chosen for further validation with the use of tomographic quantitative imaging.
To quantify tumor uptake of PDA-PEG2000 micelles, free space fluorescence diffuse optical tomography (fDOT) was used. One day after injection, the tumor uptake of PDA-PEG2000-FP730 was measured around 4.7±1.3% of injected dose per gram (%ID/g). We chose to perform dual PET/FMT measurements aiming in comparing the 3D fluorescent signal of the nanomicelles to the high [¹⁸F]-FDG internalization by the cancerous cells as given by PET. In this experiment, [¹⁸F]-fluorodeoxyglucose ([¹⁸F]-FDG) was injected 24 h after PDA-PEG2000-FP730 administration. In this experiment we found that 40±19% of the retained micelles was partially overlapping with the tumor volume visualized by PET. The fluorescent nanomicelles were found below the tumor volume where the vascularization is expected to be higher. The nano-micelle localization was monitored up over one week to confirm the effective labelling of tumors by the micelles. Since the retention of the micelle appeared favorable we further explored the potential of using the micelles as drug carriers.

Figure 3. fDOT/PET multimodal imaging of [¹⁸F]-FDG and PDA-PEG2000-FP730 micelles distribution in tumors. Left: the sagittal (a), coronal (b) and axial (c) projections of fused fDOT and PET signal in the tumor area are shown. The white arrow pinpoints the FDG accumulation in the tumor. Rainbow Look-Up Table (LUT): fDOT; Temperature LUT: FDG. Right: PET signals rendered to the envelope of the mouse corresponding to the sagittal (d), coronal (e) and axial (f) projections are shown. Pink volume: FDG inside the tumor; Yellow volume: micelles. Both volumes were extracted from the volumes of interests (VOIs) used for the quantification of each type of signal.

Deviation from Annex 1

Characterization of the quantification accuracy of FMT-XCT to resolve differential treatment levels in the Human erb-B2 animal model in the presence or absence Trastumab as it relates to histological validation. Characterization of the
quantification accuracy of FMT-alone to resolve treatment levels in the PymT xenografted animal model in the presence or the absence of Sutent (aka Sunitinib). The project is within time lines. A deviation has been the treatment the animal model that has been used in order to apply the treatment (Objective 7.2 Image targeted therapy). While in the Annex 1 it was proposed the use of Human erb-B2 animal model, we have used the PymT breast cancer model mainly because our work package is focused on the breast cancer studies.

Statement of the use of resources
There have been no deviations between actual and planned person-months per workpackage.

Revised planning
None applicable

Publications

Work Package 7- Imaging Cancer Therapy for enabling intervention
WP leader: UZH
Written by Markus Rudin (UZH) and Anikitos Garofalakis (Cea lime), March 30, 2011

Summary
WP7 will be developing imaging assays for signals downstream of HIF such as molecular targets involved in angiogenesis, anaerobic glycolysis or vasodilation. These molecular signals will be complemented by classical physiological imaging readouts of the respective process. Studies will be carried out in two disease models: i) hypoxia and induction of angiogenesis in both subcutaneous tumor xenografts and orthotopic tumor models and ii) study of hypoxic stress in models of focal cerebral ischemia in mice. We will investigate essential aspects of hypoxia induced signalling and effects of therapeutic interventions on HIF signalling and general outcome such as tumor regression/stabilization/progression (RECIST criteria) and final infarct volume in stroke. Hence, for both applications the combination of structural readouts (CT) with functional (optical and MRI) and molecular readouts (optical) readouts will be essential.

We have developed a hybrid FMT using MRI complementary to x-ray CT as a reference imaging modality. The use of MRI offers distinct advantages. 1) MRI provides high soft tissue contrast, which may facilitate tissue classification and segmentation. 2) MRI provides multimodal information, and allows in particular the assessment of tissue physiology/function. Disadvantages are in general the long acquisition time of high resolution MRI datasets. In addition the high static magnetic field associated with MRI constitutes a major technological challenge.

The FMT-MRI system was designed as an insert into a regular animal MRI system. We choose the free-beam geometry enabling flexible excitation schemes. In addition, the optical detector should be in close proximity the fluorescence source, i.e. an integral part of the MRI detector unit. In analogy to PET-MRI detectors we choose
single photon avalanche diodes (SPADs). We could demonstrate feasibility of the hybrid concept with our first prototype: there was essentially no interference between the two μδαλίτιες, spatial resolution was of the order of 1mm in plane, structures up to a depth of 3mm could be accurately assigned (reflection geometry), and the method was demonstrated to be quantitative and sensitive. Limitation of the first prototype were restrictions in field of view (8x8mm²) and limited digital resolution (32x32) of the detector as well as limited depth resolution due to the reflection geometry approach chosen. First in vivo experiments with tumor bearing mice revealed that simultaneous recording of structural and molecular information (protease activity) was feasible using this hybrid setup (Stuker et al. 2011).

Currently we are developing a second generation FMT-MRI system that addresses the weaknesses of our prototype. We have replaced the SPAD detector by a CMOS detector with a higher digital resolution (256x256) and higher field of view (35x35mm²). The setup was modified to allow for both reflection and transmission experiments. The overall engineering was modified to achieve on the one hand better mechanical stability of the setup and on the other hand facilitate operations during an experiment. First test experiment revealed an excellent performance of the CMOS camera and no interference by the MRI system. However, we observed some degradation of the MRI signal-to-noise ration when the FMT is in operation. This issue is currently being addressed. Development of the 2nd generation FMT-MRI system will be completed by 6/2011, and the system will then be used for studying biological questions.

Progress towards objectives

Objective 7.2 Image targeted therapy

As high HIF activity is associated with increased resistance to chemo- and radiotherapy, inhibition of HIF signalling appears a promising (adjuvant) therapy. We tested therefore whether pharmacological modulation of HIF signalling could be monitored using the multiple HIF readouts described (Lehmann et al. 2009). We used the compound dimethyloxalylglycinel (DMOG), an inhibitor of the enzyme prolylhydroxylase domain (PHD), which marks HIF1α for proteasomal degradation. Administration of DMOG should therefore lead to HIF1α stabilization even in normoxic areas and thus increased HIF signalling. Nevertheless, we observed that chronic DMOG administration largely suppressed HIF signalling in vivo. Interestingly we observed the same in cell culture using both bioluminescence imaging and protein analysis. There is thus evidence that chronic stimulation of HIF signalling activates a self-regulatory feedback mechanism. (Lehman et al. in prep).

Anti-angiogenic treatment on mouse breast cancer model using FMT-alone

The drug, Sutent, or Sunitinib by Pfizer, is an anti-angiogenic molecule which inhibits Vascular Endothelial Growth Factor Receptors (VEGFR). It is a multi-targeted receptor tyrosine kinase inhibitor (RTK) and is used for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumors (GIST). It has been found that there were some potentially beneficial side effects for patients suffering from breast cancer which is what we decided to investigate.

The probe of choice was Angiostamp as it allows us to visualize the integrins which was our choice as it is related to neovasculature and thus angiogenesis. The model of choice was the PymT breast cancer mouse model (see also deliverable 7. 4). The protocol called for the extraction of the cancer cells of the PymT mouse. These cells were then dissociated and then frozen. The next step called for the injection of the breast cancer cells into the mammary glands of an FVB immune-competent mouse at the level of the T4. The cells were then allowed to grow for 15 days as this has been
proven to be the time needed based on previous experiments in order to obtain an ‘ideal’ size. At this point we started treatment of Sutent at 100 µl/mouse/day for the treated group and 100 µl of DMSO/mouse/day in the control group. In order to follow the progress of the experiment, we used Planar Fluorescent imaging (Fluobeam) so track the day to day size change and also verify the presence of the probe and also the difference in presence of integrins between the treated and control groups. For quantifying the effect of treatment we also chose to measure the volume occupied by the RAFT-RGD the day 01 and the day 02 after treatment with the aid of fluorescence molecular tomography (FMT). Results (see figure below) show that there is a decrease of the neovasculature volume for the case of the treated animals.

Fig 1: Decrease of the neovasculature volume for the treated animals.

Objective 7.3 To image HIF related pathways in animals

We have developed multiple reporter assays for assessing signalling along the hypoxia-inducible factor (HIF) pathway: 1) assessment of tissue hypoxia using the ligand ¹⁸F-fluoro-misonidazole (¹⁸F-FMISO) in combination with PET imaging (not part of FMT-XCT project), 2) assessment of hypoxia-inducible factor 1α (HIF1α) stabilization using a reporter gene assay expressing HIF1α as fusion protein with either firefly luciferase or the fluorescent protein m-Cherry. We found that the the fusion protein was regulated by the proteasomal degradation machinery as the native HIF1α and that furthermore the fusion protein kept its ability to induce down-stream genes. 3) assessment of HIF activity by expressing the reporter gene firefly luciferase under the control of the HIF-responsive element (HRE; constructs with m-Cherry are currently being prepared). Finally, tumor angiogenesis was measured using established MRI assays (permeability,m tumor blood volume, vessel size index). Tumor cells (C51 colon carcinoma) have been transfected with the various constructs and injected subcutaneously into the thigh or shoulder of nude mice. Longitudinal PET and bioluminescence imaging studies were carried out for assessing the regulation of HIF stability/activity. Tumors were hypoxic throughout the observation period. This led to an initial increase in HIF1α stability and HIF activity, which later decreased to almost baseline values despite persistent hypoxia. Correspondingly there was a poor correlation between hypoxia and HIF signalling, while there was a significant correlation between HIF1α stability and HIF activity (Lehmann et al. 2009). These HIF assays are currently being translated to various glioma cell lines in order to study
orthotopic tumor models. In order to increase the flexibility of the HIF assay and to make compatible with multiple imaging modalities, and alternative reporter construct has been designed. Upon activation of HIF signalling, glycosylphosphatidylinositol (GPI) anchored avidin was expressed at the cell surface under the control of HRE promoter element. This allowed translating an intracellular signal to the outer cell membrane making in readily accessible to biotinylated reporter moieties such as fluorescent dyes, radiotracers or MRI contrast agents. Proof of principle was demonstrated in murine subcutaneous C51 tumors that were targeted using either fluorescently labelled probes (Alexa680) or 67Ga complexes for single photon emission computer tomography (SPECT studies). For both reporters, significant accumulation was observed in the tumor region (Lehman et al. 2011). The approach is attractive, as nuclear imaging readouts might be used to validate quantitative results obtained with FMT.

Multimodal imaging in hypoxia signalling.

**Highlights**

- Development of hybrid system FMT-MRI complementing FMT-XCT
- Development of molecular imaging assays for assessing HIF1α stabilization and HIF activity in tumor cells
- Multimodal HIF activity readout (HRE) for FMT imaging and as validation tool
- Pharmacological study demonstrating that assessment of drug induced modulation of HIF signalling is feasible
Next steps

- Optimization of 2nd generation FMT-MRI setup: improved reconstruction algorithms
- Development of imaging assays for studying orthotopic brain tumors
- Development of HRE-m-Cherry reporter and characterization of transfected tumor cells in vitro
- In vivo studies in C51 model using m-Cherry transfected tumor xenografts
- Visualization of pharmacological modulation of HIF expression
- First FMT-MRI measurements with subcutaneous murine tumor models; assessment of therapy response

Deviation from Annex1

A major activity relevant for FMT-XCT (though financed by another source (SNF)) was the development of a hybrid FMT-MRI system. This also involved adaptation of reconstruction algorithms. HIF assays were originally developed for bioluminescence and have now been successfully adapted for fluorescence imaging (GPI avidin). With regard to tumor models characterized, the focus at UZH is on colon cancer (C51), glioma (various murine and human lines) and breast cancer (4T1). Other breast cancer cell lines will be dealt with at HMGU and CEA.

Statement of the use of resources

There have been no deviations between actual and planned person-months per workpackage.

Publications

**Work Package 8- FMT-XCT imaging accuracy vs. PET-XCT**

**WP leader: FIHGM**

Written by: Juan Aguirre (FIHGM), Alejandro Sisniega (FIHGM), Juan F. Pérez-Juste Abascal (FIHGM), Judit Chamorro (FIHGM), reviewed by Juan J. Vaquero (FIHGM).

**Summary**

Work package 8 focuses on the validation of the FMT-XCT utility when compared with the gold-standard PET-XCT. As stated in previous reports (report 1 and 2), based on the optimal phantom design first hybrid phantoms were developed were circulated among the project partners.

**Progress towards objectives**

**Objective 8.1. To develop hybrid FMT-PET-XCT phantoms.**

**Hybrid phantoms**

As stated in previous reports (report 1 and 2), phantoms for comparing FMT-XCT with PET-XCT were already built and the PET-XCT data was acquired. Partner HMGU made the first FMT-XCT acquisition, not being able to get usable data due to reflections. On the other hand, several phantoms using the same material but with slab shapes were imaged successfully with FIHGM’s equipment. The reason of this problem must be found and corrected. In the figure below, we show a reconstruction of a cylinder inside a slab phantom made of the same material as the phantom sent to HMGU.

![Coronal views (X,Y plane)](image)

**Figure 1. Coronal slices of a cylinder of 5 mm diameter and 15 mm length, drilled in a slab phantom (5x5x1cm).**

On the other hand, and as mentioned in the previous year report (report 2), in the context of this work package we have also collaborated with the Center of Ultra-Short, Ultra-Intense Pulsed Lasers (CLPU) Salamanca (Spain) and the Universidad Politécnica de Madrid (UPM) to characterize the phantoms developed by our group. The CLPU has developed a technique for space-time reconstruction of the amplitude and phase of ultrashort pulse using interferometry, known as STARFISH. This technique allows recover the ballistic component through a sample by its coherence and polarization with a reference pulse.

The experimental setup is designed by CLPU:
Figure 2. System based on an optical fiber coupler which acts interferometer to develop spatial interferometry. The longitudinal position of the "fiber arm" controls the relative delay between reference and test. The probe beam is scanned transversely (space), with corresponding "fiber arm."

Thus, we deliver one of the diffusive phantoms developed to CLPU ($\mu_a = 0.1\text{cm}^{-1}$ and $\mu_s' = 8\text{cm}^{-1} \rightarrow \mu_s = 16-80\text{ cm}^{-1}$) with the dimensions showed in the image below:

Using the technique described previously, interference patterns were obtained for steps of 2 mm and 3 mm thick, from which we reconstruct the shape of the light pulse captured by the optical fiber.

**Results**

No enough signal was detected for steps of more than 3 mm. This result is consistent with estimates obtained by simulation, in which no ballistic photons are obtained for more than 3 mm either snake light for more than 7 mm. For the step of 2 mm thick, a significant fraction of light passes through without interaction, resulting in a significant ballistic component.
It has also tested the use of Sequoia, a high dynamic range third-order femtosecond cross-correlator (peak-nanoseconds), which allows see pulse shapes in that range. After the first step the signal intensity was not enough. Some radiation was detected IR but not enough to generate harmonics. For the 3-mm step, the similarity between simulation and observation is less obvious. Our perception is that the system is filtering the ballistic photons.

**Planning for the next year**

To try to correct this lack of signal we have built less diffusive phantoms (the same features as above but with $\mu_s' = 1$ cm$^{-1}$). These phantoms have been sent back to CLPU and are currently being characterized.

*Objective 8.2: To develop aptamers labelled with fluorescence and 18F for PET to image cancer of the same animal model and offer exact co-registration for validation purposes.*
Dual-labelled probes
Within this task, we will study together with the CEA-LIME group the viability of double-labeled fluorescence of the developed aptamers with 18F and fluorescence. Although methods for individual labelling are already established by CEA-LIME, double marking presents challenges that are currently being addressed. Moreover, as it is known, the short half-life of 18F requires that the marking is carried out close to the place where the imaging will be done. In this regard, we have a meeting next April with the CEA-LIME group which will discuss how to implement this task.

Deviation from Annex1
Task 8.2.: To develop aptamers labelled with fluorescence and 18F for PET to image cancer of the same animal model and offer exact co-registration for validation purposes is delayed and will be finished in Year 4.

Statement of the use of resources
There have been no deviations between actual and planned person-months per workpackage.

Revised planning
Not applicable

Publications
- J Chamorro, J Aguirre, J Ripoll, JJ Vaquero, M Desco. "Maximizing the information content in acquired measurements of a parallel plate non-contact FDOT while minimizing the computational cost: singular value analysis". Abstract book of European Society for Molecular Imaging (ESMI), 161, 2009
Work Package 9 - Training and Dissemination
WP leader: HMGU

Written by Veronika Erben (Project Manager, HMGU)

Training
As described in Annex 1 the proposal depends on large extend on discussion and exchange of ideas and interdisciplinary scientific knowledge between participant members. Therefore several workshops have been realized:

- Workshop “Advanced X-Rays imaging techniques”, 6-7 July, 2009, Grenoble organized by CEA Leti (for details see report 2, WP9 and deliverable 9.2).
- Workshop “Animal models/cancer imaging”, Zürich January 13, 14th, 2011:

Organization
The workshop was organized and hosted in Zürich by the Animal Imaging Center, Institute for Biomedical Engineering, University and ETH Zurich (beneficiary 6, UZH).

Participants

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
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<tbody>
<tr>
<td>Anikitos Garofalakis</td>
<td>CEA LIME</td>
</tr>
<tr>
<td>Marco Brambilla</td>
<td>CEA LETI</td>
</tr>
<tr>
<td>Markus Mronz</td>
<td>Ct imaging</td>
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<tr>
<td>Teresa Correia</td>
<td>UCL</td>
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<tr>
<td>Alejandro Sisniega</td>
<td>FIHGM</td>
</tr>
<tr>
<td>Pouyan Mohajerani</td>
<td>HMGU</td>
</tr>
<tr>
<td>Maximilian Koch</td>
<td>HMGU</td>
</tr>
<tr>
<td>Angelique Ale</td>
<td>HMGU</td>
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<tr>
<td>Markus Rudin</td>
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<td>Florian Stuker</td>
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<td>Katerina Dikaiou</td>
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Program

Thursday, 13 January 2011

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<td>The AIC research activities</td>
<td>Markus Rudin</td>
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<td>14:30- 15:30</td>
<td>Tumor imaging hallmarks of tumor progression, imaging and treatment</td>
<td>Markus Rudin</td>
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<td>approaches, animal models</td>
<td>Divya Vats</td>
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<td>15:30 -16:00</td>
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Friday, 14 January 2011

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<td>Presentation of the opti/mri setup</td>
<td>Florian Stuker</td>
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<td>09:00 – 9:30</td>
<td>Description of the goals of the experiment</td>
<td>Katerina Dikaiou</td>
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<td>FMT/MR measurement</td>
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<td>• FMT: protease activity</td>
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<td>• MR: vascular permeability/angiogenesis</td>
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<td>Future steps on opti/mri Prototype and biomedical applications</td>
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<td>14:00 -15:30</td>
<td>Presentation and hands-on demonstration on data analysis</td>
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<td>FMT reconstruction, MR data analysis</td>
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<td>FMT/MR information</td>
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<tr>
<td>15:30 -16:00</td>
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Content

**Formal presentations**

The formal presentations and demonstrations were structured around the complete workflow of biological experimentation in molecular imaging. Thus, hallmarks of tumors and animal models were presented, and a hybrid FMT/MR experiment yielding complementary information was performed on the FMT/MR system developed by UZH. Future steps of the approach and methods for histological validation were presented. In addition to UZH/ETH, partners CEA-LIME and HMGU presented the biological experiments performed so far.

**Discussion**

Planning of upcoming experiments was discussed in plenum by involved partners CEA-LIME, HMGU and UZH. As the new HMGU license for animal experimentation is expected only in spring, ex vivo experiments can be performed in the meantime. The tumor size in those animals shall, however, still conform to German animal experimentation regulations. Moreover, fluorescent probes should be excitable by the 680nm and 750nm lasers available in HMGU.
**Actions**

Partners CEA-LIME, HMGU and UZH will estimate the time needed for generation of animal models and experimentation. They will communicate the estimates and decide on prioritization of experiments.

**Dissemination**

The objective of dissemination activities is to deliver relevant project results to key target groups and to improve the relevance of results by a continuous dialogue with these stakeholders. Moreover visibility and awareness of the project are enhanced through such activities and thereby decision making is influenced. The results of the FMTXCT project were disseminated in various ways:

- Publication in leading international journals in the field and annual presentation of results at international forums of Imaging (details see deliverable 9.1 “Dissemination and Implementation document”)
- Dissemination of the FMTXCT project and the results were additionally obtained through the project website (http://www.fmt-xct.eu/) and several other websites (details see deliverable 9.1 “Dissemination and Implementation document”)
- At the conference “Scientific Challenges in European Health”, an international conference launched by the Bavarian Universities, which took place in Brussels on October 20 and 21, 2010 Vasilis Ntziachristos was selected to present the Bioimaging topic. The objective of the event was to present major themes of medical research in Bavaria and scientific achievements in these fields as well as to discuss key challenges, goals and new directions with international stakeholders from science, industry and European institutions. In the imaging presentation and the following debates one of the main focus was the FMTXCT project (Poster see deliverable 9.1 “Dissemination and Implementation document”)
- A public promotion leaflet to raise awareness for the activity and success of the project has been developed (see deliverable 9.3).
### 3) Deliverables and milestones tables

**Deliverables**

This table is cumulative and shows all deliverables from the beginning of the project. Deliverables of reporting period 3 are bold; abbreviations: Del= Deliverable, Ms=Milestone

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2 new deliverables:

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## Milestones

This table is cumulative and shows all milestones from the beginning of the project. Milestones of reporting period 3 are bold; abbreviations: Del= Deliverable, Ms=Milestone

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<td>9</td>
<td>FMT-XCT</td>
<td>WP5</td>
<td>1 HMGU</td>
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<td>YES</td>
<td>See Del. 5.4</td>
</tr>
<tr>
<td>10</td>
<td>FMT-XCT methodology for in vivo quantification</td>
<td>WP6</td>
<td>2 Cea Lime</td>
<td>32</td>
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<td>See deliverable 7.3 and deliverable 7.4</td>
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<tr>
<td>11</td>
<td>FMT-XCT methodology for quantified treatment imaging</td>
<td>WP7</td>
<td>6 UZH</td>
<td>36</td>
<td>YES</td>
<td>Correlates to deliverable 7.5 due in month 40</td>
</tr>
</tbody>
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4) Management Report

This section summarizes the management of all activities during the second reporting period of the FMT-XCT European project (01.March 10 – 28. February 11). The management structure of the project is, as illustrated below, as follows:

The Helmholtz Center Munich (HMGU) as a coordinator (scientific coordination: Prof: Dr. Vasilis Ntziachristos, administrative coordination: Dr. Veronika Erben) is directly responsible for reporting to the European Commission. The members of the executive committee are the work-package leaders in this proposal (besides partner CT Imaging). This structure facilitates allows a direct communication between partners and a common understanding of goals. Each member of the executive committee further directly supervises or steers his team, and has selected members that further allow the direct communication within work-packages. The advisory committee consists of senior external members that are well respected in scientific fields associated with this proposal. The Executive committee is interacting with Advisory Committee members on the basis of personal correspondence and private meetings at the site of the Advisory member or in international meetings. Different experienced management teams provide support in financial, legal and PR issues.
Coordination and Communication activities
The communication is working excellent- internally and externally. Additional to the project correspondence and the day-to-day requests from partners the coordination and communication within the last year included:

- **Reporting to the European Commission.** The first and the second report were accepted without any recommendations.
- **Financial reporting:** Financial information (Form Cs) were filled in by each partner and submitted via the electronic submission tool by the coordinator. After acceptance of the second report financial data had to be validated and money was transferred to the partners according to the financial plan. Several administrative problems regarding the new online reporting tools had to be solved especially since several partners did not have an ECAS account yet.
- **Preparation and finalization of report 3:** Templates were designed for collecting all information required for the activity report. Work package reports, deliverable and milestones were collected and summarized for the report. All documents were uploaded and submitted to the EC via ECAS by the coordinator under considerable administrative effort.

