PROJECT PERIODIC REPORT

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

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: 1st
PERIOD COVERED: FROM March 01, 2008 TO February 28, 2009

NAME, TITLE AND ORGANISATION OF THE SCIENTIFIC REPRESENTATIVE OF THE PROJECT'S COORDINATOR:
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DECLARATION BY THE SCIENTIFIC REPRESENTATIVE OF THE PROJECT COORDINATOR

I, as scientific representative of the coordinator of this project and in line with the obligations as stated in Article II.2.3 of the Grant Agreement declare that:

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

• The project

✓ has fully achieved its objectives and technical goals for the 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.

Name of scientific representative of the Coordinator:

Prof. Dr. Vasilis Ntziachristos

Date: 20/05/09

Signature of scientific representative of the Coordinator:

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1. **Publishable Summary**

The overall goal of this project, is to develop a truly unique imaging system, the likes of which exists nowhere by combining X-ray CT (XCT) and Fluorescence Molecular Tomography (FMT) 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 fig. 1, including two work packages (WP1 and WP9) exclusively dealing with management, co-ordination, training and dissemination activities.

Due to the technical excellence and the uniqueness of the R&D consortium, the first year has been very successful. Within the work package 2 (WP2), focused on developing the XCT technology, a complete specification defining the system architecture, safety and normative requirements, performance data and electrical design of the XCT system (deliverable 2.1 XCT design) was developed. This specification already fulfils most of the objectives of the optimal XCT design scheduled for month 24, but further optimization will occur during year 2. Based on this optimal XCT design, the
dual-energy XCT set-up was initialized and is under development now. In parallel CEA-LETI has developed an ON-BENCH-CT which can provide images of comparable quality with a final prototype system. The purpose of this development is to provide a versatile tool, in order to help the dual-energy source improvement and validation.

**WP3** and **WP4**, dealt in this first year with theoretical approaches to accomplish high imaging performance, inversion algorithms for improving reconstruction and new algorithms for fast and more efficient inversion as well as multispectral reconstructions for multiple target detection were developed and tested (deliverable 4.1 and 3.1). At the same time a large number of experimental data sets (deliverable 3.2) have already been produced. Finally, a user friendly software has been developed that can be incorporated to all FMT systems and dramatically improves usability, efficiency and attractiveness to the end user. It can be used for data analysis and visualization of results in a fast and efficient way.

According to schedule, **WP5** developed a 360-degree functional FMT prototype and commenced the development of a worldwide unique FMT-XCT hybrid prototype and the first in vivo studies are already on-going, ahead of schedule, (see technical appendix WP5). Synergistic developments within **WP6** and **WP7** dealt with in-vivo imaging strategies and preclinical imaging. WP6 successfully developed different cancer animal models which are made available for in-vivo FMT-XCT imaging (technical appendix WP6). Furthermore different targeted fluorescent probes were tested to enable the availability of the best performing probes to the consortium. In parallel, and as intended for the first year, WP7 developed reporter gene assays for HIF and HIF downstream products and in-vivo imaging feasibility has been successfully demonstrated (deliverable 7.1).

Finally, **WP8** has already identified preferred phantoms for small-animal imaging and physical properties of the ideal optical phantom have been studied. A final phantom proposal has been developed (technical appendix WP8). Based on the optimal phantom design first hybrid phantoms are under development now and will be circulated among the project partners in the following month. The project developed well during the first year and all deliverables according to Annex I have been achieved for this reporting period.
The communication between the different partners is excellent and special ties have been developed virtually between all work-packages. Two consortium meetings were already held, in parallel members of the consortium have been privatively discussing progress, issues and objectives concerning technical details of the project. A regular exchange of information by email, telephone or smaller in-person meetings between the members of the consortium and the project co-originator have taken place. There are no known problems within the network. A webpage (http://www.fmt-xct.eu), presenting the FMT-XCT European funded project to the public, was developed and is up-dated regularly.
2. PROJECT OBJECTIVES FOR THE REPORTING PERIOD 1

The project objectives starting in the first reporting period 1 (March 01, 2008 – February 28, 2009) 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
2.1 To design a micro X-ray CT system appropriate for small animal imaging.
2.2 To develop the XCT system and implement a dual energy X-ray CT system.
2.3 To optimize delivered dose using a multi-resolution CT approach.
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.

OBJECTIVES WORK PACKAGE 3 -THEORY FOR 360-DEGREE FMT
WP leader: FORTH
3.1 To implement direct inversion based on boundary removal method for media with arbitrary boundaries.
3.2 To research optimal direct inversion approach with simulations and experimental data.
3.3 To compare the direct inversion performance with conventional, previously developed FMT inversion methods.
3.4. To incorporate algorithms for multi-spectral imaging.
3.5. To develop user friendly software for inversion of XCT-FMT data based on direct inversion approaches.
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

4.1 To develop FMT inversion utilizing XCT image priors without strong anatomy function correlations.
4.2 To incorporate XCT image segmentation into the FMT code.
4.3 To calculate spatially varying optical attenuation in tissues *in-vivo*.
4.4 To develop FMT inversion based on simultaneous XCT segmentation and classification.

**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.

**OBJECTIVES WORK PACKAGE 6 - CANCER IMAGING WITH FOCUS ON BREAST CANCER**

WP leader: CEA-Lime

6.1 To provide key fluorescence probes and quantify the sensitivity and contrast achieved in the animal models developed and as a function of tumor growth.
6.2 To develop animal models of breast cancer for studying FMT-XCT performance.

**OBJECTIVES WORK PACKAGE 7- IMAGING CANCER THERAPY FOR ENABLING INTERVENTION**

WP leader: UZH

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.
**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.

Following table B 1.3.2 in Annex I, the work plan of the FMT-XCT project is reflected in the graphic 2. Objectives for the first reporting period (year 1) are highlighted in green.

![Graphic 2: Work plan of FMT-XCT system and method. Objectives for the first reporting period are highlighted in green.](image-url)
3. WORK PROGRESS AND ACHIEVEMENTS DURING THE PERIOD

Here we provide a concise overview of the progress of work in line with the structure of Annex I of the Grant Agreement except project management, which is reported separately (section 5). As illustrated in Fig. 1 and Fig. 2 the work is split into 9 work packages (WP). Please note that technical appendixes and deliverable reports explaining detailing scientific achievements are attached to this report.

WORK PROGRESS WP2: XCT DEVELOPMENT

WP leader: CEA-LETI

Please notice that a technical work package report detailing scientific achievements is attached to this report (see FMT-XCT_year 1 report_WP2_technical appendix).

SUMMARY

The global objective of WP2 is to provide an X-ray Computed tomography (XCT) module that can be integrated with a Fluorescence Molecular Tomography (FMT) module into a hybrid imaging system. Several designs and commercially available systems exist for X-ray CT of small animals. 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,
- offer 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.

The year 1 of the project is mainly concerned by the three first objectives, leading to the tasks:

TASK 2.1 “XCT DESIGN”

The goal is to issue the specifications regarding the X-ray module of the final FMT-XCT prototype that is to be integrated by VAMP within WP5. These specifications should be defined by the X-ray partners, integrating small animal imaging constraints. Compatibility with optical components should be controlled by other partners. The corresponding report constitutes the deliverable D2.1 (see Deliverable 2.1), due for month 1.

TASK 2.2 “XCT DUAL-ENERGY PROTOTYPE DEVELOPMENT”

This task consists in the development a XCT prototype and implementation of a dual-energy X-Ray acquisition protocol and corresponding data processing. Two set of angular projections should be acquired, one for each energy spectrum: low energy (LE)
and high energy (HE), then combined in both a dual-energy decomposition and reconstruction scheme to be defined.