- **Addition of Partner 8:** details see “Changes in the consortium”.
- **Preparing and post-processing of project meetings:** for details see “Meetings”.
- **Update of the project website:** All minutes and presentation and the year 2 report are available to all partners at http://www.fmt-xct.eu (Members only). A Platform for data exchange has been established at http://www.fmt-xct.eu/transfer/, login data were sent to all partners. The Year 3 report will be uploaded after approval by the EC.
Changes in the consortium

1) The small and medium-sized enterprise Vamp GmbH with its registered seat in Erlangen, Germany changed its name to CT Imaging GmbH which was approved by the European Commission (details see report 2).

2) Juan Jose Vaquero moved with its lab from FIHGM (Partner 5) to Universidad Carlos III de Madrid (UC3M). Consequently after the end of the reporting period 3 the beneficiary 5 (FIHGM) will resign and a new beneficiary (Beneficiary 8, UC3M) will be included in the project. An amendment request including the modified Annex 1 has been filed and will be submitted within the next days.

3) To accelerate the system integration of both the FMT-chain and the CT-chain HMGU and CT-Imaging worked out a concept to provide a fully functional and operational prototype. CT Imaging was commissioned by HMGU to realize the concept. Therefore they received 20,000€ more as initially planned which were subtracted from the HMGU budget. The concept comprised of basically four steps:
   - Providing a mechanical interface for the FMT components,
   - Upgrading the machine control to support some FMT components,
   - Providing a software tool enabling the user to start a standard CT acquisition
   - Providing an interface between the machine control and the existent FMT-application used by HMGU.

All tasks have been successfully accomplished by CT-Imaging and the system was delivered to HMGU (Klinikum Rechts der Isar, Munich) on March 09, 2011.

Project meetings

Several project meetings were planned and organized. The agenda and minutes were sent out in time. The advisory committee and the project officer of the EC were invited, but could due to other obligations not join. The following consortium meetings took place:

- **Kick-off meeting, Neuherberg, 14 May 2008**: Details see periodic report 1.
- **Year 1 Consortium Meeting, Munich, 24 April 2009**: Details see periodic report 1
- **Year 2 consortium meeting, IESL–FORTH, Heraklion, Greece, March 26, 2010**: Details see periodic report 2.
- **Year 3 consortium meeting, UCL, London, April 14 and 15, 2011**:
Consortium meeting for the FMT-XCT project
“Hybrid Fluorescence Molecular Tomography and X-ray Computer Tomography system and method”

EU-FP7 grant agreement no. 201792
UCL London, UK, April 14th – 15th, 2011

Participants for

1. HMGU
   Vasilis Ntziachristos (Coordinator)
   Max Koch
   Angelique Ale
   Andreas Murr

2. CEA
   Leti: Marco Bramilla
   Lime: Anikitos Garofalakis

3. FORTH
   Jorge Ripoll

4. UCL
   Martin Schwaiger
   Teresa Correia
   Vadim Soloviev
   Tim Rudge
   Simon Arridge

5. FIHGM/UC3M
   Juan José Vaquero
   Juan Abascal

6. UZH
   Florian Stuker
   Katerina Dikaiou

7. CT Imaging
   Markus Mronz

Chair of the meeting: Simon Arridge
Minutes: Andreas Murr
April 14th, 2011

WP1 & 9

Andreas Murr (HMGU) gives a short project overview, followed by notes related to the reporting and finishes with the list of deliverables and milestones for the next reporting period. The most important points are:

- The remaining project time will most importantly be dedicated to the in vivo evaluation of the FMT-XCT system in mouse models and the optimization of the developed software algorithms. This will require close coordination between the partners, also with regard to the necessary on site training sessions at the recently integrated FMT-XCT system in Munich.
- Prof. Juan Jose Vaquero moved from the “Fundacion para la Investigacion Biomedica del Hospital Gregorio Maranon”, Madrid (FIHGM) to “La Universidad Carlos III de Madrid (UC3M). Consequently, FIHGM officially exits the project on month 36 and the “new” partner UC3M (Partner 8) enters the project at month 37. A respective amendment of Annex 1 is under preparation.
- A cost neutral extension of the project is desired by the majority of the project participants. This point is discussed in more detail on day 2 of the meeting (see below).
- Animal protocols, necessary for the imminent vivo experiments, were recently submitted by the HMGU and are currently under review.

Reporting:

- The first 2 reports were accepted by the European Commission (EC)
- The current, 3rd report will be submitted end of April. Partner U3CM explains that slight delays in his reporting are due to the fact that he moved from one institution to another and therefore some signatures are still pending. This delay is likely to be ended in the near future.

Finances:

- EC pays up to 85% of the total budget (~ 3.8 m)
- 15% will be paid after acceptance of the final report (~700 k)

At the end of the project a technical review of the project will be performed by the EU. The guidance notes for this are available on the internet and are briefly discussed.

WP2

Marco Bramilla (CEA Leti) summarized the achievements within WP2. During the present year, the FMT-XCT prototype developed at FIHGM has been finished and has been made available to the consortium members for the acquisition of combined FMT-XCT data and for the acquisition of the dual-energy and contrast agent enhanced XCT datasets to be compared looking for the maximal achievable contrast between organs. Also the contrast enhancement strategy comparison and choice (Deliverable 2.6 and Milestone 5) could be completed and still has to be validated on the prototype. Markus Mronz (CT Imaging) specifies on the installation of the FMT-XCT prototype, which was delivered to HMGU at the campus of the “Klinikum Rechts der Isar” in Munich. The installation was a technical challenge due to the heavy weight of the machine and limited space between the buildings, but could be finalized without problems.
WP3

**Athanasios Zacharopoulos and Jorge Ripoll** present the FORTH contribution to fast inversion methods.

The work performed during the 3rd reporting period has been focused on the following:

FORTH has managed to create a pipeline to interface the FTM-XCT dataset to the Matrix-Free algorithm, so that the reconstructions are now less demanding in computational resources and a greater amount of data can be now used in the process. This allows FORTH to utilise the big amounts of data coming from a 360 degree acquisition system and to use more than one wavelength for the acquisition. Future work will include a deformable atlas to either segment the CT automatically or simplify the meshing process. If multispectral reconstructions are necessary they have the necessary software available and regularisation with the inclusion of more priors is possible. Subsequently it is specified in more detail on the multispectral capacity of the device. Main and very strong collaboration with exchange of data and personnel has taken place during this reporting period with UZH, UCL, CEA-LIME, HMGU and FIHGM.

WP4

**Teresa Correira** (UCL) outlines the contribution of UCL with view to image reconstruction and image priors. The theory behind FMT reconstructions using XCT image priors is explained, and the inversion algorithms are tested on both simulations and real data, provided by partners 1 (HMGU) and 5 (FIHGM). The algorithm implementation is based on the software package “Time-resolved Optical Absorption and Scattering Tomography” (TOAST) developed at UCL. Finally, the user-friendly software that incorporates the new inversion algorithm is presented.

Major findings are that images reconstructed using the anisotropic better than simple Tikhonov regularisation. The reconstructions times are in a range of within seconds. The Jacobian calculations is the most time consuming step of the image reconstructions. It depends on the number of projections and wavelets used.

WP5

**Angelique Ale and Max Koch and Markus Mronz** present the work of the last year covering

The work that has been performed for reporting period 3 is:

- Prototype including proprietary gantry has been installed (deliverable 5.4)
- Common file format has been developed for exchange of data
- Training data sets have been acquired and uploaded to common data server (task 5.3, deliverable 5.8)
- Algorithms for the assignment of optical properties were integrated in the reconstruction code (task 5.2, deliverable 5.7)
- Optimal acquisition parameters have been defined (deliverable 5.8)
- User friendly software is installed and operational on acquisition (deliverable 5.9)

WP6

**Anikitos Garafolakis** (CEA lime) presents the results of WP6 within the last year with a focus on breast cancer imaging for the use of FMT-XCT under realistic conditions:

- Available animal models and fluorescent probes were explained and discussed
- Quantitatively examined FMT performance to visualize disease processes in-vivo
• Explains FMT-XCT calibration and a combined FMT-PET-CT imaging protocol
• A cancer imaging protocol in combination with PET was explained
• He discussed the inclusion of MCF7 xenografted tumor/aptamers ACE 8 in the future studies

The clinical utility of the new system will have to be evaluated until month 48 of the project.

**WP7**
Florian Stuker and Katerina Dikaiou from UZH present results of WP7- imaging cancer therapy. They give an overview of the results achieved so far, on the available tumor models, hypoxia imaging, the combined FMT-MRI imaging and reconstruction method and an outlook on the next steps. The latter will include the setup of a second generation FMT-MRI, the establishment of an additional breast tumor model and drug studies using HIF (heat inducible factor) readout.

**WP8**
Juan José Vaquero introductorily sums up the third year activity on FIHGM/UC3M’s contributions to WP2, WP4 and WP8. Contrast agent experiments (Iopamiro 300, Fenestra VC) were performed in vivo via different routes in mice. The influence of scattering was examined and a mouse phantom with realistic scattering values for lung and liver was developed. Quantification errors were 86% for a homogenous model and 16% for a heterogeneous model.

Vasilis Ntziachristos as the coordinator ends the session with an outlook on open points to discuss next morning and thanks all participants for their great work.

**April 15th, 9-12 am 2011**

The morning session focuses on currently open technical questions and the optimization of collaboration concerning the planning and execution of the animal experiments necessary to validate FMT-XCT in vivo. The number of animals to be measured in the individual studies is defined. Also, a related training session will be organized in fall in Munich. The possible time frames for the training and the experiments are discussed.

Additionally, possible option for harmonization of the different software and data platforms are discussed. The optimal use of the XML specification file and data sets still to be put on the exchange platform on the FMT-XCT homepage are discussed.

Since a cost neutral extension of the project is desired by the majority of the project participants, this issue will be readdressed in fall 2011. Current opinion of the project officer is that a substantial change in the work program might be necessary for a prolongation.

The necessity of additional dissemination activities of the individual partners in their own countries, in order to raise public awareness of the FMT-XCT project once it is close to finalization e.g. via press releases is emphasized.

In the afternoon a guided tour through UCL imaging facilities is organized by Simon Arridge and Teresa Correira.
Beside the Workshops (details see WP9: Training and Dissemination) several telephone conferences and work package meetings (e.g. visit of Marco Bramilla (Cea Leti) at FIHGM - details see WP2, visits HMGU/ CT Imaging –details see below, visit of Vasilis Ntziachristos (Coordinator) at UCL, March 2011 and many more) have been conducted to discuss technical details.

Within the last year there was a regular exchange of information between HMGU and CT Imaging to finalize the prototype. On October 19th 2010 Markus Mronz and Mario Winterer were visiting HMGU (members HMGU joining the meeting: Vasilis Ntziachristos, Veronika Erben, Maximilian Koch). The following topics were discussed:

1) Integration of the „FMT-Chain"
2) Project plan: The FMT_XCT Gantry shall be installed at HMGU latest in March 2011
3) Demonstration of the FMT Lab-View program: Maximilian Koch demonstrated a FMT acquisition for a better understanding.
4) Discussion of hardware issues
5) Commercialization steps based on Annex 1

Max Koch (HMGU) visited CT Imaging several times within the last year. During three visits at in Erlangen the software interface between FMT- and XCT- chain was developed. In addition the mechanical and electrical integration of parts of the FMT hardware was implemented. On the first meeting the functional specification on the software interface where defined and basic technical options were evaluated. On the second meeting concrete implementations where evaluated. The hardware integration was finalized. For this purpose the FMT hardware was brought to Ct-imaging. On the third meeting the implemented software interface was finalized and tested in independent unit test. The final prototype was delivered to HMGU (Klinikum Rechts der Isar, Munich) on March 09, 2011.

At the several meetings and conferences, members of the consortium were discussing progress, issues and objectives concerning technical details of the project. Several telephone conferences have been conducted to discuss technical details concerning the integration of the XCT into the FMT system.

A regular exchange of information by email or telephone between the members of the executive committee and/or the project manager took place. Currently, there are no known problems within the network.
Project website
The domain http://www.fmt-xct.eu is hosted at the HMGU. The layout was designed to please the eye and provide a simple and functional interface. It is clearly evident that funding is provided by the European Commission under Framework 7. Beside the European map it contains the generic European flag and the FP7 logo (for more details see periodic report 1). The project website has been updated regularly. On “Members only” are available:
• Annex 1
• Presentation of consortium meetings
• Minutes of consortium meetings
• First and second periodic report including deliverables and milestones
A Platform for data exchange has been established at http://www.fmt-xct.eu/transfer/, login data were sent to all partners. Vamp (Partner 7) has been replaced by CT Imaging on the website (details see changes in the consortium). To avoid confusion due to uncoordinated entries as only contact the data of Veronika Erben are mentioned.

Ethical and animal welfare management
This call requests pre-clinical imaging, as necessary for developing a new imaging modality and creating new knowledge and algorithms. To perform animal experiments at HMGU the animal questionnaire (details see report 2) was sent to UZH and Cea Lime and animal models and probes of WP6 and 7 were integrated into the HMGU animal protocols, which should be approved within the next weeks.

Project timetable and status
One of the main tasks of the third year was to install the final FMTXCT prototype (Work package (WP) 5) at HMGU, which was finalized in cooperation with Ct-imaging. WP2 and WP 3 can be considered as completed. All deliverables and milestones for the reporting period 3 were finished. Milestone 11 (FMT-XCT methodology for quantified treatment imaging) correlates to deliverable 7.5, which is due in the reporting period 4. Therefore it was decided to postpone this milestone.

Within the last year, as described in Annex 1, one of the main focus will be to image animal models and cancer therapy (WP6 and 7) with the final FMTXCT prototype and compare FMTXCT imaging accuracy with PET CT data (WP8).
DELIVERABLE NUMBER: 2.6
DELIVERABLE NAME: IDENTIFICATION OF OPTIMAL CONTRAST-ENHANCEMENT STRATEGY

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3
PERIOD COVERED: FROM MARCH 01, 2010 TO FEBRUARY 28, 2011

Work Package: 2 - XCT Development
Date: 30.03.2011
Written by:
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1 Introduction

In conventional radiography, contrast is produced by differences in absorption of the X-rays crossing different parts of the imaged object. X-rays can be treated in this case as point particles propagating through the object along straight lines from the source to the detector. Inside the object, X-rays eventually have the possibility of interacting with the electronic shells of the atoms or the molecules constituting the object and being consequently absorbed or scattered (both elastically and non-elastically). The three main phenomena governing interaction of X-rays with matter in the energy range of $5 - 150$ keV (the energy range exploited for X-ray imaging) are the Rayleigh scattering, dominating at the lowest energies, the photoelectric effect in the intermediate energies and the Compton scattering at the highest end of that range.

For classical imaging application, x-rays absorption is described by the Beer-Lambert law:

$$ I = I_0 e^{-\mu l} , \quad (1) $$

which expresses the dependence of the X-ray intensity coming out of the object ($I$) in function of the impinging beam intensity ($I_0$) and where the $\mu$ coefficient summarises the three aforementioned effects.
The simplicity of equation 1 has permitted classical radiological imaging a widespread diffusion as an easy to perform yet powerful diagnostic technique in many application, from medicine to industrial quality inspection both in planar configuration and in tomographic modality, because the linearity of the absorption phenomena allows using a straightforward to implement and computationally not too intensive 3D reconstruction technique known since the 1910s. The main limitation of absorption based X-ray imaging is that, when the object is composed of materials with very close absorption properties such as different tissues and organs of a human or animal patients, it is no more possible to identify the single components, so that radiography is not a very good imaging modality to study, for example, soft tissues anatomy.

One technique that can greatly improve this limitation is phase contrast imaging (XPCI). Contrast in XPCI is generated by differences of index of refraction inside the sample rather than differences in the absorption coefficient and since at the surface of separation of two different materials inside the subject there is a refraction index discontinuity which is generally much higher than the corresponding absorption step, in the resulting images the contours of each subregion are greatly enhanced. The drawback is that XPCI requires a rather high degree of coherence of the impinging radiation in order to observe the edge enhancement, which means that either micro-focus sources or synchrotron facilities must be used to illuminate the object. Often, to improve coherence properties of the illumination beam, diffraction gratings are inserted along the beam path with the effect that only a small fraction of the radiation emitted by the source contributes to image formation: this means that for conventional sources, given the limited amount of available power, the exposure times to obtain an image with an acceptably low noise level are greatly increased, making XPCI definitely unfeasible for practical applications. The very high brilliance and coherence properties of synchrotron light, on the contrary, do not pose this kind of problems, even though the limited availability and the high installation and operation costs of synchrotron sources reduces convenience and applicability of the technique. Since the first implementations in 1990s [26], XPCI has been object of intense investigation to the point that many techniques have been set up for different applications using both synchrotron sources and conventional x-rays generators.

2 Physical principle of phase contrast generation

Phase contrast arises because both the amplitude and the phase of x-rays are modified as an x-ray beam propagates through an object. A detailed understanding requires the radiation be treated as a wave field rather than as simple geometrical optics. The effects on x-rays propagating through an object can be described by the complex refractive index

\[ \tilde{n} = n + ik, \]

where the parameters \( n \) and \( k \) are the usual refractive index and the extinction coefficient respectively. The real part of \( \tilde{n} \) describes phase changes, while the imaginary part is responsible for attenuation. \( k \) is related to the linear
The attenuation coefficient $\tau$ by
\[ \frac{4\pi k}{\lambda\rho} = \tau. \] (3)

The following relations show the dependence of $n$ on the object’s physical properties and the incident radiation energy [9, 25]:
\[
1 - n = \sqrt{\frac{\mu_r\varepsilon_r}{2}} \left[ \left( 1 + \frac{\sigma^2}{\omega^2\varepsilon_r^2} \right)^{1/2} + 1 \right]^{1/2}
\] (4)
\[
k = \sqrt{\frac{\mu_r\varepsilon_r}{2}} \left[ \left( 1 + \frac{\sigma^2}{\omega^2\varepsilon_r^2} \right)^{1/2} - 1 \right]^{1/2}
\] (5)

In the preceding equations $\mu_r$ and $\varepsilon_r$ represent respectively the magnetic permeability and the dielectric constant of the material and $\sigma$ is the material’s electrical conductivity, while $\omega$ is the frequency of the incident radiation. The associated phase change and attenuation on propagating at a distance $z$ are given by:
\[
\Delta \varphi = \frac{2\pi}{\lambda} \int n(z)dz \quad \text{and} \quad -\log \frac{I}{I_0} = \frac{4\pi}{\lambda} \int k(z)dz
\] (6)

where $I_0$ is the incident intensity, $I$ the final intensity and $\lambda$ the radiation wavelength. In the context of phase contrast imaging the important aspect is that differences in the real parts of the refraction index of different tissues are more than three orders of magnitude greater than the corresponding variations of the imaginary part within the diagnostic x-rays energy range [10]. In addition, $n$ diminishes much more slowly ($\sim E^2$) than $k$ ($\sim E^4$) as energy is increased [30].

Basically, phase contrast in radiograms is generated by changes in the direction of propagation of x-rays in correspondence to object edges, i.e. it is the effect of diffraction. To observe a significant pattern in the recorded images, the diffracted beams must interfere on the detector, so that the phase information is amplitude encoded because of interference. To do that a high degree of lateral spatial coherence of the illumination beam is required, like that intrinsically obtainable with synchrotron light. Phase contrast can be also observed with conventional sources, provided that some cunning is taken to obtain a (at least partial) coherent illumination of the sample: in particular, the radiation produced by a quasi-monochromatic and totally incoherent source becomes partially coherent at large distances of propagation, compared to the linear size of the source [3]: the obtainable lateral coherence length $d$ can be expressed by the following relation:
\[
d = \lambda r_{so}/f,
\] (7)

where $\lambda$ is the radiation wavelength, $r_{so}$ the source to object distance and $f$ the source spot size. It is therefore evident that to improve radiation coherence, and thus phase contrast, small focal spot size and large source to object distances are required, while temporal coherence and high monochromaticity seem to have a much lesser influence [10].
3 Experimental techniques

To put in evidence phase related intensity variations in x-rays images there are mainly three techniques which are presented in literature:

- in-line phase contrast imaging,
- diffraction enhanced imaging,
- interferometer based imaging techniques.

and they will be described in more details in the next subsections.