Two deliverables will finalize these developments: D2.2 “Functional prototype for dual energy cone beam CT” and D2.3 “Calibrated, dual energy processing software”, both due for month 15.

**TASK 2.3 “OPTIMIZATION OF DELIVERED DOSE”**

The third task should propose a multi-resolution approach, using the fact that biological tissue differentiation does not necessarily require the highest XCT resolution, aiming at reducing the dose.

**PROGRESS TOWARDS OBJECTIVES**

**TASK 2.1: XCT DESIGN**

After the kick-off meeting in May 2008, the design of the micro X-ray CT system was started by evaluating the requirements of the different modalities and determine the geometric configuration of the scanner. In this respect, the geometric configuration was based on

a) the requirements of the optical imaging (as far as these had been already known, provide enough flexibility to make subsequent alterations), and

b) the limitations of the x-ray components (magnification, active area of the detectors, available x-ray power).

A brief survey based on experience and the saturation thresholds of the detectors in question yielded a value for the necessary x-ray power of the tube. In combination with the desired values for resolution and especially the requirement of the relatively short desired scan time (as compared with the optical scan duration), a component was chosen which suited the requirements (Oxford Instruments UltraBright, 80 Watts output power, down to 13µm focal spot size). For reasons of compatibility, the detector was chosen to be similar to the one already being under investigation at CEA-LETI (Hamamatsu C7943), so the results of the base investigations with the on-bench setup shall be easily and reliably transferable to the gantry system.

Mechanical design was chosen according to the size and weight of the x-ray components including estimated values for the additional optical parts (dimensions, bearings, motors). Safety and beam manipulation devices have been incorporated into the specification (shutter, filter wheel, collimators, interlocks).

Based on these essential decisions, a complete requirements specification was produced in November 2008 (cf. Milestone 2: XCT design) by CEA-LETI and VAMP. This specification also defines the system architecture, safety and normative
requirements, performance data and electrical design of the system. It can be stated, that the existing requirements specification already fulfils most of the objectives of the “optimal XCT design” (task 2.7). This was carried out because of the relatively long times of delivery of the key components (up to 6 months for the x-ray tube), so to ensure having a working system for the research group at the end of year two, the final decision about the overall design had to be made earlier than previously planned. The issued report has been approved by HMGU. Thus task 2.1 is complete.

**TASK 2.2: XCT DUAL-ENERGY PROTOTYPE DEVELOPMENT**

A technical meeting involving VAMP and CEA-LETI was held at Erlangen in December 2008. Two deliverables (2.2 and 2.3) are due for month 15.

**DEVELOPMENT OF XCT PROTOTYPE**

At the time of this report, the XCT system is being under development at VAMP, the first parts have been constructed and the parts with long delivery times have been ordered or will be as soon as possible. The objective is to have a working system available for the research group at the end of year two.

**ON-BENCH-CT AT LETI**

CEA-LETI has developed a ON-BENCH-CT, distinct from the final industrial-grade gantry. Although this laboratory bench presents significant differences with the final one, mainly the horizontal geometry (non-gantry approach) and longer acquisition time, it should provide images with comparable quality. The purpose is to be provided with a versatile tool, in order to help the dual-energy development, improvement and validation. A new X-ray detector has been installed, that suits the requirement of the final prototype.

**DEVELOPMENT OF DUAL ENERGY PROTOCOL**

CEA-LETI has analysed the optimisation of the dual energy system, with the objective of differentiating between soft/fat and various organs tissue, and the choice of material basis. Based on both numerical simulation and experiments using the laboratory bench, preliminary conclusions have been reached but still require more validation. The preferred acquisition mode is of type step and shot, with completion of all the geometric dataset acquisition at the first energy and sequentially the full geometric data set for the second energy. This modality is technically and practically easier to implement, gives the possibility of changing both the filters configuration and the tube settings between the two acquisitions, and allows faster scans since it introduces no dead times for energy configuration switching.
The problem of the choice of the two best energy configurations for dual energy decomposition is indeed multi-parametric. To improve the contrast, the two energies should be as separated as possible one from another and both as low as possible. This requires lowering the operating voltage of the X-ray generator and using thicker filters to shape the energy spectra, consequently reducing the photon flux and increasing the detection noise. The solution needs therefore to be a compromise between energy separation and signal intensity.

It is important to remark that this optimisation has been calculated on the basis of the physical parameters of the CEA-LETI prototype. For the final prototype, the optimization needs to be performed using its own physical data and the resulting set of parameters needs not to be exactly the same.

**Task 2.3: Optimization of Delivered Dose**

Because of the choice of a planar detector, this optimization can not be done per slice, and the number of angles for the X-ray acquisitions should be fixed for the highest resolution required. We propose to develop a “regional” binning of the detector pixels, depending on the area where the highest resolution is required. Considering that this development does not present any particular problem or risk, it does not impact the XCT design, and is not a critical point for reaching the objective of WP2, it is postponed to a future period.

**REVISED PLANNING**

Dual-energy XCT development has correctly started and some preliminary conclusions have been achieved. This task (2.2) is a little late when considering the initial planning. In fact, CEA-LETI started effectively to work on the project after the kick-off meeting (in May) with an amount of charged man-power below the initially planned level for the fist months. Correct level of efforts has been put from September. VAMP also started the work 3 months after the official start in March.

The XCT system is being under development at VAMP, the first parts have been constructed and the parts with long delivery times have been ordered or will be as soon as possible (i.e. as soon as the vendor is able to approve the specified performance data), others will follow accordingly to the completion of the system. In the meantime work can be safely continued by using parts in stock, having similar interfaces, to develop and validate the software used for control of the gantry as a whole and safety related issues in particular. Notice that up to now, VAMP did still not receive positive feedback from Hamamatsu approving our specifications to the chosen detector.
Responsibility of VAMP: It is planned to have the system transferred to Munich no later than in project month 22 (December 2009) for completion and starting of the integration of the optical components. After that a design review phase will have to follow, for fine adjustment of mechanical/electrical and software interfacing. Finally, the system will be approved for further development by the HGMU group.

Responsibility of CEA-LETI: The sub-task concerning the implementation of the dual-energy approach is planned to end in June with a corresponding deliverable in September 2009 – the period September-December 2009 will be devoted to eventual required adaptation of the method to the system developed by VAMP. The adjusted and extended project plan for WP2 has been approved by the consortium at year one meeting.

Fig. 3: Detailed project plan for WP2.

For details task 2.6 “Use of X-ray contrast enhancement methods (FIHGM)” see technical appendix WP2.

WORK PACKAGE 3 – THEORY FOR 360-DEGREE FMT

WP leader: FORTH

Please notice that a technical work package report detailing scientific achievements is attached to this report (see FMT-XCT_year 1 report_WP3_technical appendix).

SUMMARY

This work package aims to develop the appropriate theory for the 360 degree FMT. During the first year of the project FORTH’s involvement has been mainly towards developing new algorithms for fast and more efficient inversion as well as multispectral reconstructions for multiple target detection. A direct inversion method based on calculating fluorescence in Fourier space was developed and tested. This algorithm has
proved significantly better than the standard algorithms when a large number of sources are used but is not convenient for FMT acquisitions where usually a small number of sources are used. Furthermore, a new algorithm for boundary removal of arbitrary surfaces has been developed and this will be used for a novel ultra-fast inversion that will be delivered in the next reporting period. The multispectral method is based on a linear unmixing algorithm and has been tested with both phantoms and in vivo studies and is capable of resolving different fluorescing and absorbing targets. At the same time a very large number of experimental data sets has been produced that are available to all partners of the consortium for algorithm comparison, optimization and finalization. Finally, a user friendly software has been developed that can be incorporated to all FMT systems and dramatically improves usability, efficiency and attractiveness to the end user. It can be used for data analysis and visualization of results in a fast and efficient way.