3.1 In-line phase contrast X-rays imaging

In-line phase contrast imaging is the simplest, from an instrumental point of view, of the three techniques and it was presented for the first time in 1995 [26], thus being the first XPCI technique actually implemented. It exploits x-rays trajectory deviation due to phase change in transversing the object to improve contrast mainly at object’s edges and allows recording an image where the effects of both attenuation and phase variation are superimposed. Figure 1 shows the principle of the technique: it is essentially the x-ray analogue of Gabor’s in-line holography principle. Initially, the coherent and monochromatic x-rays produced at synchrotron facilities were exploited, but more recently, many authors have reported of experiments with conventional micro-focus x-rays sources, [3, 4, 6, 12, 24] even if the use of synchrotron radiation still gives better performances in terms of image quality and acquisition duration [5, 19, 27]. In all the preceding papers, example images are reported of almost only objects of very small thickness: insects, leafs, small specimens of biological tissues (lamb liver, human breast tissue) and even small diamonds or zirconia micro-spheres. The only exception is [27], where phase contrast images of a rat’s and a rabbit’s lungs obtained by means of synchrotron radiation are showed. The main reason for such thin samples is that better phase contrast is obtained at very low x-rays energies (10 ÷ 20 keV), for which the penetration length of the radiation is very limited. This is particularly true for the systems using micro-focus conventional sources: according to formula 7, to obtain a highly coherent beam on the sample, a big wavelength (i.e. low energy photons) and a big distance source-object are
required, which means that the penetration length is very small and the photon flux on the sample is very low, making it impossible to image objects thicker than few millimeters. The two small animal imaging examples were obtained with higher energy x-rays (35 keV the rat and 51 keV the rabbit, respectively) and, in spite of the high brilliance of the synchrotron radiation used, exposure times are very long (30 s and 60 s for a single radiography) compared to conventional absorption imaging. Another point which is important to remark is that to obtain images of good quality it is essential to find a good compromise between x-rays energy, object-detector distance and detector’s resolution: in fact, the first two parameters influence the angle of deviation of x-rays and the size of the regions on the detector where they produce interference and edge enhancement. Too high photon energy and small object-detector distance produce very small interference regions, so, if the resolution of the detector is not sufficient, no phase contrast can be observed; on the contrary, too low energy x-rays and too big object-detector distance produce big interference regions that can hide small details, degrading image resolution. Object to detector distances can range from 0.3 m to 12 m, even if the most common range is 0.5 ÷ 1.5 m depending on the size of the object’s details that are to be put in evidence in the phase image, both for synchrotron radiation based systems and micro-focus x-ray generator based. For the last systems, the source to object distance is typically about half a meter (with a focal spot size smaller than 10 μm).

As previously stated, the image intensity at the detector is a superposition of attenuation and refraction effects. Because phase contrast imaging is particularly interesting for objects composed of materials of low atomic number, it is a good approximation to consider them as completely transparent to X-ray radiation. Moreover, also phase changes induced by the presence of boundaries inside the object are generally of very small entity. Then, developing the calculations introduced in section 2, image formation can be described as follows.

The object is supposed to be illuminated by a perfectly coherent, monochromatic plane wave of amplitude $E_0$ and is described as a complex refraction index function of the position: $\hat{n}(x) = n(x) - ik(x)$, where $k(x)$ takes into account attenuation and $n(x)$ phase shift; $x$ is the position in the object’s plane perpendicular to the x-rays propagation direction. Using complex exponential notation and avoiding to take into account explicit time dependence ($-i\omega t$) in the exponent, the electric field immediately after the object may be written as:

$$E(x,0) = E_0 \exp [i\kappa(x)z]$$

$$\kappa(x) = \hat{n}(x)\kappa(x) = n(x) + ik(x)$$

$$E(x,0) = E_0 \exp \left[ (n(x) + ik(x)) z \right]$$ (8)

For an object of constant thickness $\bar{z}$, we can resume the two terms in the exponential with two functions $\beta(x)$ and $\phi(x)$ of the position only, taking into account attenuation and phase shift respectively:

$$E(x,0) = E_0 \exp(-\beta(x) + i\phi(x))$$ (9)

Almost transparent object and small phase shifts hypothesis allow assuming $\beta(x), \phi(x) \ll 1$ so that electric field after the object can be approximated by:

$$E(x,0) \simeq E_0 \left[ 1 - \beta(x) + i\phi(x) \right]$$ (10)

5
The electric field in the image plane results being the propagation through free space of the object’s modulated electric field from the object plane to the image one. Consequently, it can be expressed, by using the Kirchhoff integral formula, as the convolution of $E(x,0)$ and the Fresnel kernel of near-field free space propagation:

$$E(y, r) = (\lambda r)^{-1/2} \exp(-ikr) \int E(x, 0) \exp(-ik(y - x)^2/2r) dx$$  

(11)

where $y$ is the coordinate in image space, $r$ the object-image plane distance and $k = 2\pi/\lambda$ the radiation wave vector. Omitting some manipulations of the above equation (see [27] for the details), the following expression of image intensity modulation due only to refraction effects can be obtained:

$$E(y, r) = E(y, 0) + \frac{ir}{2k} \frac{\partial^2 E(y, 0)}{\partial y^2}$$  

(12)

The recorded image intensity is proportional to the square of the electric field amplitude; substituting the right member of equation 10 in second term of the preceding equation, neglecting $\beta(x)$ and considering the Taylor expansion at the first order of the resulting squared electric field, we obtain:

$$I(y, r) = |E(y, r)|^2 \simeq |E(y, 0)|^2 + \frac{r}{k} \frac{\partial^2 \phi(y, 0)}{\partial y^2},$$  

(13)

which means that the contrast in the phase image is basically proportional to the second derivative of the complex part of the index of refraction of the object\(^1\).

### 3.2 Diffraction enhanced X-rays imaging

Diffraction enhanced XPCI is an improved version of free space propagation phase contrast imaging: to implement this technique, a collimator is added along the beam path as shown in figure 2, which translates phase modulations of the x-ray beam emerging from the object into amplitude modulations. The detector is placed off-axis to the illumination beam and is aligned with the analyser grating at its Bragg’s angle, where it acts as a mirror with a reflectivity dependent on the angle of incidence of the X-rays. Undiffracted rays, propagating parallel to the illumination axis, are 50% reflected, while the reflectivity for the diffracted ones depends on the direction of propagation, i.e. on their phase according to:

$$I_R = I_0 R(\theta_B + \theta),$$  

(14)

where $I_R$ is the reflected intensity, $I_0$ the intensity incident on the analyser crystal after having transversed the object, $\theta_B$ is the Bragg’s angle of the crystal and $\theta$ the angle of incidence of the x-ray. $R(\theta)$ is the analyser reflectivity (the rocking curve) and attains the maximum of 50% precisely in coincidence of the Bragg’s angle.

\(^1\)The image intensity result proportional only to the second derivative along the $y$ direction of the complex part of the index of refraction of the object because in [27] the authors consider the incident radiation coherent only in the $y$ direction and therefore refraction effects can be observed only in that direction. In case of coherent illuminating radiation in both directions, $\nabla \phi(x)$ should be considered.
3.3 X-rays imaging by grating interferometer

The second group of techniques capable of putting in evidence phase contrast information exploits a grating interferometer to obtain a sort of “dark field” X-ray imaging, in a way that closely resembles dark-field optical microscopy [30]. In figure 3 the principle of operation of a grating interferometer imaging system with coherent illumination is sketched. The key elements of the system are the two gratings $G_1$ and $G_2$: the first one is a phase grating, while the second is an absorption grating which acts basically as a transmission mask. The phase grating (of period $g_1$) produces an interference pattern which, at distance $z_t = 2g_1^2/\lambda$, is the exact image of the grating itself (Talbot effect). The $G_2$ grating is then placed at distance $z_t$ from $G_1$ with the same orientation and periodicity as the interference pattern and the detector is positioned close to $G_2$. If the absorbing grooves of $G_2$ are correctly aligned with the intensity maxima of the interference figure produced by $G_1$, then a minimum of intensity is recorded on the detector. Now, if a phase object is placed before $G_1$, it produces distortions in the wavefront impinging on this first grating that are traduced in shifts of the position of the interference maxima on the plane of $G_2$ and, in turn, translated in variations of the intensity recorded on the detector. For this scheme to work, the illumination beam must be highly coherent, otherwise no interference figure can be produced by $G_1$ [1, 28]. Moreover, since the displacements of the maxima of the interference pattern are very small, also the period of the gratings themselves must be consequently small (few micrometers). In principle
Figure 4: Grating interferometer XPCI principle with a conventional X-rays source. The \( G_0 \) grating splits the incoherent beam produced by the extended focal spot of the tube into an array of self-coherent, mutually incoherent sources.

the second grating could be removed and the displacement of the interference fringes directly measured on the detector if its pixels were sufficiently small (same size or smaller than of the fringes displacement), but practically this is not the case since current detectors have pixels of the size of some tens of microns. The requirements on the positioning of \( G_2 \) with respect to the interference pattern of \( G_1 \) are stringent only in terms of parallel alignment of the grating with the interference fringes. Relative lateral displacement, on the contrary, is not a problem because it influences only the value of the background offset. Moreover, it can be exploited by acquiring multiple images, each time changing the \( G_1 - G_2 \) relative lateral position, to recover complete and quantitative information on total phase shift induced by the object [23, 21, 29, 22].

The grating interferometer based technique illustrated is limited to imaging only refraction index fluctuation in one direction, notably that perpendicular to the direction of the two gratings grooves. Therefore the constraints on beam coherence can be a little relaxed, allowing the requirement of a good degree of coherence be satisfied only in the same direction as the recorded phase fluctuations. This allows to avoid using synchrotron sources at the price of introducing a third grating in the system (see figure 4) and adapting the distances between the gratings to a divergent beam rather than a parallel one (take into account magnification effects). The \( G_0 \) grating, which is an amplitude grating with alternatively transparent and highly attenuating grooves, splits the incoherent, diverging beam of a conventional (not even micro-focus) X-ray generator into an array of mutually incoherent line-sources. If the width of the transparent grooves is sufficiently small, each of them can be considered as a self laterally-coherent source useful for being used in the interferometer for XPCI. The resulting image in this case is the superposition of the phase-contrast images produced by each of the source grating’s grooves. The advantage of this technique over in-line XPCI is that a an X-rays generator with quite a big focal spot can be used so that a much higher photons flux is available on the sample. Moreover, the use of a grating instead of a single slit or pinhole permits to exploit the illumination beam in a much more efficient way.

From the point of view of the dimensions of the system, the overall size is comparable to that of in-line XPCI systems, both for synchrotron sources
and conventional ones, even if the detector is much closer to the sample and the source a bit more distant: for example in [23] the distances $G_0 - G_1$ and $G_1 - G_2$ are respectively 1.765 m and 27.8 mm, while in [7] the authors optimize the same system and propose 66.6 cm and 39.9 mm respectively. Finally, in [11], an improved version of the system described in [23] illuminated by synchrotron radiation, the source to object distance is 25 m, while $G_1 - G_2$ distance is comprised between 22.5 mm and 40.2 mm depending on the wavelength (14 keV to 25 keV).

The gratings used for the realization of the instruments are all silicon microstructures appositely developed and with dimensions optimized on the base of the operating wavelength and the working distances of the experimental setup. The phase grating $G_1$ is an array of parallel thin silicon plates 2 $\mu$m thick and 2 $\mu$m spaced (4 $\mu$m pitch), 20 $\mu$m tall for a total grating size of $64 \times 64$ mm$^2$. $G_2$ grating has half the pitch of $G_1$ and moreover, to make it an amplitude grating, the space between the plates if filled with gold. For X-ray tube based systems, the $G_0$ grating is basically a silicon substrate over which gold plates are deposited, with a pitch of 40 – 80 $\mu$m.

One interesting possibility that grating interferometer based XPCI offers is that, by recording multiple images while scanning the lateral position of the $G_2$ grating over one period of the grating itself, it is possible to reconstruct the full phase profile of the radiation incident on the detector. In fact, for a given pixel of coordinates $(x, y)$, let its intensity in function of the grating position be $I(x, y, x_g)$: it is related to the wavefront phase in the same position by the following relation:

$$I(x, y, x_g) = \frac{\lambda d}{g_2} \frac{\partial \varphi(x, y)}{\partial x},$$  \hspace{1cm} (15)

where $\varphi(x, y)$ is the wavefront profile function. So, the wavefront shape can be easily reconstructed by a simple integration along $x$. Moreover, being the wavefront shape related to the refraction index inhomogeneities by a linear relation, actually the reconstructed $\varphi(x, y)$ is proportional to the Radon transform of the real part of the refraction index of the object, so that also its 3D reconstruction can easily be performed by means of a filtered back-projection algorithm. The mean value of the different $I(x, y, x_g)$ is identical to a conventional radiography taken without the interferometer and it contains the absorption information and, depending on the imaging geometry, eventually also the edge-enhancing Fresnel diffraction contrast obtainable with the in-line configuration.

From the point of view of the size of the specimens and the acquisition times, it should be noted that in all the analysed papers, similarly to in-line XPCI, the sampled objects’ sizes are relatively small ($\approx 1$ cm). The exposure times are rather variable, from “…few minutes per raw image…” in [8] to about 20 minutes to obtain 9000 phase projections of full tomographic dataset reported in [11]. Such a variability depends mainly on the type of the source (for synchrotron radiation much shorter times, of course), on the pitch of the source grating $G_0$ (the higher the pitch, the shorter the exposure time) and on the type of detector used. Nevertheless, it seems that a quite high photon flux is required to have reasonable exposure times, since in all the articles either a synchrotron source was used or an industrial grade generator with 1 mm$^2$ focal spot size and very high electronic currents (25 – 30 mA @ 40 kV).
3.4 Coded aperture X-rays imaging

Coded aperture XPCI lays somehow half way between the in-line and the grating interferometer techniques described above. It is a technique conceived, patented and developed at UCL [15, 16, 17, 18, 20] and takes into account the advantages of both methods. It uses two gratings and, for illumination, either synchrotron light or conventional source x-rays, as shown in figure 5. From the point of view of the X-rays physics, this method is closer to in-line XPCI, in that the two gratings (whose pitch is bigger than that used for grating interferometer based XPCI) act basically as transmission masks, rather than diffraction ones. The $G_2$ grating is actually a mask with the same pitch of the detector’s, is placed against it and it shades the borders of each pixel, allowing the undeviated x-rays reach only the central part of the pixel itself. The $G_1$ grating, placed before the object, provides a structured illumination pattern complementary to $G_2$. An almost transparent, homogeneous object produces no deflections on the direction of propagation of the x-rays, so, just like in dark-field diffraction enhanced differential phase contrast imaging described in the preceding section, the resulting image recorded by the detector is dark or anyhow homogeneous, depending on the relative alignment of the two masks. Refraction index inhomogeneities in the sample change the direction of propagation of x-rays, locally illuminating more or less the unmasked portion of the detector’s pixels. This is directly translated into correspondingly local variations of intensity thanks of the presence of the $G_2$ grating. A certain grade of coherence of the source is necessary, but less stringent than for in-line XPCI, because the lack of coherence is compensated by edge illumination of the grids: in first approximation, when using a conventional source, the focal spot size should not exceed the masks pitch, i.e. $\sim 100 \mu m$. In January 2009, the same UCL group proposed a variation of the technique to render it sensitive to phase shifts in two direction rather than only one, substituting the usual mono-dimensional gratings with an array of “L” shaped transmission masks in place of $G_1$ and an array of square holes in place of $G_2$ (see figure 6 and [15]). Contrarily to what happens with the grating interferometers technique, scanning the relative position of the two gratings, both in the mono-dimensional case and the bi-dimensional one, does not provide a better sampling of the phase front of the radiation reaching the detector, but changing it allows adjusting the phase contrast in the recorded images. In particular, when the portion of the pixels illuminated by the un-
Figure 6: Coded aperture XPCI illumination masks. Left: a mono-dimensional mask; the same mask is used both for pre-sample beam shaping and as after-sample analyser grid. Right: bi-dimensional masks; $G_1$ apertures are "L" shaped, while $G_2$ ones are square holes. They are aligned so that only a part of the unperturbed rays illuminate the pixel behind the second mask. Phase changes inside the object make deviated rays converge either inside the square transparency or on the attenuating frame, thus coding changes in propagation direction as detected intensity changes. Rightmost sketch: illumination pattern on $G_2$ – the dark yellow central region is that illuminated by unperturbed rays; the light yellow one is the region illuminated by x-rays deviated on the left and downwards, resulting in increased detected intensity; the brown region is the one illuminated by x-rays deviated on the right and upwards, resulting in decreased detected intensity.

deviated beams is very small, a dark-field image is obtained, where the phase contrast is maximally enhanced; on the contrary, when the transparent parts of $G_1$ are aligned with those of $G_2$, the pixel is maximally illuminated, but the phase contrast is reduced. In parallel, higher phase contrast enhancement requires necessarily longer exposure times due to the reduced photon flux which is allowed to reach the detector. It is interesting to note that, passing from the condition of full pixel illumination to the opposite one, not only there is a great improvement in the sensitivity of the technique, but also that the nature of the phase signal changes. In fact, with transparent slits aligned, the phase contrast is generated only by rays deviated at big (for XPCI) angle, so that the photons that, if undeviated, would have arrived on a pixel, arrive on the adjacent ones due to phase shift. This is exactly the principle of in-line XPCI, sensitive to the second derivative of the distribution of the index of refraction inside the object. On the contrary, dark field configuration works on a principle closer to grating interferometer based phase contrast imaging, where especially small angle deviations are put in evidence and the recorded signal is proportional to the deflection angle and hence to the first derivative of the index of refraction [20].

The size of the experimental setups needed for coded-aperture XPCI are quite close to the in-line configuration, with distances between the two gratings varying in the analysed articles from 40cm to 120cm and with a distance from the source to the first mask of about 1m, when using conventional sources. Of course, for synchrotron radiation, this distance is much higher.

From the point of view of acquisition times, the coded-aperture configuration is quite interesting, in that it offers a certain adaptability at the cost of sacrificing contrast. In particular, for mono-dimensional phase sensitive setups, the presence of the grids masks about 50% of the active area of the detector (75% masking for 2D masks), with a correspondingly double (4 times) theoretical ex-
Figure 7: Sketch of the working principle of interferometer based XPCI. A first grating (S) splits the illumination beam and the M grating(s) deflect the secondary beams towards the analyser grating (A). One of the two secondary beams transverses the object and is phase-modulated by it, producing and interference pattern on the detector. Here three different possible configurations of the gratings are shown.

Exposure time needed for each image, which is fairly a good result compared to the other techniques. Nevertheless, together with the increase of phase sensitivity changing the alignment of the two masks, a reduction of the effective flux on the detector is observed. Moreover, this reduction is not associated with a reduction of the dose delivered to the sample (no quantitative relation is indicated between masks relative position, signal intensity/exposure time and dose to the object), so that an increase of the dose to the sample is to be expected with respect to the other modalities and to conventional absorption radiography.

3.5 Interferometer based X-rays imaging

This technique is based on the use of an X-ray Michelson interferometer. Basically the illumination beam is split into two components, the first of which, traveling along one of the arms of the interferometer, crosses the object; the second one, instead, propagates freely along the second arm and the two beams are re-combined on the detector to produce an interference pattern [14, 13]. Needless to say, the primary illuminating beam must have a very high degree of lateral coherence in order to observe interference on the detector; moreover, each grating crossed by the beam actually splits it in two, each of them of half amplitude (also the ones called "mirrors" in the caption of figure 7). These two conditions together make it impossible using conventional sources for interferometric XPCI because of inadequate coherence and photon flux of x-ray tubes, so that the use of synchrotron radiation is a bound choice. This technique is potentially the most sensitive of the ones presented before to phase changes, but at the same time the most complex from and experimental and practical point of view. In fact, the grating assemblies of figures 7.a and 7.b are cut from a silicon monolithic perfect crystal [13], meaning very high costs and above all, limited sample dimensions (in principle 7 cm) because of the limited maximum sizes of available silicon crystals (15 cm). The configuration of figure 7.c can in principle overcome this problem, because the two couples of gratings (S–M and M–A) are built from two separate crystals and their distance can be adjusted to host bigger samples (bigger than 10 cm × 10 cm, ideally); nevertheless alignment and stability problems emerge in this case because sub-nanoradian angular stability must be achieved. Stability problems, even if slightly less stringent, are present also in the other two configuration, to the point that the interferometers are mounted on very large granite blocks to avoid vibrations and are maintained in rooms with very well stabilized temperature and air pressure [2, 13] (tempera-
ture fluctuations induced by live samples put close to the gratings are sufficient to misalign the interferometer!)

Diffraction enhanced XPCI is similar to the grating interferometer based technique in that it is sensitive to the first derivative of the index of refraction and because, by changing the optical length of the reference arm of the interferometer, it is possible to sample the phase front of the radiation that transversed the object and reconstruct it. This way, phase projections of the sample can be obtained and tomographic reconstruction can be simply achieved by means of a filtered back-projection algorithm.

4 Conclusions

XPCI is a very interesting technique from the point of view of the attainable contrast enhancement with respect to conventional radiography: it exploits a different physical principle that has the potential to increase contrast up to $10^3$ times compared to attenuation based imaging with similar or even lower dose exposure levels. This is especially true for low Z materials, such as biological tissue, where attenuation contrast is very limited, which is something that renders the technique particularly attractive in the context of the FMT-XCT project.

Nevertheless, at the current state of the art, in spite of the great progresses made since the first implementations of phase contrast imaging, the applicability of the technique for pre-clinical applications remains rather limited for different reasons, of which, insufficient laboratory sources brilliance is by far the most important. Among the other reasons there are the necessity of quite big source–object–detector distances, insufficient resolution of current detectors and, for certain XPCI modalities, excessive experimental complexity. Some attempts have been made to overcome these limitations, such as the introduction of gratings or coded apertures, but if from one side the aforementioned problems are partly overcome, the economic cost of the required equipments is for the moment still prohibitive.