PROGRESS TOWARDS OBJECTIVES:

3.1: An inversion method for FMT based on boundary removal and back-propagating the data has been developed and is available to all partners.

3.2 A direct inversion method based on reconstruction in Fourier space has been developed and is also available to all partners.

3.3 The direct inversion has been tested and compared with the standard methods and proved to be significantly faster when a large number of sources has been used. However, for small number of sources as in the case of FMT acquisitions, it does not give satisfactory results.

3.4 A multispectral algorithm has been developed and tested for simultaneous detection of multiple fluorophores and absorbers. It will be incorporated in the FMT software in the next reporting period according to the time schedule.

3.5 A user-friendly software has been developed under Matlab and Labview environments, enabling users to analyze experimental data and visualize results in a fast and efficient way.

3.6 A large number of experimental measurements have been acquired that are available to all partners for optimization and finalization of algorithms. These measurements involve phantoms as well as in vivo experiments.

DEVIATIONS

No deviations have been necessary during this reporting period. However, a decision has been made to start a new Deliverable “Ultra – fast Inversion for FMT” that will be delivered on the next reporting period. On the meantime, the partners have access to an
inversion method which is significantly faster that the currently existing ones, based on removing the boundary and then performing ART on the now infinite homogeneous data, as also stated in Objective 3.3. This way, the flow of the project is not jeopardized until we obtain the ultra fast reconstruction method in 2010.

**REVISED PLANNING**

All objectives and deliverables have been achieved for this reporting period. A new deliverable is proposed as stated above and will be delivered on the next reporting period.

**WORK PACKAGE 4 – FMT INVERSIONS WITH IMAGE PRIORS**

*WP leader: UCL*

Please notice that a technical work package report detailing scientific achievements is attached to this report (see FMT-XCT_year 1 report_WP4_technical appendix).

**SUMMARY**

The overall goal of this work package is, similar as WP3, to improve the FMT imaging performance using mathematical and computational techniques. Therefore during year one we focused on algorithmic developments needed for inversion with priors by

- developing a FMT inversion utilizing XCT image priors without strong anatomy function correlations (objective 4.1),
- incorporating XCT image segmentation into the FMT code (objective 4.2),
- calculating spatially varying optical attenuation in tissues in-vivo (objective 4.3) and
- developing the FMT inversion based on simultaneous XCT segmentation and classification (objective 4.4).

**PROGRESS TOWARDS OBJECTIVES**

4.1 Structured priors orientating the reconstructed FMT images to have level sets parallel to those of XCT image and theoretic priors orientating the reconstructed FMT images to have maximum joint entropy with the XCT image were developed. Initial tests on simulated 3D images of mouse from a realistic atlas were performed.

**SIGNIFICANT RESULTS**

- Reconstructions in 3D depend only linearly on total number of pixels in reconstructed image and independent of the number of pixels in data.

4.2 Segmentation of XCT based on anisotropic diffusion (Peronal-Malik algorithm) and hexahedral adaptive mesh generation from XCT images were developed. Mesh reduction methods using public-domain software ISO2MESH were incorporated and
Boundary Element (BEM) and hybrid Boundary-Finite Element (BEM-FEM) methods were developed.

**SIGNIFICANT RESULTS**
- Reconstructions using FEM only for internal organs are much faster than using a complete FEM mesh.

4.3 A Non-linear reconstruction method for attenuation making use of Louiville transformation from diffusion to Schrodinger equation was developed.

**SIGNIFICANT RESULTS**
- Reconstruction of attenuation from steady-state data is dependent on good estimates of spatially varying scatter.

4.4. A combined reconstruction/segmentation method combining Gauss-Newton image reconstruction with fuzzy-kmeans image classification and a fully hierarchical Bayesian framework was developed.

**SIGNIFICANT RESULTS**
- Classification error less than 5% for simulated noisy data.

**DEVIATION FROM ANNEX1**

None.

**REVISED PLANNING**

Not applicable

3.5. **WORK PROGRESS WORK PACKAGE 5– FMT-XCT INTEGRATION**

WP leader: HMGU

Please notice that a technical work package report detailing scientific achievements is attached to this report (see FMT-XCT_year 1 report_WP5_technical appendix).

**SUMMARY**

Work package 5 exhibits a pivotal role and focuses on the development of a 360 degree hybrid FMT-XCT imaging system. Hardware, algorithmic and software developments of WP 2, 3 and 4 will be integrated to build a highly performing quantitative FMT-XCT prototype, which performance will be tested within the other work packages. The main objective for the first year was to build a functional system using non-contact measurement and 360 degree geometry. According to schedule a simplified prototype on a rotational motor stage was successfully developed, serving as a pre-platform for the final FMT-XCT integration.
PROGRESS TOWARDS OBJECTIVES

OBJECTIVES 5.1: TO DEVELOP A FULLY FUNCTIONAL MULTI-SPECTRAL FMT-XCT PROTOTYPE AND MINIMIZE XCT AND FMT INTERFERENCE

Within the first year of WP5, we implemented the prototype of a 360° free space FMT system suitable for integration onto an XCT gantry. The system was based on a custom-manufactured circular optical breadboard of 1m diameter (anodized aluminum of 1cm thickness), mounted vertically on top of a rotating motor (all delivered and manufactured by Newport Corporation, Irvine, CA, USA). On top of this gantry, a CCD camera and a scanned laser source were mounted orthogonally to the central rotation axis. A back-illuminated cooled CCD camera (Pixis 512B, Princeton Instruments, Trenton, NJ, USA) coupled to a 50mm macro lens (Carl Zeiss, Oberkochen, Germany) was selected for detection due to its high sensitivity. In front of the lens, a proprietary four-position filter wheel was mounted. The filter positions were occupied by different combinations of long pass glass filters (Schott, Mainz, Germany) and bandpass filters (Andover, Salem, NH, USA) to filter fluorescence light or the excitation wavelength. Beneath the filter wheel two electroluminescent plates are mounted to provide white light illumination of the animal when needed.

DEVIATION FROM ANNEX1

None.

REVISED PLANNING

Not applicable

3.6 WORK PROGRESS WORK PACKAGE 6– CANCER IMAGING

WP leader: CEA-LIME

Please notice that a technical work package report detailing scientific achievements is attached to this report (see FMT-XCT_year 1 report_WP6_technical appendix).

SUMMARY:

While phantoms are extensively utilized in virtually every work-package, the use of animal models of cancer is essential in order to develop FMT-XCT for its intended application, i.e in-vivo imaging. The overall goal of this work package is therefore to provide appropriate animal models, fluorescence probes and validation tools in order:

- To provide key fluorescence probes and quantify the sensitivity and contrast achieved in the animal models developed and as a function of tumor growth (Objective 6.1).
To develop animal models of breast cancer for studying FMT-XCT performance (Objective 6.2).

To develop animal models of other cancers for studying FMT-XCT performance (Objective 6.3).

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

To predict clinical utility (Objective 6.5).

**PROGRESS TOWARDS OBJECTIVES**

The year 1 of the project is mainly concerned by the three two objectives, leading to the task:

**TASK 6.1 ANIMAL MODELS OF BREAST CANCER**

The following animal models have been established at CEA-LIME, and made available for in-vivo FMT-XCT imaging.

a) mammary tumor xenografts

The following tumours have been implanted subcutaneously in mice:

- MDAMB-231 human breast adenocarcinoma cells over-expressing MT4-MMP, a metalloproteinase which does not affect in vitro cell proliferation or invasion but strongly promotes primary tumor growth and associated lung metastases in RAG-1 immunodeficient mice 8 weeks post-injection.

- primary cell cultures from human tumors that either express or do not express erb-B2, the target of trastuzumab (Herceptin®), a monoclonal antibody used in breast cancer chemotherapy; these cells have been obtained by CEA-LIME through the Paris canceropole research collaboration and implanted in nude mice. See results in below.

b) mammary tumor transgenic mice models

CEA-LIME has bred a transgenic strain of mice expressing the polyoma middle T oncoprotein (PyMT), under the control of the mouse mammary tumor virus long terminal repeat (MMTV LTR). These mice develop breast cancer with four distinct stages of tumor progression, from premalignant to malignant stages. Tumoral development is often, at late stages, associated with lung metastases. This model presents many similarities with breast cancer progression encountered in women and has been quantitatively documented using PET, SPECT and CT imaging.

**TASK 6.2 ANIMAL MODELS OF BRAIN CANCER**

Will be developed during year 2.
**TASK 6.3 FLUORESCENT PROBES**

This proposal does not aim in developing new fluorescence probes. However, successful completion, demonstration, training and dissemination of the proposed technology rely on the availability of fluorescent reporters to the consortium. To accomplish this we have taken certain steps to not only rely on commercially available fluorescent probes but enable the availability of highly performing fluorescent probes due to own developments:

- **Commercial probes**

  Angiosense 680 was tested in the PyMT model:

- **Home-made probes:**

  a) Trastuzumab, (Herceptin®) is a monoclonal antibody that we labelled with AlexaFluor® 680 for use with the human erb-B2 in expression patterns of human erb-B2 in human mammary cancer cells as per models in Task 6.1.

  b) Aptamers: CEA-LIME has developed a new method to select aptamers against whole living cells against metastatic forms of cancer. Differential whole cell-SELEX can select aptamers specific for highly lung metastatic cells (versus low lung metastatic cells) [Illan, Zueva, Tavitian, paper submitted 2009].

**NEXT STEPS:**

1. develop rodent models of brain tumours
2. optimize the bio-distribution of these aptamers in vivo and resolved them using XCT-FMT.
3. Initiate In-vivo imaging and correlation. In vivo imaging of animal models will be performed originally with the CEA-LETI for XCT validation and in FORTH for stand-alone FMT validation using commercially available probes.

**ADVANCEMENT OF PROGRAMME**

*Planned Deliverables (month of delivery planned at start of first year)*

6.1 To develop and characterize molecular probes (mo.9, 18)
6.2 Prepare and characterize mammary cancer animal models (mo.18)
6.3 Breed and making available PyMT animal models (mo.24)
6.4 Develop U87 animal models (mo.27)
6.5 Study and report the quantitative accuracy of FMT-alone and FMT-XCT in resolving tumors (mo. 36)
6.6 Study and report cancer detection performance in various organs (mo. 40)
6.7 Report the overall imaging performance. (mo.42)

*Status of achievement*
6.1-6.3 achieved and continued.
6.4: to start year 2
6.5: to start when FMT becomes available
6.6-6.7: to begin after 6.5

**DIFFICULTIES**

One breeding accident with the PyMT mice has delayed partly our programme with these animals. No changes in planned activities.

**WORK PROGRESS WORK PACKAGE 7 – IMAGING CANCER THERAPY**

WP leader: UZH

**SUMMARY**

Within WP 7 we 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.

**PROGRESS TOWARDS OBJECTIVES**

The year 1 of the project is mainly concerned by the third objective leading to the task:

**7.3: CHARACTERIZING HIF-RELATED PATHWAYS**

We have developed multiple reporter assays for measurement of HIF pathway activity:

1) hypoxia imaging using the ligand 18F-MISO in combination with PET imaging (not part of FMT-XCT project),

2) reporter assays for hypoxia-inducible factor 1α (HIF1α) expressing HIF1α as fusion protein with either firefly luciferase or m-Cherry. We have demonstrated that the fusion protein is regulated by the proteasomal degradation machinery as the native protein and that the fusion protein induces down-stream genes.

3) reporter gene assay for HIF1α activity by expressing firefly luciferase under the control of the HIF-responsive element HRE (the constructs with m-Cherry are currently
being prepared). Tumor cells (C51 colon carcinoma have been transfected using the constructs and injected subcutaneously in nude mice. A longitudinal PET and bioluminescence imaging has been carried out monitoring tumor volume as well as the regulation of HIF stability/activity (publication S.Lehmann et al. in preparation). Further proof-of-principle studies are currently ongoing modulating the levels of HIF1α through pharmacological interventions. The project developed well in the first 12 months. The principle objective, the development of reporter gene assays for HHIF and HIF downstream products has been achieved and feasibility of in vivo imaging has been demonstrated using bioluminescence imaging. The next step is adapting the HRE assay as fluorescence assay.

SIGNIFICANT RESULTS:
- Feasibility of demonstrating HIF stabilization and activity in tumor cells demonstrated
- HRE identified as most promising readout for fluorescence imaging

NEXT STEPS:
- Characterization of ODD-m-Cherrry transfected tumor cells in vitro
- Characterization of HRE-m-Cherry 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-XCT measurements with subcutaneous murine tumor models

DEVIATION FROM ANNEX1
None

REVISED PLANNING
Not applicable

WORK PACKAGE 8 – FMT-XCT IMAGING ACCURACY VS. PET-XCT
WP leader: FIHGM

Please notice that a technical work package report detailing scientific achievements is attached to this report (see FMT-XCT_year 1 report_WP8_technical appendix).

SUMMARY
Work package 8 focuses on the validation of the FMT-XCT utility when compared with the gold-standard PET-XCT. Metrics form both systems will be recorded, compared and validated in terms of the utility of the FMT-XCT system proposed in this project. To be
able to do this study it will be necessary to use a set of phantoms that will quantify relevant parameters that can quantify image accuracy in terms of sensitivity, resolution, and quantification accuracy. During the project first year partner FIHGM has worked on the definition and construction of such a phantoms.

**PROGRESS TOWARDS OBJECTIVES**

The year 1 of the project is mainly concerned by the first objective, leading to the task:

**TASK 8.1: HYBRID PHANTOMS**

This task is focused on designing and manufacturing physical phantoms that will be used for FMT-XCT system validation. The phantoms have to be hybrid in the sense that they should reveal the same imaging properties when used on a FMT-XCT system or on a PET-XCT system. This imposes a set of conditions that should be met simultaneously:

- The material used to build the phantom has to reflect the optical properties of the biological tissue, but it will also has to have attenuation and scatter properties of the biological tissue for the 511 keV characteristics radiation of the PET tracers.
- The spaces allocated for the different agent contrasts should be either disposable or easy to clean since not all the contrasts used (optical and PET) have the same decay constants.
- The phantoms should be hard, stable and easy to handle to be able to use them under different setups. They will be shipped among the different labs involved in the project.

The actions taken by FIHGM to achieve these goals have been:
- Identify the preferred PET phantoms for small-animal imaging.
- Study the physical properties of the ideal optical phantom.
- Phantom proposal.