To conclude, XPCI is not yet a mature technology for routine application and therefore, given the current state of the art, it is not to be considered a valid alternative to other x-ray contrast enhancement techniques such as the use of iodinated contrast agents or dual energy techniques.

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1 Abstract

This document is a comparison of different X-ray to mography contrast enhancement techniques that could be applied on the final FMT-XCT prototype to improve internal organ detectability, so that the obtained images, after segmentation, could serve as prior information for the optical reconstruction algorithms. Four different techniques were considered, namely a dual energy technique, four different contrast agent based techniques, a double exposure technique and phase contrast imaging technique.

Firstly, the results of the dual energy protocol defined and analyzed in the "Deliverable 2.4 – Preliminary specification for XCT design to be implemented on the hybrid prototype" are compared to those of the single, low energy part of the same protocol. Afterward, the double energy strategy is compared to four different contrast agent based techniques carried out in Madrid on Dec., 16 and 17 2010 expressly in order to compare these two strategies on the same machine and on the same mice instead of, as it was done until now, on different machines and different mice, thus avoiding inter-machine and inter-animal variabilities.

Finally, the last two techniques, double exposure and phase contrast imaging, are investigated in terms of feasibility and applicability for the FMT-XCT project.

2 Dual energy – single energy images contrast comparison

2.1 Introduction

In the following paragraphs we present a comparison between the dual energy protocol described in deliverable 2.4 with a single energy configuration in terms of achievable contrast under the constraints of acquisition times and dose delivered to the animal, showing that for the purposes of soft tissues contrast enhancement the second, high energy, acquisition is not worth the increased time and dose burden.

Data are based on dual energy scans performed on the CEA-LETI bench on sacrificed mice.

For practical purposes, the imaging protocols used for this study are recalled:

- Low energy acquisition: 40kV, 6mA, 100um tin filter; 400 projections at 0.9° angular step, with fixed frame rate of 2 images per second; projection images are then 4x software binned, dark current and flat field corrected and processed by a Feldkamp reconstruction algorithm to obtain a 3D reconstruction of the linear attenuation coefficient distribution inside the object.

- High energy acquisition: 70kV, 2mA, 100um lead filter; 400 projections at 0.9° angular step, with fixed frame rate of 2 images per second; projection images are then 4x software binned, dark current and flat field corrected and processed by a Feldkamp reconstruction algorithm to obtain a 3D reconstruction of the linear attenuation coefficient distribution inside the object.

- Combined image: images are linearly combined ($I_{\text{comb}} = \lambda_1 I_{\text{LowE}} + \lambda_2 I_{\text{HighE}}$)

2.2 Image comparison

In order to compare the contrast gain attainable with the dual energy acquisition and image processing protocols, we reported in the following images 1 and 2 respectively some slices of the low and high energy reconstructed volumes, and we plotted the related profile along the blue line in the bottom part of figure 1, that crosses three different types of tissues; muscle, adipose tissue and kidney. We also reported in the rightmost part of figure 2 the same profile plot traced on the combined image obtained as: $I_{\text{comb}} = 2.3065 \cdot I_{\text{HighE}} - I_{\text{LowE}}$, i.e. the combination that maximizes the contrast to noise ratio between adipose tissue and all the other soft tissues for those images (cfr. deliverable 2.4, pages 13-15 for details). In the images in figure 1, a little difference in the reconstructed attenuation values is present (at least between some organs and in the low energy images), as confirmed in the plots of figure 2, but there are two problems: firstly, the difference between two organs, if any, is smaller than its standard deviation (~ 0.0015cm$^{-1}$) inside the same organ; secondly, this difference is not perfectly reproducible from animal to animal. Moreover, analyzing the high energy images, they don't seem to add any particularly significant information, since they show the same kind of structures as the low energy ones, just less contrasted. This consideration is also supported by plots in figure 3, where the scatter plot of the low/high energy reconstructed attenuations are reported for the main organs. For each point of the diagram, a 3D region of interest was manually chosen inside the related organ and the mean and the standard deviation of the voxels values inside each of them were evaluated and reported on the image. What's evident is the neat distinction between bones and soft tissues, but also, interestingly enough, of adipose tissue between the soft tissues. What's more critical is the fact that the points representing heart, liver, kidneys and skeletal muscle are almost superposed, as is confirmed in the right part of the same figure, representing a zoom on soft tissues points. From the point of view of contrast evaluation, in the following table contrast for the kidney/adipose tissue, kidney/muscle and muscle/adipose tissue couples are compared in
Table 1.

<table>
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<tr>
<td>Combined images</td>
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<td>0.367 1.0741</td>
<td>0.053 0.1481</td>
</tr>
</tbody>
</table>

Figure 1. Comparison between some slices of both the low (left) and high (right) energy reconstructed data of a mouse scan. The main organs and the corresponding mean attenuation values in cm⁻¹ (low energy / high energy) are put in evidence. Organ segmentation was performed manually.

Figure 2. Plots across the blue profiles in fig. 1. The combined plot is obtained as: Comb.Img. = 2.3056*HE-LE. Inside each plot, the three tissues crossed are marked and the mean reconstructed linear attenuation coefficient is reported in red.

Figure 3. Scatter plot of the low and high energy reconstructed linear attenuation coefficients for different tissues of a mouse. The right part of the figure represents a zoom on the soft tissues data points.
Table 1. Contrast and contrast to noise ratio between the three types of tissue reported in Table 3.

Considering the contrast only, these data confirm again that high energy contrast is lower than low energy one and also that the contrast improvement obtained combining the high and low energy images is very small. It should also be considered that generally, because of muscular relaxation between the first and the second scan needed for the dual energy protocol, the mouse changes a little its shape and internal organs their position: this means that the two images are not directly superimposable, but some kind of shape matching algorithm should be envisaged (in this study low and high energy matching was performed manually).

In conclusion, the dual energy protocol, compared to a single energy one, requires twice the acquisition time because of the second energy scan, implies a higher dose to the animal, and needs a quite complex shape adaptation algorithm to correctly combine the two reconstructed 3D images. Given the poor contrast gain it provides, we suggest to avoid the implementation of the full dual energy protocol on the final FMT-XCT prototype for routinely animal imaging, but rather to use the sole low energy acquisition. This last, with respect to imaging protocols used on commercial scanners (that are similar to the high energy one), allows obtaining some global contrast gain, at the sole price of a slightly increased animal radiation dose.

3 Low energy – contrast agent protocols comparison

3.1 Introduction

In this chapter we analyze and compare the contrast enhancement obtainable with both the low energy protocol described above and different contrast agent based protocols. Since the CEA-LETI bench is not suitable for extensive animal imaging, all the experiments subsequently described were carried out in Madrid using the facilities and the prototype X-rays scanner provided by FIHGM: this way all the uncontrollable variabilities involved in comparing images obtained on different machines were avoided. This machine is not exactly the same as the FMT-XCT final prototype, but differences are limited and the results should be at a great extent valid also for the latter.

3.2 Machine description

The machine at FHGM in Madrid is a gantry-mounted prototype x-ray scanner coupled with a FMT chain. The animal is held on a carbon fiber bed, z-axis-translatable in order to simplify the positioning of the animal and, eventually, change the imaged region of the body. During the experiments no FMT data were acquired, the only interest being in X-rays, so the optical chain will not be described.

The micro-focus X-ray source is a modified version of the commercial Hamamatsu L9631, in which the control unit has been split from the high voltage source and the X-ray tube, thus significantly reducing the size and the weight of the device. The size and weight reduction obtained simplifies to a great extent the integration of the source in a moving gantry. The X-ray source has a power of 50W with a focal spot size depending on the applied power and ranging from ~15um to ~80um, an anode voltage range between 20kV and 110kV, a maximum electron current of 800uA.

The detector (Hamamatsu C7940DK-02) is composed by a needle-shaped CsI:Tl scintillating layer, directly deposited on top of an array of CMOS photo-diodes. The field of view is 120mmX120mm and, with 2400x2400, 50um sized square pixels. The input window is a 1mm thick carbon fiber sheet, particularly suited for small animal given its very low attenuation properties that maximizes sensitivity. The detector is mounted on the gantry by means of a motorized translation stage that allows changing imaging magnification.

To stop the X-ray flux outside the acquisition system, the prototype is equipped with a shielding box integrated in the rotating gantry that blocks also the scattered radiation that can be harmful for the FMT acquisition components and the light reaching the FMT field of view.

3.3 Experimental setup

Four different experiments were carried out on 2 mice on 2 days, changing at each experiment the contrast agent or the way it was administered to the animal. For each experiment the following protocol was adopted:

1. each mouse was imaged with the dual energy protocol;
2. contrast agent was injected;
3. contrast agent imaging protocol applied.

In the following section the imaging configuration of the dual energy protocol and of the contrast agent acquisitions are specified.
Imaging configurations

The generator working point and filter material and thickness for the dual energy protocol were adapted by means of software simulation to the physical and geometrical characteristics of the bench, as described in "deliverable 2.4 – Preliminary specification for XCT design to be implemented with the hybrid prototype". The settings for the contrast agent acquisitions were determined on previous experience of the FIHGM group.

All images were acquired with a fixed integration time of 125ms, at 4x binning, with 720 projections. The source-to-isocenter and source-to-detector distances were set to 155mm and 215mm, respectively. For the two modalities, only the X-ray generator working point and filters were changed.

**Dual energy protocol, low energy acquisition:**
- 35kV, 175μA
- 50μm Tin filter

**Dual energy protocol, high energy acquisition:**
- 50kV, 25μA
- No filter

**Contrast agent acquisition:**
- 40kV, 150μA
- 1mm Al filter

In order to minimize reconstruction artifacts, dark (for dark current noise subtraction) and flat (for the calculation of attenuation data) images were acquired at each change of the x-ray generator working point.

**Flat images acquisition procedure:**
- series of 8 repetitions of 40 images. Last 40 images are taken with same current as imaging settings. The first 7 are taken at half current, a quarter current etc... for better detector's non-linearity correction. For each 40-images series, the mean image is calculated and stored. For reconstructions with CEA-LETI reconstruction engine only the last image (at the same working point as the actual acquisitions) was used.

**Dark images acquisition procedure:**
- 40 images are acquired without X-ray illumination to measure dark signal of the detector. The mean image is calculated and then stored for subsequent calculations.

During the experiments the full dual energy protocol was applied for completeness of data acquisition, but in the discussion that follows we will use only the low energy data for comparison against the contrast agent images, because of two main reasons already treated into the first part of this document and also in "Deliverable 2.4 – Preliminary specification for XCT design to be implemented with the hybrid prototype": firstly, adding the second scan for the high energy image improves the protocol duration and the dose to the animal, while it does not improve significantly the combined image contrast; secondly, mouse muscular relaxation between the first and the second acquisition considerably change the shape of the animal and the position of internal organs, so that the superposition of the low and high energy images is difficult and definitely non optimal.

### 3.4 Choice of the contrast agents

Four different approaches for soft tissue contrast enhancement were explored using two different commercial contrast agents. The two selected contrast agents are Iopamiro 300 (Bracco Imaging s.p.a., Milan, Italy) and Fenestra VC (Advanced Research Technologies Inc. Montreal, Canada).

The contrast agent Iopamiro is a water-soluble iodinated contrast agent similar to those used in clinical practice for applications such as head and body contrast-enhanced computed tomography, excretory urography or angiography. Its composition is based on Iopamidol, with a concentration of 61.2%. This kind of contrast agents are rapidly cleared by the animal kidneys when they are injected intravenously, providing great contrast capabilities in kidneys and bladder some minutes after injection. They can also be administered intraperitoneally, providing negative contrast between the abdominal organs and background tissue that can be used to delimitate the animal organs from its surrounding fat tissue.

To assess the contrast enhancement achievable by the use of Iopamiro in every situation, a third administration technique was tested. Iopamiro was orally administered to the mouse. This way, an increase in the contrast between the gastrointestinal track and the surrounding tissue is expected.

On the other side, Fenestra VC is a iodinated contrast agent (50 mg iodine/ml) which is not water-soluble, feature that enlarges to a great extent the time the contrast agent stays in blood, providing great vascular contrast capabilities. After some minutes it is cleared through the liver and accumulates in it, obtaining a contrast enhancement between this organ and the surrounding tissue.
3.5 Experiments description

Measurements procedures adopted for each experiment are the ones described above. In this paragraph the contrast agent protocols are described.

Four different approaches were used, according to the discussion in the previous section. For all of the contrast agents experiments, the animals were anesthetized using Isoflurane and a common animal preparation technique was used, consisting of the steps summarized below.

1. Anesthetize the animal using the anesthesia concentration for induction during ~45 seconds or less if the animal is properly anesthetized.
2. Reduce the anesthesia concentration to that for catheter placing.
3. For those experiments in which the contrast agent is administered intravenously, expand the tail vein with a 100W red light bulb placed 15cm far from the tail. Light is applied for ~1 minute.
4. Apply some alcohol to the injection area with a sanitary napkin and insert the needle of the catheter, except for the case of oral Iopamiro.
5. Place the animal on the bed of the imaging system together with the necessary devices to maintain anesthesia and set its concentration to the maintenance level.
6. Start the injection of the contrast agent using the catheter at the recommended flow (or start the oral administration in the case of orally administered Iopamiro) and record the start time for the injection.

The timing for the different acquisitions is summarized below.

1. intravenous Iopamiro
   a. 1st acquisition 5’ after injection
   b. 2nd acquisition 15’ after injection
2. intravenous Fenestra
   a. 1st image taken ~5’ after injection
   b. 2nd image taken ~20’ after injection
   c. 3rd image taken ~50’ after injection
3. intraperitoneal Iopamiro
   a. 1st image taken ~2’ after injection
   b. 2nd image taken ~10’ after injection
4. oral ingestion Iopamiro
   a. 1st image taken ~5’ after ingestion
   b. 2nd image taken ~15’ after ingestion

In every case where two acquisitions are performed, the whole experiment delivers ~100 mGy to the animal under study.

3.6 Experimental results

In this section, we present the images obtained for each of the experiments described above, analyze contrast and put in evidence advantages and disadvantages for the different applied protocols.

3.6.1 Intravenous Iopamiro

Iopamiro rapidly diffuses from the blood flow to the kidneys, that are readily put in evidence in the image with an absorption coefficient closer to bones rather than to soft tissues. The diffusion from circulation to kidneys is so quick, that in the image taken 5’ after injection the kidneys seem saturated with contrast agent. Interestingly, the image 15’ after injection shows an even better contrast, putting in evidence also the internal structure of the kidneys (at least for what resolution and image quality allow to see) probably thanks to a partial wash out of the contrast agent.
Figure 4. Comparison of the images obtained with the low energy configuration of the dual energy imaging protocol (a) and with the contrast agent protocol (b and c). Image b was acquired 5' after CA injection, image c 15' after CA injection.

Figure 5. Contrast comparison between low energy image and intravenous iopamiro contrast agent protocol. On the right the profile plot of the reconstructed signal and on the left the images and the corresponding profile.

Also the low energy image (figure 4.a) allows to clearly distinguish kidneys, but the achievable contrast in not comparable at all with the one obtained in contrast agent images.

Quantification of the contrast is performed by tracing a profile plot of the reconstructed x-ray linear attenuation coefficient as reported in figure 5.

The profile was plot across three significant tissue regions: a kidney, an adjacent back skeletal muscle and the adipose tissue between them. In the following table 1, the mean attenuation values inside each region are reported together with the corresponding standard deviations.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Muscle</th>
<th>Adipose</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Energy</td>
<td>0.074</td>
<td>0.051</td>
<td>0.068</td>
</tr>
<tr>
<td>5' after injection</td>
<td>0.063</td>
<td>0.050</td>
<td>0.088</td>
</tr>
<tr>
<td>15' after injection</td>
<td>0.064</td>
<td>0.053</td>
<td>0.087</td>
</tr>
</tbody>
</table>

Table 1. Linear attenuation coefficient and standard deviation inside some representative tissues in the mouse abdomen. Values are reconstructed from data obtained with the low energy protocol and with 2 contrast agent protocols (intravenous iopamiro 5' and 15' after injection). Values are in cm⁻¹.
In table 2 (and in all subsequent contrast calculations) the relative contrast between the couples of tissues and the related contrast to noise ratio are reported. They are calculated as:

\[
C_{rel} = \frac{|\mu_{i1} - \mu_{i2}|}{\min(\mu_{i1}, \mu_{i2})} \quad \text{CNR} = \frac{|\mu_{i1} - \mu_{i2}|}{\max(\sigma_{i1}, \sigma_{i2})}
\]

Compared to a noise level of about 0.003 cm\(^{-1}\), the three protocols allow distinguishing the three tissue types, but when contrast agent is present the contrast level is increased significantly. Particularly difficult is to find a contrast between muscular and kidney tissues with the low energy protocol, since the contrast is only a few times bigger than noise.

### 3.6.2 Intravenous Fenestra

Fenestra, contrarily to iopamiro, remains confined into blood circulation for rather a long time (several hours) and therefore allows obtaining images where contrast is based on blood perfusion. Therefore, contrast is not so specifically concentrated onto a specific organ (the kidneys, in the case of iopamiro), but is globally distributed all over the whole body. Conversely, the contrast improvement is much less neat. With reference to figures 6b and 6c, the aorta in the middle of the chest and abdominal region is the most evident feature of the image, but intestines walls are distinguishable as well, even if with a resolution of 130 µm, it is difficult (at least in a 2D representation) to clearly outline single organs or intestine’s shape because of the closeness of separation surfaces. Compared to the low energy image, some improved contrast is present but amelioration does not seem to be dramatic. In order to quantify contrast, only the low energy image and the the contrast agent one 5' after injection will be considered, since no difference is appreciable between this last and the image acquired 15' after injection.

In figure 7, the difference between the low energy and the contrast agent protocols is clearly evident both from a visual and quantitative point of view. Visually, the low energy image is brighter but more noisy, because the spectrum of the illumination beam is so shifted towards lower energies that most of the signal is absorbed into the object, resulting in higher attenuation and lower photon flux on the detector. The contrast agent image has instead a lower intrinsic contrast, but the presence of the iodinated contrast agent in the blood puts in evidence soft tissues details thanks to their perfusion. This is particularly evident for the heart, where the presence of the contrast agent allows distinguishing visually the two atria, information that is confirmed in the profile plot on the right part of the same figure.
10

Figure 8. Contrast comparison between low energy image and intravenous Fenestra contrast agent protocol. On the right the profile plot of the reconstructed signal and on the left the corresponding images and the profile position.

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Adipose</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Energy</td>
<td>0.073</td>
<td>0.053</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td>0.004</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>Contrast agent</td>
<td>0.040</td>
<td>0.026</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.007</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3. Linear attenuation coefficient and standard deviation inside some representative tissues in the mouse abdomen reconstructed from data obtained with the low energy protocol and with the contrast agent protocol (Fenestra intravenous). Values are in cm$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>Muscle-Adipose</th>
<th>Muscle-Kidney</th>
<th>Adipose-Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Energy</td>
<td>0.377</td>
<td>0.042</td>
<td>0.243</td>
</tr>
<tr>
<td>Contrast agent</td>
<td>0.538</td>
<td>0.050</td>
<td>0.615</td>
</tr>
</tbody>
</table>

Table 4. Contrast between the three types of tissue reported in table 3.

In the abdominal region, both in the low energy and in the contrast agent images, the details are less contrasted. For the C.A. image, this is due to a diffuse increased contrast almost everywhere, while, for the low energy one, to the bad quality of the image itself (curiously, this particular image is more noisy and of poorer quality with respect to what is normally obtained with the same protocol. This is evident also comparing it to the other low energy images presented in this report). In any case, the contrast values obtained in this case always for the muscle-adipose, muscle-kidney and adipose-kidney couples of tissues are in general lower than those obtained in the preceding section. The presence of contrast agent does not contribute to internal organs separation since probably abdominal organs perfusion is more homogeneous.

3.6.3 Intraperitoneal Iopamiro

Intraperitoneally injected, Iopamiro diffuses inside the interstitial spaces between abdominal organs, thus producing a negative contrast, with interstitial spaces brighter than organs themselves. In figure 9.b, a short time after injection, contrast of interstitial spaces is clearly visible due to the presence of the contrast agent; in figure 9.c, 15’after injection, the contrast starts to become less evident with an overall increment of attenuation, probably because of penetration of the contrast agent inside organs. In general, with respect to the low energy image (fig. 9a), more details and inter-organ spaces seem to be evident, even if no contrast increase should be expected in the mean inter-organs attenuation values. In the following figure 10, the usual profile plot across kidney, adipose tissue and muscle is reported.
In this case, the low energy protocol, exploiting a less energetic illumination beam, generates a contrast between the three types of tissues that is not present in none of the contrast agent images. In fact, with reference to figure 10.II, the plot across the red profiles of figure 10.I shows a clear contrast of muscular and kidney tissues against adipose tissue, but only in the low energy image a difference in the reconstructed attenuation coefficients of muscle and kidney is present (even if faint). The situation is almost the same for the green profiles of image 10.I, plotted in figure 10.III (only the 5’ after injection image is analyzed, since there is no difference from the 15’ one), where a thin film of contrast agent is interposed between the right kidney and the surrounding soft tissues: this does not induce a difference in the values of attenuation coefficients of the two organs, but their separation in the contrast agent image is clearly evident as shown in figure 10.III. For the sake of internal organs segmentation in the X-CT volumes this should be a very helpful element. For completeness, in table 5 the contrast values for plot of figure 10.II are reported.

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Adipose</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Energy</td>
<td>0.075</td>
<td>0.002</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Contrast agent</td>
<td>0.060</td>
<td>0.041</td>
<td>0.059</td>
</tr>
<tr>
<td>05’ after</td>
<td>0.003</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>injection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrast agent</td>
<td>0.060</td>
<td>0.043</td>
<td>0.061</td>
</tr>
<tr>
<td>15’ after</td>
<td>0.002</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>injection</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Linear attenuation coefficient for some representative tissues in the mouse abdomen reconstructed from data obtained with the low energy protocol and with the contrast agent protocol (Iopamiro intraperitoneal). Values are in cm⁻¹.