**TASK 8.3: FMT-XCT IMAGE PERFORMANCE VALIDATION WITH PET-XCT IMAGING**

Although this task is scheduled for the fourth year of the project, FIHGM is starting to assemble an FMT-XCT prototype that will facilitate the performance evaluation in situ before distributing the phantoms.

**NEXT STEPS**

In the following months FIHGM will make the first hybrid phantoms. On the completion of these prototypes validation, the phantoms will be circulated among the project partners to gather some feedback on their use and define modifications for the next interaction.
DEVIATION FROM ANNEX1
None.

REVISED PLANNING
Not applicable

WORK PACKAGE 9—TRAINING AND DISSEMINATION
WP leader: HMGU

This workpackage is intended to contribute significantly to the rapid diffusion of scientific knowledge that is being generated within this project by
   1) training scientist on FMT-XCT and underlying technologies,
   2) dissemination activities within partners and to scientific, industrial and public sector and
   3) technology transfer activities.

Training and particularly dissemination activities will become more relevant towards the end of the project. A training session on XCT is planned as described in Annex1 (WP2 and WP9) and will be an opportunity of training in advanced XCT use. It should be housed by CEA-LETI at Grenoble in June or July 2009. A workshop mainly focussed on WP3 and WP4 activity, image reconstruction algorithms and the use of priors is planned in London in September 2009. To ensure dissemination to the public, a project website is available at http://www.fmt-xct.eu (for more information see project management (5).
## 4. Deliverables and Milestones

### Table 4.1 Deliberables

<table>
<thead>
<tr>
<th>Del. no.</th>
<th>Deliverable name</th>
<th>WP no.</th>
<th>Lead beneficiary</th>
<th>Nature</th>
<th>Dissemination level</th>
<th>Delivery date from Annex I (proj month)</th>
<th>Delivered Yes/No</th>
<th>Actual / Forecast delivery date</th>
<th>Comments</th>
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<td>HMGU</td>
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<td>RE</td>
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<td>Pu,Re</td>
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<td></td>
<td>Described in 5.5 Development of the project website (management)</td>
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<td>1.4</td>
<td>Program of the consortium meetings</td>
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<td>CEA Leti</td>
<td>R</td>
<td>PP</td>
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<td>See “FMT-XCT_del.2.1_XCTdesign.pdf”</td>
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<td>3.1</td>
<td>Direct inversion algorithm</td>
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<td>O</td>
<td>PU</td>
<td>9</td>
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<td>See “FMT-XCT_del.3.1_direct inversion algorithm.pdf”</td>
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Decision for a new deliverable “Ultra-fast Inversion for FMT” (month 24).
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<thead>
<tr>
<th>Deliverables and milestones</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Deliverable</th>
<th>Objective</th>
<th>Participants</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
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<td>Experimental training set measurements</td>
<td>Forth</td>
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</tr>
<tr>
<td>4.1</td>
<td>Inversion algorithms</td>
<td>UCL (differing from B 1.3.4 Annex 1*)</td>
<td>OPU 9 YES</td>
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<td>5.1</td>
<td>360 degree FMT prototype</td>
<td>HMGU (differing from B 1.3.4 Annex 1*)</td>
<td>OPU 12 YES</td>
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<td>6.1</td>
<td>To develop molecular probes</td>
<td>CEA- Lime</td>
<td>OPU 9 YES</td>
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<td>7.1</td>
<td>Preparation of HIF transfected breast cancer cells</td>
<td>UZH</td>
<td>OPU 9 YES</td>
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<td>8.1</td>
<td>Construct imaging phantoms</td>
<td>FIHGM</td>
<td>OPU 12 YES</td>
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*The different lead different lead beneficiaries for deliverable 4.1 and 5.1 were due to a typo in annex 1 table B.1.3.4.*
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<th>Milestone name</th>
<th>Work package no.</th>
<th>Lead beneficiary</th>
<th>Delivery date from Annex I</th>
<th>Achieved Yes/No</th>
<th>Comments</th>
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</thead>
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<td>Consortium agreement</td>
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<td>1 HMGU</td>
<td>1</td>
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<td>Signed document &lt;br&gt;See “FMT-XCT_del.1.1 signed CA.pdf”</td>
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<tr>
<td>2</td>
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<td>2</td>
<td>2 CEA-Leti</td>
<td>1</td>
<td>YES</td>
<td>Executive committee agreement &lt;br&gt;See “FMT-XCT_del.2.1_XCTdesign.pdf”</td>
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<td>Optimal Free-space FMT system</td>
<td>5</td>
<td>1 HMGU</td>
<td>12</td>
<td>YES</td>
<td>Functional Prototype &lt;br&gt;see “FMT-XCT_1year report_WP5_technical appendix.pdf.”</td>
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5. PROJECT MANAGEMENT

This section summarises the management of all activities during the first reporting period of the FMT-XCT European project (01. March 08 – 28. February 09). The management of this project required extensive organisation, as outlined in Annex 1 WP 1. All the information related to the project had to be selected, coordinated and communicated between the consortium, the advisory committee and the administrative departments to enable appropriate information flow between participants and the EU (Fig. 4). Therefore a project manager was hired exclusively for management purposes, starting from July 08.

![Management Diagram]

Fig. 4: Management overview of FMT-XCT. This project starting in March 08 is funded under the 7th Framework program by the European Commission.

5.1 COORDINATION AND COMMUNICATION ACTIVITIES

The communication is working excellent- internally and externally. As outlined above all the information related to the project had to be selected, coordinated and communicated between the different departments/institutions. This included the coordination of the work packages, the collection and dissemination of management and financial information, the preparation of contractual documents and reports related to the project, the conduction of financial surveys, the financial settlements,
banking activities and the financial reporting. Beside the daily work related to the project several management/coordination activities should be mentioned:

- Preparation and finalization of the Grant Agreement
- Preparation and finalization of the Consortium Agreement (5.2)
- Organization of the kick off meeting (5.3)
- Organization of the year 1 consortium meeting (5.4)
- Development of the project webpage (5.5)
- Preparation and finalization of the first periodic activity report
- Organization of the prefinancing including request/requirement of the bank account information of all beneficiaries
- Assistance in the establishment of the advisory committee
- Preparation and finalization of the first periodic financial report *

*Due to the preliminary version of FORCE, where only the coordinator gets access to Form Cs, all data had to be transferred by the project manager to FORCE and submitted to the Commission. The pdf version of the full set of Form Cs had to be signed by the financial department of each beneficiary because for the moment only the signed paper version counts. It was announced by G. Zisimatos, Head of Administration and Finance Unit, F6 within the Training on FP7 reporting in Bruxelles March 13th 2009, that it is not allowed to distribute the log-in data to the beneficiaries. This demanded an enormous administrative effort for the project manager.

5.2 Contractual Issues
A Consortium Agreement between the Helmholtz Zentrum München and the beneficiaries was drafted and finalised. In this document the rights and obligations of the individual partners are defined and regulated. The consortium agreement was signed by every partner. (see Deliverable 1.1). There were no changes in the consortium and to the legal status of any of the beneficiaries.

5.3 Financial Issues
As agreed by the executive committee the prefinancing (160% of the average EU funding per period) was split in first (100%) and second (60%) payment to enable a positive cashflow.
5.4 MEETINGS

Consortium meetings were planned and organized. The agenda and minutes were sent out in time. The following meetings took place:

**Kick-off meeting, Neuherberg, 14 May 2008**

With the exception of Prof. Markus Rudin, all project leaders were present. Representative for the University Zürich Dr. Thomas Müggler and Florian Stuker joined the meeting. Dr. Jürgen Ertel, Department for Program Planning and Management (PPM), gives an overview of the administrative, financial and management aspects. He states that the financial issue is very important and presents a plan for the distribution of the pre-financing made by the Commission which is accepted by all partners. Prof. Dr. Vasilis Ntziachristos gives an overview of all tasks and the guidelines according to Annex 1. He describes in brief the work packages with their objectives and deliverables. Every partner gives a presentation introducing organization and scientific work. An ethical expert, Dr. Marta Zientowska, who has several years experience in performing animal experiments, was participating. For detailed information see deliverable 1.2, minutes of the kick-off meeting).

**Year one Consortium Meeting, Munich, 24 April 2009**

The Year 1 consortium was held in Munich on April 24th. Although originally planned in Grenoble, the Central location of Munich to the partners and the availability of an international, easy to access airport made it easier for partners to organize the trip, compared to Grenoble that is served by fights to Paris/Lyon and then a bus service that makes it difficult to access. For each beneficiary one or more representatives were attending. Prof. Dr. Vasilis Ntziachristos welcomes all partners and introduces Dr. Veronika Erben, who as the project manager, started with an overview explaining administrative and financial issues. Subsequently, every partner presented scientific work of the first year, following the structure of Annex 1 (for more details see deliverable 1.4, program of the year 1 consortium meeting). The next meeting is planned in Herakleon, Crete, although other candidate cities include London UK. This meeting will be co-localized together with a workshop on free space FMT (mo24). All presentations are available at [http://www.fmt-xct.eu](http://www.fmt-xct.eu) (Members only).
Private communication at meeting and conferences
At the several meetings and conferences, members of the consortium were privatively discussing progress, issues and objectives concerning technical details of the project. A regular exchange of information by email or telephone between the members of the executive committee and/or the project manager took place. There are no known problems within the network. A technical meeting involving VAMP and CEA-LETI was held at Erlangen in December 2008.

5.5 DEVELOPMENT OF THE PROJECT WEBSITE
The domain name, bought at domaindiscount 24.com, is hosted at the HMGU. The webpage (http://www.fmt-xct.eu) is based on Typo 3, which as a free open source content management offers full flexibility and extendability while featuring an accomplished set of ready-made interfaces, functions and modules (http://typo3.com/).

5.5.1 LAYOUT OF THE WEBSITE
The layout was designed to please the eye and provide a simple and functional interface. To ensure continuity on our website as well as to make the navigation easier, the same information was positioned in the same areas on each page (menu left, header middle, logo right, contact right). The heading of every page contains the project acronym, the full title and the project logo.

Fig. 5: Website of the FMT-XCT project.
5.5.2 Structure of the Website

The technical details of the project are based on Annex 1 of the grant agreement.

Home

The home page of the website briefly describes the aim of project. 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.

The Project

Motivation

To understand the motives of the project the webpage starts with some background information about imaging of biomarkers and different detection methods. It further explains the pros and cons of traditionally used methods and closures with pointing out the potential of FMT.

Summary

A summarized technical description of the project is given.

Objectives

The overall long-term objective of this project are listed. The internal link shows the drawing of the FMT-XCT prototype.

Methodology

A technical description how to successfully achieve the best performing optical imaging method is specified.

Work plan

A figure shows the work plan, which is spitted into 9 work packages.

Partners

All partners are marked on a European map. A detailed description of the project partners can be viewed by clicking on the links in the map.

Members only

The internal log in domain is reserved for project partners only. The members area is pass word protected (username: fmt-xct, password: eu_proj_2008) and intended to be dynamic, incorporating working material, documents uploaded for project meetings, or for internal distribution.

Following documents were uploaded by the project manager:

- Description of work: Annex 1
- Presentations and minutes of the kick-off meeting
- Presentations and minutes of the year one consortium meeting
• After submission and approval by the European Commission the year one report including technical appendixes, deliverables and milestones will be uploaded.

File transfer
As discussed by the consortium at the year one meeting, a password protected platform (Username: fmt-xcttransfer; password: get4data) for data transfer was established.

Sitemap
The sitemap represents the architecture of the web site so that the visitors can access all content on the site.

Additional features:
• Create print ready pages
• Vary the size of the web page
• Imprint and Disclaimer

5.6 TRAINING ACTIVITIES ON FP7 REPORTING
For being up-to-date concerning FP7 reporting and to ensure a smooth reporting process, the following training sessions were visited:
• How to negotiate and administer framework 7 grant agreements,
  Dr. Sean McCarthy, Hyperion, 26th November 2008, Helmholtz Zentrum München
• Training on FP7 reporting and amendments. Use of the FORCE IT tool.
  Directory Health, Unit F6, 13th March 2009, Auditorium of the MADOU building, Buxelles.

5.7 PROJECT TIMETABLE AND STATUS
All deliverables have been achieved for this reporting period. Only minor changes in the time plan.
Deviation from Annex1
None.
Revised planning
Not applicable.
6. EXPLANATION OF THE USE OF THE RESOURCES

Total direct costs per beneficiary are coherent with the direct costs claimed in Form C.

Personnel, subcontracting and other major direct cost items for beneficiary 1 (HMGU) for the period 1

<table>
<thead>
<tr>
<th>Work Package</th>
<th>Item description</th>
<th>Amount</th>
<th>Explanations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4, 5, 6, 7, 8, 9</td>
<td>Personnel costs</td>
<td>153,099,55€</td>
<td>Salaries of 2 postdoctoral students (both 9.5 person month), 1 postdoctoral student (2 PM), 1 postdoctoral student (1.5 PM), 1 PhD student (4 PM) 1 project manager (8 PM)</td>
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<tr>
<td>Investments (depreciation rate)</td>
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<td>463,05€</td>
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<td>Travel</td>
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<td>Project meeting Heraklion, Prof. Vasilis Ntziachristos</td>
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<td>Remaining direct costs</td>
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<td>496,89€</td>
<td>Catering costs Kick-off meeting</td>
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<td><strong>TOTAL DIRECT COSTS</strong></td>
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<td><strong>15,448,132€</strong></td>
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Personnel, subcontracting and other major direct cost items for beneficiary 2 (CEA) for the period 1

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<tr>
<td>2, 6</td>
<td>Personnel costs</td>
<td>72,712,39 €</td>
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<td>Subcontracting</td>
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<tr>
<td>Consumables</td>
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<td>Investments</td>
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33/36
### Personnel, subcontracting and other major direct cost items for beneficiary 3 (FORTH) for the period 1

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### Personnel, subcontracting and other major direct cost items for beneficiary 4 (UCL) for the period 1

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<td>Remaining direct costs</td>
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### Personnel, subcontracting and other major direct cost items for beneficiary 5 (FIHGM) for the period 1

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<td></td>
<td>Consumables</td>
<td>6,664,94€</td>
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<td>Investments</td>
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<tr>
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<td>Travel and subsistence</td>
<td>3,629,07€</td>
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<td></td>
<td>Publication</td>
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### Personnel, subcontracting and other major direct cost items for beneficiary 6 (UZH) for the period 1