<table>
<thead>
<tr>
<th></th>
<th>Muscle-Adipose</th>
<th>Muscle-Kidney</th>
<th>Adipose-Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Energy</td>
<td>0.442</td>
<td>0.087</td>
<td>0.327</td>
</tr>
<tr>
<td>5’ after</td>
<td>0.463</td>
<td>0.017</td>
<td>0.439</td>
</tr>
<tr>
<td>injection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15’ after</td>
<td>0.395</td>
<td>0.017</td>
<td>0.419</td>
</tr>
<tr>
<td>injection</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Contrast between the three types of tissue reported in table 5.

### 3.6.4 Oral iopamiro

![Figure 11. Comparison between low energy image (a) and orally injected iopamiro images, respectively 5’ after ingestion (b) and 15’ after ingestion (c).](image)
After oral ingestion, lopamido floods the first part of the digestive tract and allows putting in evidence mainly the stomach and the highest part of the intestines as is clearly evident in figure 11, parts b and c. The contrast is actually very localized to the digestive tract, but the improvement with respect to low-energy image (figure 11.a) is evident. To quantify such improvement, in figure 12.a we reported the profile plot across the red profiles of parts a and b of the preceding figure: as is evident, in the low energy image it is possible to identify the three different crossed regions once again only thanks to the presence of the interstitial space between stomach and intestine; this, with a lower absorption coefficient with respect to surrounding tissues, is the separation marking element. In the lopamido image, thanks to the presence of the contrast agent especially on the walls of stomach and intestine, the borders of these two organs are much more clearly marked. We pass from contrast ratios of 0.364 (stomach-interstitial space) and 0.391 (interstitial space-intestine) for the low energy image to 1.979 and 0.75 respectively for the lopamido enhanced image.

On the contrary, looking at figure 12.b, we can see that where no contrast agent is present, the contrast improvement obtained with the low energy protocol allows distinguishing intestine features that are not apparent in the contrast agent image (even if with a faint contrast of 0.146).

### 3.7 Differences between the used scanner and the FMT-XCT final prototype

As anticipated in the introduction, the machine used for the experiments described is slightly different from the final FMT-XCT prototype, even if the differences are smaller than those between the CEA-LETI bench and the FMT-XCT machine. The most evident differences concern once both the X-rays source and the detector. Differences between the sources of the two machines is rather minimal from the imaging performances point of view, the greatest being in the maximal output power (50W for the FIHGM machine, 80W for the FMT-XCT one). Output window and high voltage range are similar, so we expect the spectra of the emitted beams to be quite close at equivalent working points.

Concerning the detectors of the two machines, they are almost the same model, but with a significant difference: the FIHGM detector has a carbon fiber window, while the FMTXCT one an aluminum cover. This will make a bit of difference in the spectra of the measured x-rays, with the FMTXCT detector measuring a narrower, higher energy shifted spectrum.

The impact of these peculiarities on the contrast agent protocol should be minimal, since for these imaging techniques the presence of an aluminum filter between the source and the object already cuts lower energies: if we consider the carbon fiber window as completely transparent (indeed a reasonable approximation), the aluminum one on the FMTXCT detector should represent no more than an attenuation factor of about 1.275 of the energy reaching the detector and a negligible beam hardening.

On the contrary, the impact on the low energy protocol can reasonably be expected to be a little higher, since for this one the less energetic photons are the ones that mostly contribute to contrast improvement and the aluminum cover will probably cut a part of them away. Nevertheless, the CEA-LETI detector is exactly the same as the FMT-XCT one and the images presented in the first part of this report taken with the aluminum cover do not show flagrant contrast degradation with respect to those obtained with the FIHGM detector.

### 4 Double exposure protocol

**Development of double-exposure techniques for soft-tissue contrast enhancement**

Contrast enhancement methods that do not involve dual-energy X-ray exposure are under study and development at FIHGM. These techniques extend the effective dynamic range of the Flat-Panel detector to obtain better quality images when the sample has both low and high attenuation areas. (Flat-Panel are the preferred X-ray detectors for cone-beam geometries, even when the dynamic range is not as good as other semiconductor detector technologies). The need of a wider dynamic range on the detector arises when a dense material, such as a metal probe (used for gating, monitoring,
etc.) is placed inside the body of the animal under study or when a soft tissue surrounded by a thick bone needs to be imaged. In those sample areas where the number of photons reaching the detector is similar to the dark current level of the detector pixel, the projection data is strongly degraded by the quantization noise. The developed method involves the acquisition of two different datasets with different radiation exposure durations, but the same spectral configuration. Then, both scans are appropriately combined taking into account the detector and sample properties.

During the last year, several combination algorithms were developed and implemented and their results were compared in order to obtain the optimal combination technique. The final algorithm is based on a maximum likelihood estimation that makes use of the information present in both datasets to obtain the pixel value of the high-dynamic-range projection image.

The quality of the dual-exposure data was compared with that achieved using a regular acquisition protocol delivering the same radiation dose to the sample. The dual-exposure data show better signal-to-noise ratio, contrast-to-noise ratio and low contrast resolution. The enhancement is larger in the high attenuation regions of the sample, as expected. Thanks to the better quality of the dual-exposure data, the analysis of the different tissues is easier and those structures masked by quantization noise are unmasked.

The enhancement on low-contrast resolution and signal to noise ratio is shown in figure 13. Special attention must be paid to the enhancement in the profile data shown in figure 14.

![Figure 13](image.png)

**Figure 13.** Slices of a PMMA phantom with inserts filled with water acquired using a regular protocol (left) and the dual-exposure protocol (right). Note the better definition of the smallest inserts in the dual-exposure data.

![Figure 14](image.png)

**Figure 14.** Profile data measured across the white lines shown in figure 3 for the regular data (left) and for the dual-exposure data (right).

5 **X-ray phase contrast imaging**

In the context of improving the reconstructed volumes contrast for better image segmentation, CEA-LETI investigated also the feasibility of using in the FMT-XCT project an x-rays imaging technique called *phase contrast imaging* (XPCI).

With this technique, image contrast is not due to variations of absorption properties in different parts of the object, but rather on differences of refractive index. The great advantage is that image contrast due to a given refractive index discontinuity in the object is about $10^3$ times stronger than that produced by the corresponding attenuation generated one, especially for low atomic number materials.

The drawback is that, being a sort of interferometric technique, it needs object illumination by means of quite highly coherent x-ray beams, requirement that is currently very difficult to satisfy with laboratory sources.

In what follows, the most interesting aspects of this technique are resumed, while a more extended analysis is reported in annex I – “X-ray Phase Contrast Imaging review”.

5.1 **Different XPCI techniques**

The first attempts at obtaining phase contrast based X-rays imaged date back to the beginning of year 1990ies. Since then, a number of different techniques have been presented in literature. The main families of technique are:

- in-line xpci
• diffraction enhanced x-ray imaging
• grating interferometer based x-ray imaging
• coded aperture x-ray imaging
• interferometer based x-ray imaging

Of these, the inline, grating interferometer and coded aperture techniques are the most interesting in the FMT-XCT context and, between them, the inline technique is by far the simplest, the least expensive and the most practical to be possibly implemented for the project. For this reason, only inline xpci will be presented, leaving the description of the others to the annex I.

5.2 In-line XPCI

In-line phase contrast imaging is the simplest, from an instrumental point of view, of the three techniques and it was presented for the first time in 1995, thus being the first XPCI technique actually implemented. It exploits x-rays trajectory deviation due to phase change in crossing the object to improve contrast mainly at object’s edges and allows recording an image where the effects of both attenuation and phase variation are superimposed. Figure 15 shows the principle of the technique: it is essentially the x-ray analogue of Gabor’s in-line holography principle. Supposing that the impinging x-ray beam is perfectly coherent, the phase shifted wavefront exiting the object propagates to the detection plane and there the generated interference figure is recorded. By means of the Kirchhoff integral formula, under the hypothesis of coherent illumination, it is possible to calculate the detected field’s amplitude, that comes out to be proportional to the second derivative of the refractive index along the directions perpendicular to the x-ray propagation axis.

Figure 15. In-line phase contrast imaging principle.

The oldest papers reporting of the development of this technique made use of the highly coherent and monochromatic x-rays produced at synchrotron facilities; more recently, it has been discovered that the beam monochromaticity is not an essential requirement for the formation of the xpci interference image, while spatial coherence is and many authors have reported of experiments with conventional micro-focus x-rays sources, even if the use of synchrotron radiation still gives better performances in terms of image quality and acquisition duration for its high spatial coherence and high brilliance.

To consider a micro-focus spot as point-like, the source to object distance must be much bigger than the focal spot size itself, and generally the one reported in literature is about 50cm. Moreover, in order for the phase image to have details detectable by a detector with 50um sized pixels, the object to detector distance should be in the order of 50cm-150cm, thus making the whole assembly a bit bigger than the currently planned FMT-XCT prototype system.

Since the amplitude of the phase signal decreases for increasing x-ray energy, it is preferable to use very low energy illumination beams (15keV-20keV); nevertheless at so low energies, also biological tissues are strongly attenuating, so that the penetration depth is limited to several millimeters and the most frequently reported specimens are tree leaves, insects or very thin portions of biological tissues. Small animal imaging is rather difficult with laboratory sources, whose brilliance would impose very long exposures to obtain a good signal to noise ratio, while it is relatively easier with synchrotron sources.

In figure 16 an example 1D phase contrast signal is showed that was calculated by means of a simulation software expressly developed at CEA-LETI to evaluate the feasibility of in-line XPCI for the FMT-XCT project.

Figure 16. In-line XPCI signal amplitude for different illuminating X-ray energy (beam supposed monochromatic) for an object to detector distance of 1m and a detector’s pixel size of 5um. The simulated object (left image) is a plastic cylinder of 0.5mm diameter immersed into water.
The plot shows the signal obtained simulating a 0.5 mm diameter cylinder made of plastic immersed in water. The two materials have almost the same linear attenuation coefficient, so in a conventional radiography they could not be distinguishable. Nevertheless, the small difference in their refractive index generates a strong signal in correspondence of the refractive index discontinuities at the borders of the cylinder. Unfortunately, the signal amplitude rapidly decreases with increasing energy and the width of the oscillations is so narrow that, to avoid damping due to the limited resolution of the detector, a very small pixel has been simulated (5 μm).

5.3 XPCI: Concluding remarks

XPCI is a very promising technique, that has the potential to increase to a great extent the sensitivity of x-ray imaging. Nevertheless, at the current state of the art, the lack of very brilliant laboratory x-ray sources and the limited resolution of detectors greatly limits the possibilities of this technique. In particular, the technique would be hardly implementable on the FMT-XCT prototype and, more importantly, would be impossible to use for routinely practice.

6 Conclusions

In this report we compared different contrast enhancement strategies for application on the FMT-XCT final hybrid prototype. In particular, 5 different techniques have been analyzed and compared:

- single energy protocol,
- dual energy protocol,
- four different contrast agent based protocols,
- double exposure technique,
- in-line x-ray phase contrast imaging.

Of these, in-line x-ray phase contrast imaging was demonstrated to be unfeasible for the FMT-XCT project due to the fact that the technology is not yet mature for practical applications. For the remaining techniques, each of them has advantages and disadvantages that make it more suitable than the others depending on the situations.

The full dual energy protocol and the low energy only one allow obtaining a general, whole body improvement of contrast with respect to imaging protocols commonly used in the pre-clinical practice (such as those suggested for small animals commercial scanners), which are very similar to the one used for all the contrast agent acquisitions. In certain situations, this improvement is sufficient to detect anatomical features not detectable with protocols using more energetic X-rays, nevertheless, the entity of this contrast improvement is globally rather faint and does not allow to ameliorate significantly the possibility of identifying all the internal organs of the mouse. Between the two protocols, the full dual energy one adds some complexity and is more time requiring with respect to the low energy only protocol and the contrast gain it offers is limited, so the latter seems to be more suitable for routinely use.

The four contrast agent based imaging protocols we tested showed a neat increase of contrast thanks to the presence of the iodine-based contrast agent, whose linear attenuation coefficient is much stronger than that of all soft tissues. The main drawback we could remark for such protocols is anyhow the fact that better results are obtained when a specific organ is targeted by the contrast agent: in this case the target organ undergoes a very significant contrast enhancement with respect to the surrounding tissues (as it was the case for example for kidneys with intravenous injection of iopamiro or the stomach for oral ingestion of the same product). In the other cases, where the drug was supposed to enhance contrast more globally (intravenous Fenestra or intraperitoneal iopamiro), the contrast agent diffusion dynamics, the very complex anatomy of the mouse and/or the limited resolution of the obtained images do not allow obtaining so brilliant results.

The last technique we compared (double exposure) is a strategy to extend the dynamic range of the detector and could be useful to put in evidence low contrast details in highly attenuating parts of very heterogeneous objects when in low attenuating areas the detector dynamic range is saturated.

In conclusion, our sensation is that when for a given study a very specific organ is of interest and a suitable contrast agent and/or a related administration protocol are available to target it, the attainable contrast enhancement is by far superior compared to what can be obtained without a contrast enhancing product. On the contrary, when this is not possible, the low energy configuration is a good technique to push to the maximum the imaging performances of the machine in use.

* On this point we also recall that other more complex protocols are cited in literature and used for small animal imaging (but also on human patients) that combine the use of a contrast agent with a dual energy technique to further improve contrast when a contrast enhancing drug is to be used. See for a review: A.Graser, T.R.C.Johnson, H.Chandarana, M.Macari, "Dual energy CT: preliminary observations and potential clinical applications in the abdomen", Eur. Radiol. 19 (2009)
DELIVERABLE /MILESTONE NO: 3.6

DELIVERABLE/MILESTONE NAME: QUANTITATIVE EVALUATION OF ULTRA-FAST INVERSION WITH HYBRID DATA

GRANT AGREEMENT NUMBER: 201792

PROJECT ACRONYM: FMT-XCT

PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3

PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
Introduction

In this part of the WorkPackage our Main Objective was to train the Matrix-Free Algorithm for Reconstructions on Fluorescence Molecular Tomography (FMT) to work with the data acquired from the FMT-XCT tomographer in Munich. The matrix free method provides a very fast way to reconstruct for FMT images that is extremely cheap in computational resources such as memory and computational time, allowing for a large amount of data, that a 360 degrees rotation of the specimen would create.

The main point of focus in this work is the interfacing between the .xml data format, used as a standard in the project, and the creation of the necessary input for the matrix-free algorithm. This effort requires the extraction and creation of a Tetrahedral mesh from the XCT images provided during the acquisition with the FMT-XCT apparatus and the projection of sources and detector positions, as well as the choice of the data to be used on the surface of the specimen under imaging.

More specifically, in this note we will follow the necessary steps taken on this direction based on two examples of datasets acquired from the FTP server of the project. The first set “KOCH10_Agar2_exchange” is a cylinder phantom with two fluorescence tubes and the second “NeckProsense” a mouse with a neck tumour. For a description of the experiments see Deliverable 5.5. All the steps are accounted for and the resulting reconstruction is given at the end.

Mesh Generation

The first step in our effort included the creation of a tetrahedral mesh that would be suitable for the use of the matrix-free algorithm. Since the algorithm is based on the TOAST FEM solver, tetrahedral elements were chosen as the optimal geometrical discretisation method since they can be flexible in describing a complicated geometry without an great increase on the size of the mesh. In addition there are many available tools for manipulation of tetrahedral meshes. In our case we used the Tetgen (http://tetgen.berlios.de/) mesh Generator which is free for research and non-commercial uses and the Octave/Matlab wrapping for Tetgen from the iso2mesh package (http://iso2mesh.sourceforge.net/cgi-bin/index.cgi). For the segmentation and manipulation of the actual mouse XCT image (figure 2), we used the 3DMed (http://www.mitk.net/) medical imaging software and Amira a commercial equivalent. The result of the segmentation of the bone and lungs region of the mouse can be seen in figure 3. The final meshes used had 15800 nodes and 92873 elements for the mouse (figure 5) and 11006 nodes 47868 elements for the cylinder phantom (figure 6).
Figure 1: slices of the XCT image of the specimen 1: cylinder phantom.

Figure 2: slices of the XCT image of the specimen 2: mouse with neck tumour.

Figure 3: Bones and lungs mesh from segmented mouse XCT.
Figure 4: Mouse experimental setup. The Green squares represent the 18 positions of the camera, while the green lines are the direction of viewing for the camera. Red circles represent the sources positions.

Figure 5: Surface of the mouse model: with red cycles representing sources positions and magenta squares the projected on the surface sources. The red line represents the axis of rotation.

Figure 6: Surface of the Cylinder model: with red cycles representing sources positions and the green x's the normals of the sources used for the projection on the surface of the cylinder.
Data Acquisition and manipulation

Once the meshes describing the geometry of the specimen are created the next step is to find the sources and detector position on the surface of the objects that will be used. The xml data format save the position and the normal to the camera and detectors plane so this information has been used to project the camera and sources to the surface of the object. A simple algorithm that calculates intersection of lines with the triangles of the surface of the objects has been created and the projections are stored. For the cylinder model we used 162 sources and 11684 detector positions while for the mouse model 161 sources with 6497 detectors as seen in figure 7. The images of fluorescence and excitation wavelength were mapped on the positions of the camera and the positions of visible detectors for each camera was projected back on the images. To eliminate the effect of noise in our chosen data points, averaging over a small neighbourhood was used for each detector point.

Figure 7 : Surface of the mouse model: The green dots represent the chosen detector position on the surface, red cycles the sources positions and magenta squares the projected on the surface sources. The red line represents the axis of rotation

Looking on the sum of the ratios of fluorescence divided by excitation data used for the algorithm in the case of the cylinder (figure 9) we noticed the effect of the holding rods of the experimental setup in the data. We successfully removed the data that are affected by the holding rods as seen in figures 10 and 11.
Figure 8: The ratios of fluorescence divided by excitation data used for the algorithm projected on the surface of the object. Notice the red lines occurring from the two rods holding the cylinder in place on the lower side of the cylinder.

Figure 9: The ratios of fluorescence divided by excitation data used for the algorithm projected on the surface of the object, after removing the effect of two rods holding the cylinder in place on the lower side of the cylinder.

Figure 10: The ratios of fluorescence divided by excitation data used for the algorithm projected on the surface of the object, after removing the effect of two rods holding the cylinder in place on the lower side of the mouse.
Matrix Free

Once the data were converted on the format necessary for the matrix free algorithm and a good quality meshes were created, we were ready to proceed with the reconstruction. We choose a Tikhonov regularization method since we were not using any prior constrains about the positions of the fluorochromes inside the specimen. The reconstruction in the case of the mouse used less than 600mb of memory and took approximately 5 minutes for the inversion in a Pentium Laptop. The resulting images can be seen in figures 11 and 12. For the case of the cylindrical Phantom the results can be seen in figures 13 and 14 and the inversion took about 3 min in a Pentium laptop.

Figure 11: Matrix-free reconstruction on a mouse. Blue is the reconstructed fluorochrome concentration

Figure 12: Matrix-free reconstruction on a mouse. Blue is the reconstructed fluorochrome concentration
We have successfully managed to create a pipeline to interface the FTM-XCT dataset to the Matrix-Free algorithm, so that the reconstructions are now less demanding in computational resources and a greater number of data can be now used in the process. This allows us to utilise the big amount of data coming from a 360 degrees acquisition system and even to use more than one wavelength for the acquisition.

References


DELIVERABLE 4.5
DELIVERABLE QUANTIFICATION OF ALGORITHMIC PERFORMANCE

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
1. Summary

Our inversion algorithm based on prior information is tested on experimental data, provided by partners 1 and 5. The algorithm implementation is based on the software package Time-resolved Optical Absorption and Scattering Tomography (TOAST) developed at UCL.

2. FMT inversion using XCT image priors

Consider the forward problem to be \( y = F(u) \), where \( F : U \rightarrow Y \) is an operator mapping from parameter to data space and \( y \) is the measured data, which is considered to be \( y\text{fluor}/y\text{exc} \), where \( y\text{fluor} \) are the fluorescence measurements and \( y\text{exc} \) the excitation measurements. The image reconstruction involves minimising the following objective function:

\[
\begin{align*}
\text{Minimise } E(u) &= \frac{1}{2} \| y^{\text{meas}} - F(u) \|^2 + \tau \int_{\Omega} \psi(|\nabla u|) d\Omega \\
&= L(y^{\text{meas}}, F(u)) + \tau \Psi(u),
\end{align*}
\]

where \( \tau \) is the regularisation parameter. The term \( \Psi \) is the prior function, which in the case of multimodality imaging, is constructed in terms of an auxiliary reference image. In this particular project, the XCT data is used as the reference image.

3. Quantification of algorithmic performance using experimental data

3.1. Phantom

Data were provided by partner 5. The phantom consisted of a resin slab with a small capillary tube filled with a fluorescent dye, which was located close to imaging surface. The optical and CT images were acquired simultaneously. A total of 42 projections were used in the image reconstructions (the CCD camera was static and only the source position varied). Figure 2 a) shows the reconstructed fluorescence using Tikhonov regularisation. Figure 2 b) shows the reconstruction with anatomical prior and Perona-Malik function and figure 2 c) the reconstruction with total variation.
3.2. Mouse

Optical data and CT images of an in-vivo mouse with a brain tumour were obtained by partner 1 at 184 different source-camera positions. Their FMX-XCT system has the x-ray tube and detector mounted onto the rotating gantry and, on the perpendicular axis is the laser and CCD camera. The gantry rotates around the mouse placed in the centre. The CCD images consist of 512x512 pixels, where the pixel size is 0.073 mm. Only 29 projections were used in the image reconstruction. Figure 3 a) shows the mesh cross-section. Figure 3 b) shows the reconstruction obtained using Tikhonov regularisation and figure 3 c) shows the reconstruction obtained using the anatomical prior and Perona-Malik function.

The images reconstructed using priors clearly show an improvement in the image quality compared to images reconstructed using a simple zero-order Tikhonov regularisation.
DELIVERABLE 4.6

DELIVERABLE NAME: USER FRIENDLY SOFTWARE

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
1. Summary
We developed a Matlab-based software, which reconstructs FMT images using prior information. We provide a demo that reconstructs FMT images from experimental data provided by WP 1.

2. Software: FMT-XCT reconstruction using priors


Add the software folder to your Matlab path and type:

````
>> FMT_XCT_UCL
```

This will open a MATLAB GUI window. If you press the Help button you should see the following image:

This image explains what each field represents.

2.1 Load files

*Mesh*: Load mesh file. It has to be a msh extension file. The mesh is usually generated from the CT images.

*QM*: Load QM file. It has to be a qm extension file. This file contains the sources and detectors positions.
Fluorescence: Load the fluorescence images. The images must be resized to 128x128 pixels, and the file should contain all the measurements, ie, the file should be 128x128x number of projections.

Excitation: Load the excitation images. Dimensions are the same as the fluorescence file.

CT: Load the CT images. Images should be resized to 128x128x100 and smoothed, for example, using Gaussian filter.

2.2 Prior

Tikhonov: One of the following edge preserving functions can be selected: Tikhonov, total variation, Perona-Malik, exponential Perona-Malik, Huber, Tukey and Bayesian.

Threshold: Threshold parameter of the edge preserving function. Values below this threshold value are smoothed out. It takes as an input a value between 0 and 1.

Step size: Controls the influence of the prior term in the image reconstruction. It is usually < 50.

Iterations: Number of iterations of the inner loop. It is usually < 20.

CT threshold: Threshold value of the Perona-Malik edge function used to find the edges of the anatomical prior. An image showing the edges of the anatomical prior is displayed a few seconds after. If the threshold value is changed the image is updated.

2.2 Reconstruction

Projection pixel size: pixel size of the optical images.

Hyperparameter: regularisation parameter can be inserted manually or calculated using the L-curve method.

Number of wavelets: number of wavelet coefficients to keep. It represents the level of data compression. If 128*128 wavelet coefficients are used no compression is applied (do not try this, it will not work).

Display slice: Slice displayed on the top images. It is a number from 1 to 100.

Iterations: Number of iterations of the outer loop.

Calculate Jacobian? If left unchecked the user will be asked to load a Jacobian and data file. Otherwise, a new compressed Jacobian and data file will be calculated (can take some time, depending on the number of projections and wavelets). A window will pop-up asking the user if the optical properties used to calculate the Jacobian are homogeneous.
If yes, the user will be asked to insert their values. Otherwise, the user will be asked to load files with the optical properties.

*L-curve?:* Calculate the regularisation parameter using the L-curve method. The user will be asked to insert the number of regularisation parameters, the starting and final values necessary to calculate the L-curve:

Without CT prior?: Check box to reconstruct images without anatomical prior.

3. DEMO

The demo reconstructs FMT images from phantom data provided by WP1.

Files: DEMO_MESI.msh
 DEMO_QM.qm
 DEMO_FLUO18.mat (18 projections)
 DEMO_EX18.mat
 DEMO_CT.mat
 DEMO_DATAnw64
 DEMO_Jnw64

Note that the parameter values are not necessarily the same when using the different edge preserving functions. Here are some examples of parameters that return reasonable reconstructions (other parameters were kept the same):

- TV : Riter=25 Piter=10 step=3
- Pme : Riter=25 Piter=5 step=5
- PM : Riter=25 Piter=10 step=5
- Tukey : Riter=25 Piter=10 step=5
- Huber : Riter=25 Piter=15 step=2
- B : Riter =25 Piter=8 step=1
DELIVERABLE 5.4

MILESTONE no: 9

DELIVERABLE/MILESTONE NAME: FUNCTIONAL FMT-XCT PROTOTYPE

GRANT AGREEMENT NUMBER: 201792

PROJECT ACRONYM: FMT-XCT

PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3

PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes the FMT-XCT prototype, as was already described in report 2, task 5.1, but now including the self-developed gantry which offers faster acquisition times through minimization of FMT/XCT interference and providing more flexibility for acquisition parameters by having full access to all devices.

**Hardware Description**

**Hardware overview and block diagram:**

```
(SOURCE: ct-imaging)
```

**XCT Chain**

Description of the XCT related Hardware:

<table>
<thead>
<tr>
<th>Type</th>
<th>Specification</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector</td>
<td>C7942ca-22</td>
<td></td>
</tr>
<tr>
<td>X-Ray tube</td>
<td>Oxford UltraBright 96004</td>
<td></td>
</tr>
<tr>
<td>Geometry</td>
<td>Distance of focal spot to detector: 400mm detector to object: 200mm</td>
<td></td>
</tr>
<tr>
<td>Field of view</td>
<td>56 mm</td>
<td>Due to a smaller active</td>
</tr>
</tbody>
</table>
X-Ray tube shutter max speed | 100ms
---|---
X-Ray collimator | Collimates in the longitudinal direction
X-Ray prefilter | Can hold up to 3 filters
PLC | Based on a 16 Bit (Infineon C167 MCU) and WAGO modules [http://www.frenzel-berg.de/](http://www.frenzel-berg.de/)
CCD filter | 7 changeable filters. One serves as a shutter
Accuracy of gantry angle | Set: <0.1°
| Read: <0.01°
Gantry rotation speed | Max 40°/s
Animal bed | Travel up/down: 25 mm
| Travel forward/backward: 500 mm
FMT laser stage | KDT 105
FMTXCT Acquisition PC | Spectra RACK R411
Interlock | Front doors, object lid, Laser and X-Ray tube, CCD shutter

(Source: ct-imaging)

**FMT Chain**

Hardwar description of the FMT chain:

**Camera**
The used camera is a “ProEM 512B eXcelon”( Princeton Instruments, Trenton, NJ, USA) With a resolution of 512x512 Pixels on a back-illuminated frame-transfer CCD Sensor. The photoactive aria of the sensor measures 8.2 x 8.2 mm² (→ 16µm Pixels). The Camera is providing „eXcelon coating“ which tries to prevent the etaloning effect which occurs on back illuminated CCD sensors. The Frame Transfer feature gives the advantage to not needing a mechanical shutter and enabling shorter acquisition times without imagecorruption cause by meachnical shutter speeds. (>33 fps in full frame modus). A second advantage is to be able to do image readout and acquisition of the next image in overlapping time frames . The camera is attached using GigaBit Ethernet to the acquisition PC.

![Figure 1Quantum Efficiency Curve (Source: Princeton Instruments)](image-url)
Lens
The mounted Lens is a “XENOPLAN 1.4/23MM COMPACT” (Jos. Schneider Optische Werke GmbH, Bad Kreuznach, Germany) (C- Mount) with a fixed focal length of 23 mm what results in a field of view of approximately 5 cm. This lens is coated and corrected for wavelengths from 400nm to 1000nm. Main advantage in comparison to the F-mount solution in our first prototype is the enhanced mechanical stability by the better mounting (treaded) and the small size and weight.

Lasers
Two power adjustable continues wave (0 … 400 mW ) fibre coupled laser modules (B&W Tek, Inc, Newark, DE, USA) in the wavelengths 750nm and 680nm are provided. The Laserpower is adjusted by analog outputs connected to the PLC.

x-y Stage
Several Laser collimators are mounted on a piezo driven x-y stage with high accuracy (50nm) incremental linear encoders for feedback. (KDT 105, Feinmess Dresden GmbH, Germany)

Filter Slider
Ct-imaging designed a custom filter slider with currently 6 free filter positions (32 mm free aperture) and one additional lead made X-ray shield in an central position. The filter slider is easy accessible through service doors and could be changed to enable more different sets of filters for different wavelengths.

Software Interface
The interface between the FMT acquisition software and the scanner hardware has been developed in cooperation with ct-imaging (VAMP). To ensure full access to all required hardware devices on the scanner a software interface was defined. To be more concrete ct-imaging provided a DLL to access all the devices which are required by both, the FMT and the XCT acquisition. This DLL can be integrated in different acquisition programs and especially well integrates into the current Labview™ acquisition code.
As not all of the FMT Chain containing hardware was connected to the PLC, some of the components are directly connected to the acquisition PC and do not appear on the defined Interface:

- `bool Init(void);`
  The method initialized the communication with the plc
- `bool IsCalibrated(bool &xIsCalibrated);`
  The method returns the scanners calibration state
- `bool Calibrate();`
  This method starts the calibrating routine on the scanner.
- `bool GantrySetPosition(const float fPosition);`
  This method starts the moving of the gantry to the position.
- `double GantryGetPosition();`
  This method returns the current position of the gantry (based on optical angle measurement)
- `bool FilterWheelSetPosition(int iPosition);`
  This method sets the position of the filterslider
- `bool BedSetPosition(const float fYPosition, const float fZPosition);`
  This method sets the position of the animal bed. The animal bed is moveable in transversal and coronal direction
- `bool DigitalOut(unsigned char ucOutByte);`
  This method set the 8 boolean values for the 8 digital out chanels
- `bool AnalogOutCh1(const float fChannel1);`
- `bool AnalogOutCh2(const float fChannel2);`
- `bool AnalogOutCh3(const float fChannel3);`
- `bool AnalogOutCh4(const float fChannel4);`
  This methods are setting the value for the corresponding analog out channel. The methods where separated for improving temporal performance at this point.
- `bool ReleaseReference(void);`
  Stop the communication to the plc

XCT Acquisition is performed by a standalone application provided by ct-imaging. The acquisition parameters are adjustable by configuration files (intuitive xml file format). The standalone acquisition routine can be called with some adjustment parameters and returns its status and progress in recurrent pulses. In addition, a XCT preview version of this standalone application gives the possibility to acquire only one X-ray projection of the current object. This gives the possibility to offer a comfortable and intuitive method to the user for positioning the animal. Both, X-ray projections and optical images are usable here. Both of the XCT acquisition programs can be integrated seamlessly in the FMT-XCT acquisition GUI.

FMT Acquisition Software:
The FMT acquisition program described in “FMT-XCT periodic report 2; Work Package 5“ is extended to be able to acquire data from this scanner. For this purpose a hardware abstraction layer was introduced. This user-friendly acquisition software is also described in deliverable 5.9
Advantages since report 2, task 5.1:

- Full access to the all necessary hardware through well defined software interfaces. This makes different acquisition strategy feasible, for instance the calculation of optimal source position pattern using XCT projection images.
- Prototype accessible through maintenance doors for filter changes and modifications
- Less mechanical restrictions due to lack of space
- The hardware is prepared for operating up to four different lasers wavelengths. This enables fast adoption and implementation of measurement protocols for different fluorescent markers.
- Camera with frame transfer sensor ensures faster acquisition times (theoretical enhancement is over 50%) at similar image quality
- Better stability to gravity because usage of lightweight c-mount lens and better mechanical mounting of camera and lens
DELIVERABLE NO 5.5

DELIVERABLE/MILESTONE NAME: DATA-SETS TO BE USED AS TRAINING DATA SETS FOR ALGORITHMIC DEVELOPMENTS

GRANT AGREEMENT NUMBER: 201792

PROJECT ACRONYM: FMT-XCT

PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3

PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes the training data sets that were acquired with the prototype for algorithmic development. The selected training data sets reflect different types of measurements.

Three data sets were acquired:
- a measurement of a raisin phantom provided by FIHGM
- a measurement of an agar phantom made at HMGU
- and a measurement of a nude mouse with a breast cancer tumor and a fluorescent probe that targeted the tumor.

All datasets were uploaded to the transfer server that is accessible for all partners. The datasets consisted each of the FMT data and the X-ray CT data acquired by the system. The FMT data was uploaded in xml file format, the file format selected by all partners as the most convenient file format to use for exchange.

1. FIHGM phantom data set

The FIHGM phantom data set consisted of a 360° measurement of the raisin phantom with 3 tubes of 1 mm diameter, as described in deliverable 8.2.

The tubes were filled with a mixture of intralipid, ink, water and alexa 750, that mimicked the optical properties of the raisin phantom as specified by FIHGM.

FMT measurements were acquired every 20°, and the laser was scanned over the phantom in a pattern of 12 source positions for every gantry angle. Intrinsic acquisition time was set to 0.2s, minimum emission acquisition time to 0.1s and maximum acquisition time to 10 seconds. The figures below show the acquired X-ray CT volume, and examples of the white light, excitation, emission and normalized images.
This data is accessible at the transfer drive:
http://www.fmt-xct.eu/transfer/files/KOCH10_PHANTOM_3TUBE_004_transfer.zip

2. Agar phantom data set

The agar phantom data set consisted of a 360° measurement of an agar phantom with two tubes of 3 mm diameter.

The agar phantom was made of a mixture of intralipid, ink and water, modeled to achieve an absorption coefficient $\mu_a = 0.1\ \text{cm}^{-1}$ and scattering coefficient $\mu_s = 8\ \text{cm}^{-1}$. (These optical parameters correspond to deliverable 8.2) The mold consisted of a cylindrical syringe of 25 mm diameter. Cavities were made of two transparent tubes of 2.5 mm diameter, filled with the same mixture of intralipid, ink, water and additionally alexa 750.

FMT measurements were acquired every 20°, and the laser was scanned over the phantom in a pattern of 3x3 source positions for every gantry angle. Intrinsic acquisition time was set to 0.1s, minimum emission acquisition time to 0.4 s and maximum acquisition time to 6.0 seconds. The figures below show the acquired X-ray CT volume, and examples of the white light, excitation, emission and normalized images.
This data is accessible at the transfer drive:
http://www.fmt-xct.eu/transfer/files/KOCH10_Agar2_exchange.zip

3. Breast cancer tumor data set

The third data set consisted of data from a mouse model with a subcutaneous tumor from a breast cancer cell line. The activatable fluorescent probe ProSense was used to target the tumor.

FMT measurements were acquired every 20°, and the laser was scanned over the mouse in a pattern of 9 source positions for every gantry angle. Intrinsic acquisition time was set to 0.2s, minimum emission acquisition time to 0.1s and maximum acquisition time to 10 seconds. The figures below show the acquired X-ray CT volume, and examples of the white light, excitation, emission and normalized images. Additionally we uploaded cryoslice images for the neck tumor study that could be used as validation images.
This data is accessible at the transfer drive:
DELIVERABLE NO: 5.6
DELIVERABLE NAME: MULTISPECTRAL CAPACITY

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
Introduction
FORTH has developed and evaluated the implementation of multi-spectral capabilities to a custom-built FMT system. Firstly an unmixing algorithm was developed and tested in vitro and subsequently applied in vivo, with the aim of obtaining quantitative data from two or more fluorophores simultaneously. To this end the unmixing algorithm was calibrated according to the spectral emissions of two distinct pairs of fluorophores: 1) CFSE and ATTO590 and 2) GFP and DsRed. CFSE and ATTO590 were used in mouse phantom studies, while GFP an DsRed were used for an immunologic in vivo model. The multispectral capacity is fully incorporated to the matrix free algorithmic approach, presented in Deliverable 3.6, which has been evaluated with test data from the FMTXCT prototype.

As has been reported also in the 2nd Periodic Report spectral unmixing of fluorophore emission is based on the fact that the detected fluorescence signal can be expressed as a linear combination of the different fluorescent components present in the sample. Hence, for each detection channel a linear equation can be derived that is comprised of the sum of the concentration of the fluorescence emitters multiplied by a weighting factor corresponding to the strength of the emission in that channel. If the number of detection channels is equal to the number of the investigated fluorescence targets a completely defined system of equations can be derived and solved to calculate the unknown concentrations.

Fig. 1: Characteristic spectra obtained with our Spectral/FMT system and the corresponding fittings for the calculation of the spectral contributions of the two fluorophores. a) A schematic of the measurement geometry with the positions where the spectra were collected (solid stars) in respect to the position of the tubes (green for CFSE and red for ATTO590). b) and c) Spectra for 488nm and 514nm excitation respectively (green and red triangles) and the corresponding fittings (black squares).

Spectra were collected for each illumination point through the spectrograph, and then data were plotted after calibration with a commercial Hg lamp (HG-1 Mercury Argon Calibration Source, OceanOptics, Dunedin, USA). As an example, Figure 1 shows the spectra corresponding to two different illumination points (indicated by the solid stars Figure 1a) on the phantom containing the two fluorophores placed 3mm apart at a depth of 6mm. From these spectra the contribution of each fluorophore was calculated by fitting each acquired spectrum to the known spectra of CFSE and ATTO590 and obtaining the relative strengths for each spectral region as follows:

\[
U_{fiti} = g_i(\lambda)G_i(\lambda) + r_i(\lambda)R_i(\lambda) + bkgrd_i
\]

where \(U_{fiti}\) is the fitted spectrum, \(g_i(\lambda)\) and \(r_i(\lambda)\) are the fitted spectral contributions of the fluorophores for the excitation wavelength \(i\) as a function of wavelength \(\lambda\) and \(G_i(\lambda)\) and \(R_i(\lambda)\) are the known spectra of the fluorophore obtained independently. The parameter \(bkgrd_i\) corresponds to any other spectral contribution such as non-specific autofluorescence, its initial value corresponds to the minimum value recorded and is effectively subtracted in each spectrum. The index \(i\) corresponds to the excitation wavelength. By means of a least squares algorithm the error parameter of Equation 2 is
minimized and the spectral contributions $g(\lambda_{\text{min}}, \lambda_{\text{max}})$ and $r(\lambda_{\text{min}}, \lambda_{\text{max}})$ over the detection bandpass region of the filters $[\lambda_{\text{min}}, \lambda_{\text{max}}]$, are obtained as shown in Equations 3 and 4. Index $x$ corresponds to the fluorophore, in our case CFSE and ATTO590.

$$
err = \sqrt{\frac{\sum (U_i - U_{\text{fit},i})^2}{N_u}}
$$

(2)

where $U_i$ is the in situ recorded spectra, in the case of Figure 2 the green and red spectra corresponding to the two excitation wavelengths.

$$
g_{\text{CFSE}}(\lambda_{\text{min}}, \lambda_{\text{max}}) = \int_{520\text{nm}}^{560\text{nm}} g_{488}(\lambda)G_{488}d\lambda \quad \text{and} \quad r_{\text{CFSE}}(\lambda_{\text{min}}, \lambda_{\text{max}}) = \int_{514\text{nm}}^{570\text{nm}} g_{514}(\lambda)G_{514}d\lambda
$$

(3)

$$
g_{\text{ATTO590}}(\lambda_{\text{min}}, \lambda_{\text{max}}) = \int_{520\text{nm}}^{560\text{nm}} r_{488}(\lambda)R_{488}d\lambda \quad \text{and} \quad r_{\text{ATTO590}}(\lambda_{\text{min}}, \lambda_{\text{max}}) = \int_{514\text{nm}}^{570\text{nm}} r_{514}(\lambda)R_{514}d\lambda
$$

(4)

These numbers correspond to the spectral contributions of the fluorophores (one for each excitation/filter pair). They are then used to unmix the 3D FMT reconstructions in order to retrieve the 3D unmixed images of each fluorophore’s concentration solving the following matrix equation for $C$ which corresponds to the unknown 3D concentrations of the fluorophores.

$$
[U] = [s] \times [C]
$$

(5)

This approach has been incorporated to the matrix free algorithm (Deliverable 3.6) were the spectral contributions of the fluorophores are implemented in a complete Jacobian as blocks for the different wavelengths and fluorophores. The algorithm is thus ready to be used with multispectral FMTXCT data.

**Results**

The *mouse phantom* experiments were performed by maintaining the CFSE concentration constant at 4μM and varying the ATTO590 concentration at 5μM, 10 μM and 15 μM. The pattern used for the data acquisition was of 5x8 sources covering an area of 8x13mm². All measurements were performed in non-contact reflection geometry, reproducing the experimental conditions of most of our biologically relevant studies where superficial targets such as lymph nodes are targeted. The CFSE and ATTO590 containing tubes were inserted subcutaneously in the upper torso area via two small incisions on the skin of the animal. The whole procedure was performed under terminal Isoflurane anesthesia.

Results are presented in Figure 2 from which both qualitative and quantitative conclusions can be extracted. Figure 2a) depicts the quantification results of the unmixing procedure performed in two ways: calculating the spectral strengths by *i)* fitting the in situ collected spectra, represented by the open and solid squares for CFSE and ATTO590 respectively (measured) and *ii)* by using the known spectra obtained from a fluorimeter, represented by the open and solid down triangles for CFSE and ATTO590 (literature). Both data are fitted to a linear regression model with $R^2 = 0.997$ for case *i* and $R^2 = 0.979$ for case *ii*. The corresponding slopes are 1.21 for case *i*) and 0.933 for case *ii*). In order to visualize the increase in quantification accuracy obtained by spectral unmixing, Figure 2b presents the ratios of the recovered concentrations of ATTO590 over CFSE for the measured and literature spectra cases (solid squares and open triangles, respectively), as well as the calculated known concentration ratios (solid circles), illustrating the improved accuracy that is achieved when measuring and fitting the spectra in situ. The ratios obtained with the 2D standard method (open circles) are off scale from the true calculated ones and thus the corresponding data were not included in Fig. 2a.
Fig. 2: a) Quantification results for the recovered concentrations of ATTO590 and CFSE for the two methods of obtaining the spectral strengths (see text for details). b) Ratios of the recovered concentrations of ATTO590 over CFSE (see text for details).

Finally, an overlay of the 3D reconstructions for 10μM ATTO590 and 4μM CFSE from the in-vivo data is shown on a schematic outline of the mouse in Figure 3. The images were obtained using the measured spectral fitting method described above. Figure 5a shows the reconstruction obtained with the raw data before the unmixing is performed, while Figures 3b and 3c depict the unmixed 3D reconstructions of the two fluorophores clearly separated.

Fig 3: Coronal views of the 3D reconstructions of the CFSE and ATTO590 fluorescence signal overlaid on a schematic outline of the mouse. The inset shows the axial view of the same reconstructions. a) the mixed reconstructions, b) the unmixed ATTO590 reconstruction and c) the unmixed CFSE reconstruction.