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<th>Item description</th>
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<th>Explanations</th>
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<td>1, 6, 7</td>
<td>Personnel costs</td>
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<td>Salary of 1 PhD student for 12 months</td>
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<tr>
<td></td>
<td>Subcontracting</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Consumables</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Investments</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Travel</td>
<td>1,120,94 €</td>
<td>Consortium Meeting Munich (2 participants)</td>
</tr>
<tr>
<td></td>
<td>Remaining direct costs</td>
<td>0€</td>
<td></td>
</tr>
<tr>
<td>TOTAL DIRECT COSTS</td>
<td></td>
<td>30,216,46 €</td>
<td></td>
</tr>
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</table>
Personnel, subcontracting and other major direct cost items for beneficiary 7 (VAMP) for the period 1

<table>
<thead>
<tr>
<th>Work package</th>
<th>Item description</th>
<th>Amount</th>
<th>Explanations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Personnel costs</td>
<td>31.922,95 €</td>
<td>Salaries of 1 PhD application developer (Biologist) and 1 PhD scientist (Physicist) working part time on the project, as well as 1 mechanical engineer for construction in Q IV/08</td>
<td></td>
</tr>
<tr>
<td>Subcontracting</td>
<td>0€</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Consumables</td>
<td>0€</td>
<td>None yet, will be listed in next period</td>
<td></td>
</tr>
<tr>
<td>Investments</td>
<td>0€</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Travel</td>
<td>142,70 €</td>
<td>Kick-Off meeting 05/2008 Munich</td>
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</tr>
<tr>
<td>Remaining direct costs</td>
<td>0€</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

| TOTAL DIRECT COSTS | 32.062,65 € |

7 CERTIFICATES

No certificate on the financial statements is due for this period, because the expenditure threshold has not been reached by any of the beneficiaries.
TECHNICAL APPENDIX WP2: XCT DEVELOPMENT

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: 1ST
PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
This document is a technical appendix to the "Year one periodic report", that details scientific achievements of WP2 at month 12 of the project.

Two sub-tasks are concerned: XCT design, and Dual energy protocol, involving mainly two partners, CEA-LETI and VAMP. Other partners are involved in WP2, but in future sub-tasks (see below Task 2.6: Use of X-ray contrast enhancement methods).

INTRODUCTION

One activity report was due at month 12 of the project, aiming at summarizing progress towards objectives and deviances of planning. We thought that it is an opportunity to present to the Consortium what has been done at that date. This document does not correspond to a deliverable, and even not to a complete task achievement. It presents a current state of the work.

We remind hereafter the two sub-tasks concerned by the first year of the project:

TASK 2.1 “XCT DESIGN”

The goal is to issue the specifications regarding the X-ray module of the final FMT-XCT prototype that is to be integrated by VAMP within WP5. These specifications should be defined by the X-ray partners, integrating small animal imaging constraints. Compatibility with optical components should be addressed. The corresponding report constitutes the deliverable D2.1.

TASK 2.2 “XCT DUAL-ENERGY PROTOTYPE DEVELOPMENT”

This task consists in the development a XCT prototype and implementation of a dual-energy X-Ray acquisition protocol and corresponding data processing. Two set of angular projections should be acquired, one for each energy spectrum: low energy (LE) and high energy (HE), then combined in both a dual-energy decomposition and reconstruction scheme to be defined. Two deliverables will finalize these developments: D2.2 “Functional prototype for dual energy cone beam CT” and D2.3 “Calibrated, dual energy processing software”, both due for month T0+15.

TASK 2.1 “XCT DESIGN”

This paragraph is a summary of the report “WP2: XCT design, D2.1”.

The proposed system will integrate a micro-focus X-ray tube and a solid state digital X-ray image sensor for the image acquisition, implementing cone-beam geometry. A computer will operate the X-ray source, an X-ray stopping shutter and the frame grabber to acquire the projections.

The design of the micro X-ray CT system was started by evaluating the requirements of the different modalities and determining the geometric configuration of the scanner. From this point of view, the geometric configuration was based on a) the requirements of the optical imaging (as far as these had been already known and to provide enough flexibility to make subsequent alterations), and b) the limitations of the x-ray components (magnification, active area of the detectors, available x-ray power). A brief survey based on experience and the saturation thresholds of the detectors in question yielded a value for the necessary x-ray power of the tube.

Technical specifications of the prototype are summarized in the following figure and subsequent table.
The following table summarizes the X-ray components characteristics and acquisition parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance X-Ray source to X-Ray detector</td>
<td>400 mm</td>
</tr>
<tr>
<td>Distance X-Ray source to object</td>
<td>200 mm</td>
</tr>
<tr>
<td>Distance object to X-Ray detector</td>
<td>200 mm</td>
</tr>
<tr>
<td>Maximum diameter of the examined object</td>
<td>60 mm</td>
</tr>
<tr>
<td>Resolution of reconstructed object</td>
<td>0.08 to 0.1 mm</td>
</tr>
<tr>
<td>X-Ray source collimation</td>
<td>Cross collimation</td>
</tr>
<tr>
<td>Source filtering</td>
<td>Filter wheel with at least 3 Positions, one empty.</td>
</tr>
<tr>
<td>Detector pixel size</td>
<td>0.08 to 0.1 mm</td>
</tr>
<tr>
<td>Detector area</td>
<td>$\geq 120 \times 120 \text{mm}^2$</td>
</tr>
<tr>
<td>Frame rate</td>
<td>$\geq 5$ frames/s</td>
</tr>
<tr>
<td>Anti-scatter grid</td>
<td>Provisions integrated; Grid tbd</td>
</tr>
<tr>
<td>Shutter</td>
<td>Integrated, System tbd</td>
</tr>
<tr>
<td>X-ray tube focal spot</td>
<td>$\leq 0.08$ to $0.1$ mm</td>
</tr>
<tr>
<td>X-Ray source power</td>
<td>$&gt; 50$ W and $&lt; 100$ W</td>
</tr>
<tr>
<td>X-Ray source type</td>
<td>Forced air cooling</td>
</tr>
<tr>
<td>X-Ray source energy range</td>
<td>35/40kVp to 80/100kVp</td>
</tr>
<tr>
<td>Scan time</td>
<td>Min 4 minutes</td>
</tr>
<tr>
<td>Number of acquisitions angles</td>
<td>$\geq 1000$</td>
</tr>
<tr>
<td>Data volume per set</td>
<td>Approx. 3-4 GB</td>
</tr>
</tbody>
</table>

In particular, concerning the X-ray detectors and generators available on the market suiting the above requirements have been identified, the recommended ones being:
- **X-rays generator**: Oxford instruments UltraBright (80 Watts output power, 20-90kV, down to 13µm focal spot size), is the microfocus X-rays generator available on the market that gives the best compromise between the needs of high brightness (to avoid too long scan times), small focal size (to retain a high spatial resolution) and reduced mechanical dimensions (compatible with the instrument's design constraints).

- **Detector**: Hamamatsu C7943 (CsI scintillator + cMOS photodiode array, 100µm pixel size, 1248x1248 pixels, 7 frames per second). This detector is the proposed one since, while respecting the resolution constraint of less than 100µm per voxel in the reconstructed volume, is the one which gives the best performances in terms of noise, sensitivity, dynamic range and saturation charge.

The detector under investigation at CEA-LETI is of the same family of the proposed one (only with higher resolution), so the results of the base investigations with the laboratory bench shall be easily and reliably transferable to the gantry system.

Mechanical design was chosen according to the size and weight of the x-ray components including estimated values for the additional optical parts (dimensions, bearings, motors). Safety and beam manipulation devices have been incorporated into the specification (shutter, filter wheel, collimators, interlocks). The collimator will allow limiting irradiation to the needed field of view of the considered object. To deal with the X-Ray scattering that will be generated by the conical geometry and which will limit the contrast in the recorded radiographs, the positioning of a suitable anti-scatter grid in the system will be considered and fully evaluated.