Before proceeding to the realistic in vivo model involving GFP and DsRed Tcells we have tested our method with an in vitro model where the same cells were imaged at increasing concentrations. Specifically, each cell type was resuspended at 2x10^5 cells/μl and then diluted. Volumes of 1, 2.5, 5, 7.5 and 9 μl of GFP and DsRed cells were mixed to a total and fixed sample volume of 10μl. The 10μl cell culture samples were plated in a 24-well culture plate and imaged, multispectral data was acquired using two distinct emission filters. Quantification accuracy was determined by linear regression analysis on the data before and after multispectral unmixing. The relationship between intensity and cell concentration was found to be linear both before (r²= 0.97 and 0.99) and after spectral unmixing (r²= 0.96 and 0.95). However, the quantitative comparison between DsRed and GFP intensity values, previous to the unmixing step, can only be performed through a calibration step, since the linear relationship between concentration and intensity shows significantly different parameters (slope and intercept). Following the unmixing step the slope of the GFP and DsRed linear regression analysis are practically identical and the intercepts are within the error limits of the fits. Therefore, following spectral unmixing the intensity values of two distinct fluorophores can be quantitatively compared.
Figure 4: In vitro quantification of colocalised GFP and DsRed cell culture plates. Increasing concentrations (0.2-1.8x10^5 cells/μl) of GFP and DsRed cells were mixed to a total volume of 10μl and imaged. The intensities recorded with the multispectral instrument were fitted to a linear regression model. a) intensity values before (mixed) and b) after unmixing (unmixed) show excellent correlation to the concentration of cells in the specified volume. The values reported in the table indicate the change in linear relationship between intensity and concentration induced by spectral unmixing and demonstrates that GFP and DsRed intensities are, in quantitative terms, directly comparable between each other only following spectral unmixing.

The multispectral capabilities were then applied to a biologically relevant context where the dynamics of two populations were observed over time. GFP and DsRed were used as fluorescent reporters of two distinct but co-localised T cell populations and imaged during the mounting of an immune response. T-cells in fact function as active sentinels through the circulatory and lymphatic systems. The mechanisms driving stages of T-cell activation, differentiation, migration and clonal expansion depend on crosstalk between different cell populations and represent an ideal in vivo imaging model. In particular, T-cell proliferation is a fundamental parameter for a vigorous and successful immune response against pathogens and is also a diagnostic/prognostic marker in autoimmune diseases and vaccine designs. T cell antigen recognition was monitored in vivo using multispectral quantitative imaging and the concentration values recovered for GFP and DsRed were validated with cell sorting. CD2/GFP and F5/DsRed T cells were adoptively transferred in Rag1-/- immunodeficient mice. In brief, an immune response was induced by the injection of a synthetic NP peptide to which only the F5/DsRed population is sensitive to, within the same time frame the CD2/GFP population is not expected to respond. Multispectral data was collected over a time frame of 5 days to illustrate the kinetics of antigen recognition. As expected, the F5/DsRed population undergoes significant proliferation (+44%) over 4 days whereas the homeostatic control mechanisms that drive the CD2/GFP cells maintain the polyclonal T cell pool stable. Following the last imaging session (day 4) the mice were sacrificed and left and right lymphnodes analysed using cell sorting. The percentage of FACS positive fluorescent cells was plotted against the intensity values recovered with multispectral imaging. Linear regression analysis, before and after unmixing, shows that GFP and DsRed are quantitatively comparable with very good correlation only after multispectral unmixing, as demonstrated by the linear correlation between the two fluorescent populations. Statistical analysis showed a significant increase in F5/DsRed population between days 0 and 4, whereas the variation in CD2/GFP cell population was not significant. This result evidences the NP-specific antigen response of the F5/DsRed T cell population, when compared to polyclonal CD2/GFP T cells and illustrates one of the many applications that could benefit from multispectral imaging.
Figure 5: In vivo application of multispectral unmixing in a biologically relevant context. GFP and DsRed were used as reporter fluorophores for two T cell populations that carry different specificities. The colocalised T cell populations were imaged in the cervical lymph nodes using multispectral imaging and the intensity values were compared to percentage positive fluorescent cells, for each fluorophore, using cell sorting (FACS %). Linear correlation for the intensity values was compared (a) before and (b) after unmixing. The linear fit was significantly improved following the unmixing step, demonstrating that GFP and DsRed populations can be, in quantitative terms, directly compared using multispectral unmixing. (c) An immune response was induced by injecting NP synthetic peptide and the response of the two colocalised populations was monitored daily. Specifically the F5/DsRed cells recognise the NP and respond by clonal expansion, whereas polyclonal CD2/GFP do not respond to this stimulation. (d) Antigen recognition was assessed by statistical analysis where only the change in DsRed population between days 0-4 was significant.

References
DELIVERABLE NO: 5.7
DELIVERABLE NAME: COMPUTE AND ASSIGN OPTICAL ATTENUATION VALUES

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes the calculation of optical attenuation values to be used for the reconstruction of hybrid fmt-xct data, and the way the optical properties are assigned.

**Introduction**

The optical attenuation values that are to be determined are the absorption and scattering coefficients for different anatomical regions of the mouse. The focus in this investigation was on the thorax region.

In deliverable 4.4 an automatic feature extraction algorithm was described for the segmentation of a mouse in the anatomical regions: lung, bones, heart and remaining tissue. The optical properties for these regions were investigated.

Assignment of the optical properties was implemented as a step in the calculation of the forward finite element model.

**Calculation of optical properties**

As the basis for the determination of optical properties we took the properties that were measured using a frequency domain system in 2006 (1), see below table.

<table>
<thead>
<tr>
<th>Region</th>
<th>Absorption (cm⁻¹)</th>
<th>Scattering (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung region</td>
<td>0.2-0.3</td>
<td>25-35</td>
</tr>
<tr>
<td>Heart region</td>
<td>0.3-0.4</td>
<td>20-25</td>
</tr>
<tr>
<td>Liver region</td>
<td>0.4-0.6</td>
<td>10-15</td>
</tr>
</tbody>
</table>

Midrange optical properties were extracted from the table similar to the optical properties used in (2).

<table>
<thead>
<tr>
<th>Region</th>
<th>Absorption (cm⁻¹)</th>
<th>Scattering (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung region</td>
<td>0.25</td>
<td>30</td>
</tr>
<tr>
<td>Heart region</td>
<td>0.35</td>
<td>23</td>
</tr>
<tr>
<td>Bone region</td>
<td>0.1</td>
<td>20</td>
</tr>
<tr>
<td>Tissue region</td>
<td>0.3</td>
<td>10</td>
</tr>
</tbody>
</table>
Initially we used the midrange optical properties for the calculation of the forward model. The assignment of optical properties for the calculation of the forward model was done by linking the segmentation of the mouse to the nodes in the finite element mesh. For each node it is determined in which anatomical segment the node is situated. Optical properties were then assigned corresponding to the different segments.

In order to optimize the optical properties used, we varied the optical properties used in the reconstruction in a structural manner until an optimal reconstruction was found. This resulted in the following values for the absorption and scattering coefficients to be assigned to the anatomical regions:

<table>
<thead>
<tr>
<th>Optical Region</th>
<th>Absorption (cm$^{-1}$)</th>
<th>Scattering (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung region</td>
<td>0.25</td>
<td>27.5</td>
</tr>
<tr>
<td>Heart region</td>
<td>0.35</td>
<td>17.5</td>
</tr>
<tr>
<td>Bone region</td>
<td>0.2</td>
<td>15</td>
</tr>
<tr>
<td>Tissue region</td>
<td>0.3</td>
<td>15</td>
</tr>
</tbody>
</table>

**Assignment of optical properties**

The determined optical properties were subsequently assigned to the nodes in the finite element mesh using the segmentation as described above: for each node it is determined in which anatomical segment the node is situated. Optical properties are assigned corresponding to the different segments, and used in the finite element calculations of the forward model according to the diffusion equation:

\[
-\nabla \left[ D_s(r) \nabla \Phi_x(r) \right] + \mu_{ax}(r) \Phi_x(r) = S_x(r)
\]

\[
-\nabla \left[ D_m(r) \nabla \Phi_m(r) \right] + \mu_{am}(r) \Phi_m(r) = -\Phi_x(r) n(r)
\]

In which \( \mu_a \) is the absorption coefficient and \( D=1/(3\mu_s) \), with \( \mu_s \) the scattering coefficient, accounts for the scattering in the medium. The forward model including attenuation coefficients is then used for the inversion. See also deliverable 6.5 for a comparison of the reconstructions using the forward model with and without heterogeneous optical properties.

DELIVERABLE 5.8

DELIVERABLE NAME: FUNCTIONAL SPECIFICATION OF OPTIMAL ACQUISITION AND OPERATIONAL PARAMETERS

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes the functional specification of optimal acquisition parameters. The parameters described include: mouse positioning, source positioning, angular coverage and illumination times.

**Mouse positioning**

The implemented mouse bed consists of two carbon rods with strings in between. The mouse is placed on the bed. During mouse placement the following needs to be considered:

- *The nose of the mouse* should be placed inside the anesthesia cap in a stable position.

- *The arms of the mouse* should be placed in a way that makes the shape optimal for modelling with the finite element method and diffusion equation. For example: skin flaps are difficult to model accurately, they should be avoided. Wrinkels should be avoided. The arms should be placed as close to the animal as possible so that there are no air gaps between the arms and the body of the mouse, because air is not taken into account in the diffusion equation. When imaging the thorax area, arms should be placed upwards, when imaging the head or neck area it can be better to place them downwards. See the images of different arm positioning below.

- *The strings* of the bed should be placed such that they do not run over the area of most interest. The strings are flexible and the user can move them to the desired position. The main area of interest should not contain a wire. See an example of the strings below.
Source positioning

In general it is advisable to keep the measurement as short as possible. Shorter measurements are preferable for the animal and ensure stability of the probe. We have investigated regular source grids. In case of subcutaneous tumors the area to image is clear and a grid of 3*3 sources with a spacing of 2.5 mm will be sufficient. When the lung area is imaged, the region of interest is more difficult to define, also due to breathing effects of the animal. A grid of 6*3 sources with a spacing of 2.5 mm will generally be sufficient, more sources could be needed depending on the resolution that is required.

Angular coverage

We generally scan the animal every 20°. This results in 18 angular positions equally spaced over the full 360° range.

Illumination times

There are three parameters that can be varied for the illumination times: the intrinsic illumination time, the base fluorescent illumination time and the maximum fluorescent
exposure time. The illumination time for the intrinsic measurement is generally set to 0.2s. For small animals, 0.2s can be too long and the images will get saturated. In that case we reduce the intrinsic illumination time to 0.1s. The laser power is automatically adapted to an optimal value based on the intrinsic acquisition. The illumination time for the fluorescence acquisition was set at first to a maximum of 10s. We have reduced the maximum illumination time to 6s, this is sufficient. The base exposure time of the fluorescence acquisition should be selected as large as possible, but small enough to prevent saturation of the image for different source positions. The range we use is between 0.2s and 1s, depending on the mouse or phantom that is imaged.

The optimal settings are summarized in the table below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Advised setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse – Arms</td>
<td>Close to body</td>
</tr>
<tr>
<td>Bed – strings</td>
<td>Not covering region of interest</td>
</tr>
<tr>
<td>Sources - distance</td>
<td>Grid with 2-3 mm spacing, covering area of interest</td>
</tr>
<tr>
<td>Sources - n</td>
<td>9-24 sources</td>
</tr>
<tr>
<td>Angles</td>
<td>Every 20 degrees</td>
</tr>
<tr>
<td>Intrinsic illumination time</td>
<td>0.1-0.2 seconds</td>
</tr>
<tr>
<td>Base fluorescent illumination time</td>
<td>0.2-1 seconds</td>
</tr>
<tr>
<td>Maximum fluorescent illumination time</td>
<td>6 seconds</td>
</tr>
</tbody>
</table>
DELIVERABLE NO: 5.9
DELIVERABLE: FUNCTIONAL USER FRIENDLY OPERATIONAL SOFTWARE

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes the operational software that is installed on the acquisition computer of the fmt-xct system.

The main user interface is programmed in labview with an interface to matlab and has the following appearance:

![Software Interface](image)

When the program is started, the CCD camera will start cooling until the optimal temperature is reached and Xray shielding is automatically initiated.

**Experiment section**

**Button 1**
The first step in the set up of an acquisition is the selection of the desired wavelength. In this example 750 nm.

**Button 2**
The second step is the selection of imaging sequence, either start with xct standalone, fmt standalone or hybrid acquisition.

**Button 3**
The gantry needs to be initialized in order to determine the exact position and end points of the rotation.

**Button 4**
Then the animal can be placed into the system. First the animal is placed onto the animal bed. The bed will slide into the middle of the system. Additional positioning is possible to make sure the animal is in the middle of the image and the desired part of the animal is in the field of view.
*Button 5*
When the animal is in the desired place, the experiment can be configured. The configuration screen displayed has the following appearance:

The main functionalities of the configuration are described here. Optimal values for the acquisition parameters have been described in deliverable 5.8.

**Study data section**
First a folder is created in which the acquisition data is saved. The study should be given a specific name related to the experiment.

The user can now choose the acquisition parameters.

**Rotation section**
The rotation defines the angles, and angle increment for the rotation of the gantry. With an increment of 20 degrees over the 360 degree range this results in 18 acquisition angles.

**Exposure times section**
At each angle a white light image is taken, and a grid of sources is scanned over the mouse. For each source position an intrinsic and fluorescence image is taken. The illumination times and laser power can be selected in the Exposure times section of the interface.

**Adjustment toolbox**
The figure window can be used to show a white light image of the mouse. First the user can select a gantry position defined on the right, then with “move” move the gantry to that angle, grab an image, and view the mouse on the bed. Based on this image, the user can define an area in which the sources should be located.
Laser settings section
In the laser settings section, the positions of the sources can be defined. The middle of the animal across the animal will be defined automatically. From the middle, the user can define the max and min offset and the distance between the sources. In this example this ranges from -2.5 to 2.5, with an increment of 2.5mm. This results in 3 sources per line. Along the animal the user can also define the max and min position on the animal and an increment. In this case from 12 to 4 mm with a spacing of 2.5 mm, resulting in 7 lines of sources. In total there will be a grid of 7*3=21 source positions for each of the 18 selected angles of the gantry.

Button 7
After the experimental parameters are set by the user, the indicators next to the performed steps will turn green and the measurement can be started, by pressing “run acquisition”.

This will open another window, showing the acquired images directly after acquiring. In this way the user can monitor the progress and image quality during the measurement. The last window will close automatically when the acquisition is finished and the labview program can be stopped and closed down.
DELIVERABLE NO: 6.4
DELIVERABLE: Develop U87 animal models (mo.27)

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
We set up an orthotopic animal model for glioblastoma in nude mice, nude rats and Fischer, rats. The model is based on intracranial injection of tumour cells (human U87, human Gli36, human U251 or rat 9L glioma cells) using a stereotactical device (Stoelting Inc.) Injection of $1 \times 10^5$ or $2 \times 10^5$ cells is performed into the striatum of nude mice or rats, respectively. Tumour growth can be detected by PET imaging from day 8 on (9L in Fischer rats), and is clearly visible in nude mice at day 14 post injection. We also generated different cell lines expressing fluorescent proteins or firefly luciferase. U87, Gli36 or 9L cells expressing the firefly luciferase gene could be visualised by bioluminescence imaging early (3 days) after intracranial implantation in nude mice and 6 days post injection in rats.
**Figure 1a.** PET image nude mouse 9L intracranially 14 days post injection

**Figure 1b.** PET image Fischer rat 9L intracranially 8 days post injection

**Figure 1c.** Bioluminescence imaging of Fischer rat bearing i.c. 9L-luc tumor 6-20 days post injection
DELIVERABLE : 6.5

DELIVERABLE: Study and report the quantitative accuracy of FMT-alone and FMT-XCT in resolving tumors (mo. 36)

GRANT AGREEMENT NUMBER: 201792

PROJECT ACRONYM: FMT-XCT

PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3

PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
FMT-alone

Preceding the evaluation of the performance of the hybrid FMT-XCT, measurements have been performed by using FMT-alone. The studies concerned the use of custom-made probes for the specific and the passive targeting of tumours. The specific targeting was based on the use of nucleic acid sequences called aptamers that are developed to bind specifically target. The procedure for the development of the aptamers is named SELEX (systematic evolution of ligands by exponential enrichment).

On the other hand we have tested nano-micelles that lately have attracted attention as potential carriers for drug delivery. One of these nano-micelles has been selected for evaluation by performing FMT-alone measurements.

1. Development and evaluation of an aptamer for the labelling of the MCF-7 breast cancer model.

In the previous reports we have presented a new aptamer developed (ACE8) in our lab called able to bind membrane proteins with high affinity. By looking microscopically, we showed that the ACE8 aptamer binds to the breast cancer cell line MCF-7 first at the membrane and then it gets internalized. The next step was to perform in-vivo small animal measurements. We used the FMT technique to quantify the tumor uptake of ACE8 in the MCF-7 tumor xenografts. Measurements were performed at 3 hours post-injection using the TomoFluo3D prototype (Fig. 3). We recently calibrated the TOMOFLUO 3D using nuclear imaging and demonstrated that it can non-invasively quantify fluorescent probes concentration in small animals (Garofalakis et al, *In vivo calibration of free-space Fluorescence Tomography using Nuclear Imaging*. Optics Letters 35(18) p. 3024-3026 (2010)). The contrast obtained with fDOT was higher than the contrast obtained with epiluminescence fluorescence imaging and revealed ten times more aptamer ACE8 in the tumor compared to a control sequence (0.67 ± 0.16% of injected dose compared to 0.07 ± 0.06%, respectively)(Figure 2).

Development of a new nano-micelle for the passive targeting of the MDA-MB-231 breast cancer model.
Nano-particles have the potential of carrying contrast agents and drugs to tumours. They take advantage of the leaky vasculature surrounding tumours to get trapped based on a phenomenon which is known as Enhanced Permeation and Retention (EPR). We have developed nano-micelles that have very small size in order to improve their diffusion deeper inside tumours. We have three tested polymerized polydiacetylene (PDA)-micelles of different coatings; either nitrilotriacetic acids (NTA) and different poly(ethylene glycol) (PEG) chain lengths.a. PDA-NTA, b. PDA-PEG350 and c. PDA-PEG2000 micelles

These nano-micelles have been for in-vivo imaging after being labelled with the 730 a infra-red (NIR) fluorescent dye FluoProbes® 730 (FP730). We used Epi luminescence fluorescent imaging which is a fast screening method for the selection of the best of the three nano-micelles. The results showed that the micelle PDA-PEG2000 micelles had the best tumor to target ratio and thus it was chosen for further validation with the use of tomographic quantitative imaging..

To quantify tumor uptake of PDA-PEG2000 micelles, free space fluorescence diffuse optical tomography (fDOT) was used. One day after injection, the tumor uptake of PDA-PEG2000-FP730 was measured around 4.7±1.3% of injected dose per gram (%ID/g). We chose to perform dual PET/FMT measurements aiming in comparing the 3D fluorescent signal of the nanomicelles to the high [18F]-FDG internalization by the cancerous cells as given by PET. In this experiment, [18F]-fluorodeoxyglucose ([18F]-FDG) was injected 24 h after PDA-PEG2000-FP730 administration. In this experiment we found that 40±19% of the retained micelles was partially overlapping with the tumor volume visualized by PET. The fluorescent nanomicelles were found below the tumor volume where the vascularization is expected to be higher. The nano-micelle localization was monitored up over one week to confirm the effective labelling of tumors by the micelles. Since the retention of the micelle appeared favorable we further explored the potential of using the micelles as drug carriers.

The drug, Sutent, or Sunitinib by Pfizer, is an anti-angiogenic molecule which inhibits Vascular Endothelial Growth Factor Receptors (VEGFR). It is a multi-targeted receptor tyrosine kinase inhibitor (RTK) and is used for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumors (GIST). It has been found that there were some potentially beneficial side effects for patients suffering from breast cancer which is what we decided to investigate.

Written by: Angelique Ale, Max Koch, PhD students
Comparison of fmt alone vs fmt-xct in resolving tumors

This document describes the comparison of fmt alone vs fmt-xct in resolving tumors.

For this investigation we have imaged several mice with subcutaneous tumors.

Three mice prepared by CEA-LIME were imaged at HMGU. The mice had subcutaneous U87 brain tumors and were injected with different probes. See images below.

![Images of mice with tumors and different probes](image1)

In a similar experiment we imaged a mouse with a subcutaneous breast cancer tumor in the neck, injected with prosense to target the tumor. This mouse is part of the common training dataset, described in deliverable 5.5.

Reconstructions of the data were performed using two approaches: 1) reconstruction without using information from xct, 2) reconstruction with using information from xct.

1) Reconstruction without information from xct.

The reconstruction without information from xct consisted of first calculating a forward model of light propagation. This model was based on the diffusion equation combined with the finite element method and normalized born approximation, see (1, 2) for a detailed explanation. In this case it was assumed that the mouse is homogeneous, and homogeneous optical properties were assigned (\( \mu_a=0.3 \text{ cm}^{-1}, \mu_s=10 \text{ cm}^{-1} \)) to all the nodes in the forward model. This results in a weight matrix that can be used for linear inversion. Inversion of the matrix was done using the LSQR algorithm with standard tikhonov regularization.

2) Reconstruction with information from xct.

The reconstruction without information from xct consisted of also first calculating a forward model of light propagation, with the difference that in this case it was assumed that the mouse is not homogeneous. The mouse was first segmented in anatomical regions (bones,
tissue), and optical properties corresponding to anatomical segment were assigned to the nodes in the forward model, see also deliverable 5.7. Inversion of the matrix was done using the LSQR algorithm with a anatomically structured regularization matrix, see (1, 2) for a detailed explanation.

Results

The results of using prior information from xct compared to stand alone FMT are displayed in the figure below for the tumor study. Both priors are important and the final reconstruction is significantly more accurate compared to stand alone FMT. The improvement is expected to be even more substantial for more complex imaging geometries, see (1, 2) for more examples of the improvements achieved by using the prior information from x-ray ct into the reconstruction.

DELIVERABLE  7.3
MS10
DELIVERABLE/MILESTONE NAME: FMT-XCT VS HISTOLOGICAL CORRELATES IN TUMORS

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes the ability of FMT-XCT in resolving tumors.

Experiment description

Cells from a breast cancer cell line were implanted in the next of a nude mouse, see also deliverable 5.5. The fluorescent probe used for the experiment was prosense. Prosense targets Cathepsin B, L, S and Plasmin. It is an activatable probe. This has the advantage that there is not much background fluorescence, only very local signals are observed. The FMT-XCT acquisition was followed by hybrid fmt-xct reconstruction, subsequently cryoslicing, and H&E staining.

FMT-XCT reconstruction

For the reconstruction we used a hybrid algorithm that includes xct based priors. The reconstruction using information from xct is shown in the first image below. In the background a corresponding slice from the xct volume is shown. It shows the bones in white and tissue in gray. The brighter signals overlaid on the slice are the recovered fluorescence signals. A clear signal from the bones and tumor is visible in the reconstruction. (Tumor is the top most signal close to the skin of the mouse).

Histological correlates

The reconstruction result was validated against two histological methods:

Validation by Cryoslicing

The first validation method that we used was the cryoslicer, as described in (1). The cryoslicer is a recently developed modality for validation. With this validation methodology it is possible to obtain an image of transversal slices of the mouse that are directly comparable with the reconstruction result.
DELIVERABLE 7.4
MILESTONE NO 10
DELIVERABLE/MILESTONE NAME: FMT-XCT VS HISTOLOGICAL CORRELATES IN PYMT TUMORS

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
This document describes FMT-XCT of Pymt mice vs histological correlates.

**FMT-XCT imaging**

Several transgenic Pymt mice with tumor sites in a metastatic stage were imaged with the fmt-xct system. Two fluorescent probes were injected 24h prior to euthanasia. The probes used were Prosense 750 and Integrisense 680. Prosense is an activatable probe that is activated by Cathepsin B, L, S and Plasmin. IntegriSense is a targeted probe that targets avb3.

The images obtained with the FMT-XCT for one of the imaged mice are shown below.

In the white light image and xct rendering several tumors and metastases are visible. One of the tumors is indicated with an arrow.

![White light image and Xct rendering](image)

The images obtained at the 680 nm channel correspond with IntegriSense 680. The images below show an excitation, emission and normalized image. The normalized transmission image shows signal from around the tumor areas.

![Excitation, Emission and Normalized images](images)

The images obtained at the 750 nm channel correspond with ProSense 750. The images below show an excitation, emission and normalized image. The normalized transmission image shows signal from the tumor areas as well.

![Excitation, Emission and Normalized images](images)
These results suggest that integrisense accumulates around the tumor sites, and prosense is activated at the tumor sites. Both probes are well detectable with FMT-XCT.

**Histological correlates**

The PymT tumors have been histologically studied in CEA-LIME. The aim of the study was to validate the presence of fluorescence signal of the Angiostamp 680 inside the tumors and establish the location of the fluorescence with respect to the location of the tumor cells. For the microscopical imaging of the PymT tumors we followed a protocol where the animals were sacrificed after in-vivo imaging and the tumor was removed. The tumor has been cut in small sections of 5um in a cryostat. Fluorescent microscopic measurements have been performed to directly assess fluorescence activity. The results are shown in the image below. In the image A, strong fluorescence signal of Angiostamp was found inside the tumor. By fusing with the tumor cell nuclei shown in image B we can compose the image C where we can see the distribution of the Angiostamp with respect to the tumor cells. The Angiostamp680 is found to be in the extracellular matrix. Angiostamp680 contains the RGD ligand which can specifically bind the avβ3 integrin. The latter plays an important role in the development of the neovasculature and thus is expected to be localized in the connecting tissue as it is shown in the image below.
The reconstruction of the in-vivo measurements correlates well with the signals observed in the cryoslice: the cryoslice shows similar signals in the tumor region and bone areas.

*Validation by H&E staining*

The second validation method was a more conventional validation method: H&E staining of the same area as was imaged with the cryoslicer.

Also in the H&E stained slice we could identify the tumor area, corresponding to the region that is reconstructed as a bright signal, and is also clearly visible in the cryoslice, see images below. This confirmed that the fluorescent signals observed subcutaneously in the reconstruction and cryoslice were indeed related to the breast cancer cell tumor.

DELIVERABLE NO: 9.1
DELIVERABLE: Dissemination implementation document

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
The objective of dissemination activities is to deliver relevant project results to key target groups and to improve the relevance of results by a continuous dialogue with these stakeholders. Moreover visibility and awareness of the project are enhanced through such activities and thereby decision making is influenced. The results of the FMTXCT project were disseminated in various ways:

1) Publication in leading international journals in the field and annual presentation of results at international forums of Imaging, such as

- Ale A, Schulz RB, Sarantopoulos A, Ntziachristos V
  Imaging performance of a hybrid x-ray computed tomography-fluorescence molecular tomography system using priors
  MEDICAL PHYSICS 37(5); 1976-1986 (2010)
- Freyer M, Ale A, Schulz RB, Zientkowska M, Ntziachristos V, Englmeier KH
  Fast automatic segmentation of anatomical structures in XCT images to improve FMT reconstruction.
- Schulz R, Ale A, Sarantopoulos A, Freyer M, Soehngen E, Zientkowska M, Ntziachristos V
  Hybrid System for Simultaneous Fluorescence and X-ray Computed Tomography.
  IEEE TRANSACTIONS ON MEDICAL IMAGING 29(2); 365-73 (2010)
- M. Simantiraki, R. Favicchio, S. Psycharakis, G. Zacharakis and J. Ripoll,
- Saras A, Favicchio R, Birk U, Zacharakis G., Mamalaki C, and Ripoll J.,
- C. Panagiotou and S. Somayajula and A. P. Gibson and M. Schweiger and R. M. Leahy and S. R. Arridge,
- J Chamorro, J Aguirre, J Ripoll, JJ Vaquero, M Desco. "Maximizing the information content in acquired measurements of a parallel plate non-contact FDOT while minimizing the computational cost: singular value analysis". Abstract book of European Society for Molecular Imaging (ESMI), 161, 2009

**Book chapters:**

• “Applications of optical tomography in biomedical research”, by Ana Sarasa-Renedo, Alex Darrel and Jorge Ripoll, to be published in “Handbook of photonics for Biomedical Science” edited by V. Tuchin.


More publications are listed at the end of each WP report.

**Invited Talks Vasilis Ntziachristos:**

• Society of Nuclear Medicine 57th Annual Meeting, Salt Lake City Utah 2010 “Photonic imaging advances and complementarities with nuclear imaging”

• European Conference of Biomedical Optics (ECBO) SPIE/OSA/CLEO Munich, 2009 Plenary speaker: “The new era of mesoscopic and macroscopic photonic imaging”

• Topics in Molecular Imaging (TOPIM) 2009 Les Houches, France 2009 “Brining the best out of light: Multi-modality photonic imaging”

• 24th Trans-antlantic Airway Conference, Lucern, 2009 “Imaging of pulmonary disease with optical molecular imaging”

• European Society for Molecular Imaging (ESMI) Barcelona, Spain 2009 “State of the art optical imaging”

• World Molecular Imaging Conference, Nice, France 2008 “Advances in Photonic Imaging”

• 4th NCRI Cancer Conference, Birmingham, UK 2008 'Imaging: from molecules to patients'

• Informa Life Sciences Global Imaging Summit, Cologne 2008 Plenary speaker: 'Molecules to Models: Imaging in Drug Discovery and Development’

2) Dissemination of the FMTXCT project and the results were additionally obtained through the project website ([http://www.fmt-xct.eu/](http://www.fmt-xct.eu/)) and several other websites, such as

- [http://www.helmholtz-muenchen.de/forschung/forschungsprojekte-und-forschungsverbuende/index.html](http://www.helmholtz-muenchen.de/forschung/forschungsprojekte-und-forschungsverbuende/index.html)

- [http://www.helmholtz.de/forschung/eu_projekte/zusammenarbeit/gesundheit/fmt_xct/](http://www.helmholtz.de/forschung/eu_projekte/zusammenarbeit/gesundheit/fmt_xct/)


3) At the conference “Scientific Challenges in European Health”, an international conference launched by the Bavarian Universities, which took place in Brussels on October 20 and 21, 2010 Vasilis Ntziachristos was selected to present the Bioimaging topic. The objective of the event was to present major themes of medical research in Bavaria and scientific achievements in these fields as well as to discuss key challenges, goals and new directions with international stakeholders from science, industry and European institutions. In the imaging presentation and the following debates one of the main focus was the FMTXCT project (see poster attached).

4) A public promotion leaflet to raise awareness for the activity and success of the project has been developed (see deliverable 9.3).
Biomedical Imaging – an emerging key technology

Global Market Overview: Medical Technology

The relevance of imaging to the practice of modern medicine will continue to increase in the future and is driven by several factors, one of the most significant of which is the rapid aging of the global population and the subsequent rise in prevalence of many age-related diseases such as cancer, cardiovascular disease, and neurological disease.

Emerging Imaging Technologies

Hybrid Fluorescence Molecular Tomography and X-ray Computed Tomography (FMIT-XCT)

Principle: FMIT-XCT allows for simultaneous fluorescence molecular tomography (FMIT) and X-ray computed tomography (XCT) in a hybrid, non-concurrent imaging approach. The system combines both imaging modalities to provide functional and anatomical information in a single examination.

Multi-Spectral Optoacoustic Tomography (MSOT)

Principle: MSOT combines optical and acoustic imaging to provide high-resolution images of biological tissues. It is based on the absorption and subsequent release of heat in the tissue, which generates acoustic waves. These waves are detected by transducers and processed to form an image.

Real Time Intraoperative Fluorescence Imaging (RTIFI)

A novel fluorescence imaging system developed for real-time intraoperative applications. The system combines fluorescence imaging with image-guided surgery to provide real-time visualization of surgical sites.

Publication Highlights

1. Nitschke, M., Nitschke, H., \( \text{Fluorescence plectolipid imaging of \textit{Ex Vivo} murine myocardium with fluorescent nanoparticles} \), Functional Heart Imaging, 1(1), 232-242 (2010).

Health Cooperation Programme

- FTI: Hybrid Fluorescence Molecular Tomography and X-ray Computed Tomography (FMIT-XCT), Collaborative Project
- Eucomed: Innovation Network for Medical Imaging, Partner
- ERC Advanced Grant 2008: Biomedical Imaging, Partner
- Marie Curie Individual Fellowships: Dr. Nitschke, Partner
- Erasmus Intensive Programmes: Optical Imaging, Partner
- Molecular Imaging: Industrial context, state of the art, multidisciplinary Imaging, Partner
DELIBERABLE NO: 9.3
DELIBERABLE: PUBLIC PROMOTION LEAFLET

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT) – X-ray Computed Tomography (XCT) method and system
PERIODIC REPORT: 3
PERIOD COVERED: FROM March 01, 2010 TO February 28, 2011
Work Package: 9
Work Package title: Training and dissemination
Date: March 2011
Written by: Angelique Ale, Veronika Erben

Outside of folder

FMT - XCT
Functional Magnetic Resonance Imaging and X-ray Computed Tomography: system and method

Alims

The FMT-XCT project aims to combine functional magnetic resonance imaging and X-ray computed tomography into a hybrid, quantitative imaging system. The project is a joint venture of researchers from several institutions across Europe. The technology is based on combining two complementary imaging modalities that provide functional and structural information of biological tissues.

Why perform Multi-Modality imaging with the FMT-XCT?

Combines two different imaging modalities in one device

Combines the advantages of different modalities to gather the most relevant information

Combines functional and structural information

Combines quantitative functional (fMRI) and structural imaging modalities

Processes high-resolution images to provide functional and structural information

Inside of folder

Methodology

The FMT-XCT system integrates the two different imaging modalities to provide functional and structural information simultaneously. The system combines high-resolution structural imaging with functional imaging techniques. The data is processed to provide detailed information about the biological tissues.

More Information

The FMT-XCT system provides high-resolution images of biological tissues. It is designed to be used in conjunction with other imaging modalities to provide comprehensive information about the biological structures. For more information, please contact the project team or visit the project website.
MILESTONE NO:  5
MILESTONE NAME: XCT DUAL ENERGY VS. CONTRAST ENHANCEMENT

GRANT AGREEMENT NUMBER: 201792
PROJECT ACRONYM: FMT-XCT
PROJECT TITLE: Hybrid Fluorescence Molecular Tomography (FMT)
               – X-ray Computed Tomography (XCT) method and system

PERIODIC REPORT:  3
PERIOD COVERED:    FROM March 01, 2010 TO February 28, 2011
Introduction

Initial objective and planning

Milestone M5 consists in the selection of an appropriate XCT technology to use for integration in the FMT-XCT prototype and is a significant milestone for the final system. It is mainly covered by WP2. The need to develop a specific X-ray CT system comes from the diverse needs of the hybrid approach to produce an XCT design that is not only appropriate for small animal imaging but also:
- provides adequate accommodation of the optical components,
- eliminates X-ray interference with optical components,
- offers improved contrast between organs as is important for the optimal utilization of X-ray CT information as priors in the FMT inversion procedure, as explained and performed in WP4.

Following the initial technical annex, M5 should be achieved in two steps: “A preliminary recommendation will be performed in month 18 by the executive committee, and the technology and decision will be officially presented in month 24”. During Year 2 meeting, it was decided to postpone the final decision to month 30. The annex states that the milestone should present a comparison between dual energy XCT and contrast agent XCT strategies, based on “quantitative image analysis on experimental data”.

Effective work

Milestone 5 is covered by the different WP2 tasks, and mainly corresponds to the following deliverables:

- D2.4 - Preliminary technical specification for XCT design to be implemented with the hybrid system
- D2.7 – Final technical specification for XCT system
- D2.6 - Comparison of contrast enhancement strategies

D2.4 and D2.7 were produced in the second year (April 2010) and D2.6 in the third year of the project (March 2011). Thus Milestone M5 can be considered as completed. Conclusions are remarked afterwards.

The reason of the delay (Month 36 instead of 24) is detailed in the Periodic Activity Reports. The main point was the lack of availability of a prototype usable for X-ray experiments on living mice: CEA test bench is not suitable for extensive tests on living mice and differs significantly from final prototype; FIHGM system is close to final prototype but was available only in late 2010; final XCT prototype (recently transferred to HMGU Munich), once its construction completed, immediately underwent integration with optical system thus was not available for X-ray experimentation using mice. Consequently, the conclusions of M5 are based on experiments using FIHGM system. When the final prototype will be available, additional experiments would usefully allow the completion of the evaluation.

Final technical specification for XCT system (D2.7)

A complete specification was produced. It explains the choice of the X-ray components (detector, X-ray tube) and the geometry. It also describes the system architecture, the mechanical design including safety and beam manipulation devices, and the final gantry. Safety and normative requirements, performance data and electrical design of the system are addressed. These specifications have been used as input for WP5 (integration).
Technical specification for XCT design (D2.4)

The main achievement is the implementation of a dual-energy X-Ray acquisition protocol and corresponding data processing. Two set of angular projections are acquired, one for each energy spectrum: low energy (LE) and high energy (HE), then can be optionally combined in a single, high contrast image. A reconstruction algorithm is applied. For protocol optimization we used both experiments on the CEA-LETI bench and numerical simulation. This optimization is a difficult problem due to the X-rays absorption properties of different kinds of soft tissues that are very similar (few percent or less in difference) in the energy range used. Remind that the goal is enhancing internal organs contrast, and not contrast between tissues and bones. The resulting acquisition protocol is presented in the following figure.

![Optimal configuration for prototype](image)

This protocol for LETI bench has been validated on various phantoms and living mice. An example is shown hereafter: CT slice in both low and high energy, and plots representing various organs in dual-energy axis set, with associated variance (bones not represented because out of scale).

![Comparison between the low (left) and high (right) energy reconstructed data of a mouse scan. The main organs and the corresponding mean attenuation values in cm⁻¹ (low energy / high energy) are plotted. Organ segmentation was performed manually.](image)
The experiments that we performed show that it is easy to distinguish between bones and soft tissues, feasible to distinguish between adipose and other soft tissues, but almost impossible to identify organs.

In conclusion, the dual energy protocol, compared to a single energy one, requires twice the acquisition times because of the second energy scan, implies a higher dose to the animal, and needs a quite complex shape adaptation algorithm to correctly combine the two reconstructed 3D images. Given the poor contrast gain it provides, we suggest to avoid the implementation of the full dual energy protocol on the final FMT-XCT prototype for routinely animal imaging, but rather to use the sole low energy acquisition. This last, with respect to imaging protocols used on commercial scanners (that are similar to the high energy one), allows obtaining some global contrast gain, at the sole price of a slightly improved animal radiation dose.

**Optimization of contrast enhancement strategies on XCT system (D2.6)**

In this task we analyzed and compared different X-ray tomography contrast enhancement techniques that could be applied on the final FMT-XCT prototype to improve internal organs detectability, so that the obtained images, after segmentation, could serve as prior information for the optical reconstruction algorithms. Four different techniques were considered, namely a dual energy technique, four different contrast agent based techniques, a double exposure technique and phase contrast imaging technique. A particular attention was turned to the comparison of the contrast enhancement obtainable with both the dual energy protocol and different contrast agent based protocols. Since the CEA-LETI bench is not suitable for extensive animal imaging, all the experiments were carried out in Madrid using the facilities and the prototype X-rays scanner provided by FIHGM. The objective was to directly compare the two contrast enhancement strategies on the same machine and on the same mice instead of, as it was done until now, on different machines and different mice, thus avoiding inter-machine and inter-animal variability. This machine is not exactly the same as the FMT-XCT final prototype, but differences are limited and the results should be to a great extent valid also for the latter.

Experimental system and protocols are detailed in deliverable D2.6, as well as the resulting CT images and contrast evaluation. An example based on Intravenous Iopa miro is given hereafter. In this protocol, contrast agent rapidly diffuses from the blood flow to the kidneys, that are readily put in evidence (figure 4.b & c) in the image with an absorption coefficient closer to bones rather than to soft tissues. Also the low energy image (figure 4.a) allows to clearly distinguish kidneys, but the achievable contrast in not comparable at all with the one obtained in contrast agent images. Quantification of the contrast is performed by tracing a profile plot of the reconstructed x-ray linear attenuation coefficient as reported in figure 5. A quantitative analysis of the obtained contrasts shows that the three protocols allow distinguishing the three tissue types, but when contrast agent is present the contrast level is increased significantly. Particularly difficult is to find a contrast between muscular and kidney tissues with the low energy protocol, since the contrast is only a few times bigger than noise.

![Figure 4. Comparison of the images obtained with the low energy configuration of the dual energy imaging protocol (a) and with the contrast agent protocol (b and c). Image b was acquired 5' after CA injection, image c 15' after CA injection.](image_url)
Similar experiments were conducted using others contrast agent based imaging protocols. All the tested protocols showed a neat increase of contrast thanks to the presence of the Iodine-based contrast agent. The main drawback we could remark for such protocols is anyhow the fact that better results are obtained when a specific organ is targeted by the contrast agent: in this case the target organ undergoes a very significant contrast enhancement with respect to the surrounding tissues. In the other cases, where the drug is supposed to enhance contrast more globally, the contrast agent diffusion dynamics, the very complex anatomy of the mouse and/or the limited resolution of the obtained images do not allow obtaining so brilliant results.

**Investigation of other contrast enhancement strategies**

In the context of improving the reconstructed volumes contrast for better image segmentation, CEA-LETI investigated also the feasibility of using in the FMT-XCT project a recent x-rays imaging technique called **phase contrast imaging (XPCI)**. This novel technique, exploiting the wave nature of X-Rays, allows putting in evidence very low contrast features at material boundaries, especially when dealing with low Z material and low energy. The challenge is to implement this technique using a laboratory X-Ray source rather then a synchrotron one.

A short report has been issued (attached to deliverable D2.6) presenting a review of the different XPCI techniques and preliminary evaluation. No experiment has been performed, neither the FMT-XCT generator nor the detector being suitable. Our conclusion is that XPCI is a very promising technique, that has the potential to increase to a great extent the sensitivity of x-ray imaging. Nevertheless, at the current state of the art, the lack of very brilliant laboratory x-ray sources and the limited resolution of detectors greatly limit the possibilities of this technique. In particular, the technique would be hardly implementable on the FMT-XCT prototype and, more importantly, would be impossible to use for routinely practice.

Contrast enhancement methods that do not involve dual-energy X-ray exposure are under study and development at FIHGM, especially **dual exposure method**. This technique extends the effective dynamic range of the Flat-Panel detector to obtain better quality images when the sample has both low and high attenuation areas. This technique could be useful to put in evidence low contrast details in highly attenuating parts of very heterogeneous objects when in low attenuating areas the detector dynamic range is saturated.

**Conclusions**

Different contrast enhancement strategies have been compared for application on the FMT-XCT final hybrid prototype.

X-ray phase contrast imaging was demonstrated to be unfeasible for the FMT-XCT project due to the fact that the technology is not yet mature for practical applications.

The full dual energy protocol and the low energy only one allow obtaining a general, whole body improvement of contrast with respect to imaging protocols commonly used in the pre-clinical practice (such as those suggested for small animals commercial scanners). In certain situations, this improvement is sufficient to detect anatomical features not detectable with protocols using more energetic X-rays, nevertheless, the entity of this contrast improvement is globally rather faint and does not allow ameliorating significantly the possibility of identifying all
the internal organs of the mouse. Between the two protocols, the full dual energy one adds some complexity and is more time requiring with respect to the low energy only protocol and the contrast gain it offers is limited, so the latter seems to be more suitable for routinely use.

In consideration of the four contrast agent based imaging protocols tested, our conclusion is that when a very specific organ is of interest for a given study and if a suitable contrast agent and/or a related administration protocol are available to target it, the attainable contrast enhancement is by far superior compared to what can be obtained without a contrast enhancing product. On the contrary, when this is not possible, the low energy configuration is a good technique to push to the maximum the imaging performances of the machine in use.

If additional experiments on living mice could be performed using the final prototype, they would usefully allow the completion of the evaluation of the obtainable contrast by XCT techniques.

Optimized contrasted tomographic volumes of mice can now be provided to the Consortium and especially to WP4 (and Task 4.2 XCT segmentation) for segmentation and extraction of prior information for FMT inversion.