In order to keep moderate the complexity of the prototype, the gantry will operate in non-continuous rotation mode. Acquisition time for each projection should be about 0.2s (1200 acquisitions in 4mn). For dual energy CT, this acquisition time has to be multiplied by two. Also the acquisition mode plays an important role; step-and-shoot requires the shutter to open and close, and the gantry to reach the next position between projections. The expected scan time will therefore be of minimum 4 minutes per single energy XCT scan.

As the maximum size of the objects will be of 60mm and the expected resolution of about 0.1mm, leading to a number of acquisition angles of about 1200 to 1500. This will lead to raw data volumes of about 3.5 GB per projections set (7 GB per dual energy set).

Based on these essential decisions, a complete requirements specification was produced (cf. Deliverable 2.1, XCT design, Milestone 2). This design specification also defines the system architecture, safety and normative requirements, performance data and electrical design of the system.

**Task 2.2 “XCT dual-energy prototype Development”**

**Laboratory bench at LETI**

The laboratory bench developed at LETI is quite different from the final prototype described in the previous paragraph. The aim of this bench is to be a very flexible system, with performance similar to the final prototype, intended for testing and optimizing mainly the dual energy protocol and the delivered dose.

The laboratory bench is arranged so that the tomographic axis is vertical and the X-rays generator and the detector are horizontally aligned with the imaging chamber. This last is placed on a rotating stage, allowing a complete examination of the animal along 360°, while detector and generator are kept motionless. The X-Ray generator is a monoblock X-Ray source, manufactured by Gilardoni (Italy) with a CW power of 500W. The kV ranges from 40 to 140 allowing Xray emission with an energy spectrum ranging from 10 to 140 keV. The flux of the radiation emitted by the tungsten anode is tuned by the electrode current, ranging from 1 to 7 mA. The Xray detector is a flat panel detector (model C7942CK by Hamamatsu, Japan) composed by a matrix of 2240x2344 pixels of 50µm for a total active area of 120x120mm². A picture of the system is reported below (Figure 2).

A software implementing the Feldkamp cone beam reconstruction algorithm is used to process the projection data and perform the volumetric reconstruction.
**Figure 2:** Photograph of the LETI laboratory bench.

**BRIEF REVIEW OF DUAL ENERGY TECHNIQUES**

Dual energy decomposition is a well established technique especially exploited for non-destructive control for example in the fields of luggage control for airport security, or inspection of composite materials parts, and, for medical applications, in bone densitometry. It consists essentially in acquiring two images (or set of projections, for tomography) of the same subject at two different energies of the impinging x-rays. The differences between the absorption spectra of the various materials of which the object is composed allow also the recovery of information about its composition, other than simply its global attenuation properties.

A typical x-ray imaging configuration consists of a source (of intensity $\Phi_0$) of X-rays which are attenuated while passing through the object, and which are finally collected (intensity $\Phi$) by a suitable detector. The radiation intensity measured by the detector (supposing the x-ray beam being monochromatic) can be expressed by the Beer-Lambert law:

$$\Phi = \Phi_0 \cdot \exp(-\int \mu(l)dl)$$  

Assuming a linear response of the detector, one can deduce that the ratio between the intensity measured without ($\Phi_0$) and with ($\Phi$) the object is a measure of the x-rays attenuation, or a x-ray projection of the object along the direction $l$. In practice, the logarithm of such a ratio is used and a projection (also called attenuation measurement) is defined as:

$$p = \int \mu(l)dl = -\ln\left(\frac{\Phi}{\Phi_0}\right)$$

Actually, $\mu(l)$ depends on the chemical properties of the material being crossed (i.e. material present at position $l$) and on the x-rays energy, in particular:

$$\mu = \mu(E, \rho, Z) = \rho \cdot \tau(E, Z),$$

where $\rho$ is the material density (volumic mass), $E$ the x-ray energy and $Z$ the atomic number of the material. $\tau$ is called total mass attenuation coefficient and depends only on the energy of the incident photons and on the chemical nature of the material. It is called total because it resumes the contribution of four fundamental types of interactions between x-rays photons and matter, namely the elastic scattering (or Rayleigh scattering), the inelastic scattering (or Compton scattering), the photoelectic effect and pair generation. For x-rays in the energy ranges of medical interest, the elastic scattering term and the pair generation term can be neglected, and retaining only the photoelectric and Compton parts, $\tau$ can be rewritten as:

$$\tau(E, Z) = \tau_p(E, Z) + \tau_c(E, Z)$$

Combining this expression with the previous definition of $\mu$:
\[ \mu = \rho \cdot \tau(E, Z) = \rho \cdot \left( \tau_p(E, Z) + \tau_c(E, Z) \right) \]  

Different authors (the first being Alvarez in 1976) propose to rewrite \( \mu \) according to a slightly different linear decomposition:

\[ \mu(E) = a_p f_p(E) + a_c f_c(E) \]  

which is still based on the distinction between fundamental interactions, but where the \( a \) coefficients take into account all (and only) the properties of the materials and the \( f(E) \) functions take into account the energy dependencies of each interaction type.

This way, the projection of an object composed of \( N \) slabs of different materials of length \( l_i \) and produced by a monochromatic x-rays beam at energy \( E_m \) can be written as:

\[ p_{E_m} = \int \mu(l) dl = f_p(E_m) \sum_i l_i a_p(\rho_i, Z_i) + f_c(E_m) \sum_i l_i a_c(\rho_i, Z_i) \]  

The terms \( \sum_i l_i a_p(\rho_i, Z_i) \) and \( \sum_i l_i a_c(\rho_i, Z_i) \) are called respectively Photoelectic projection (\( A_p \)) and Compton projection (\( A_c \)). In principle, carrying out two measurements of the same object at two different energies \( (E_1, E_2) \) and supposing to know the functions \( f_p(E) \) and \( f_c(E) \), the photoelectric and Compton projections of the object can be obtained solving a non-linear system of 2 equations with 2 unknowns. Practically, the photoelectric and Compton projections are never used, since they are quite exotic concepts and because the solution of the system with polychromatic x-rays beams (which is almost always the case) is difficult.

But, generalizing the principle of base functions decomposition of the measurements, any two independent functions can be chosen. In particular, using the absorption spectra of two given materials \( (\mu_1(E), \mu_2(E)) \) inside Eq.(g) in place of \( f_i(E) \), the terms \( A_1 = \sum_i l_i a_{m_1}(\rho_i, Z_i) \) and \( A_2 = \sum_i l_i a_{m_2}(\rho_i, Z_i) \) are called material-1 and material-2 equivalent thicknesses of the object along the direction \( l \). Two measurements at two different energies can therefore be written as:

\[ \begin{align*}
    m_{E_1} &= -\log \left( \int_{E_1}^{\Phi_0(E)} e^{-\mu_1(E)A_1(l)} e^{-\mu_2(E)A_2(l)} dE \right) \\
    m_{E_2} &= -\log \left( \int_{E_2}^{\Phi_0(E)} e^{-\mu_1(E)A_1(l)} e^{-\mu_2(E)A_2(l)} dE \right)
\end{align*} \]  

This system, resolved for the \( A_1 \) and \( A_2 \) unknowns, gives the equivalent thicknesses of the object in terms of the two basis materials: i.e. if in place of the actual object there was only an object composed of two slabs of material 1 and 2, to observe the same attenuations at both energies they should have had respectively thicknesses \( A_1 \) and \( A_2 \). The attenuation curves represent one of the possible basis of the space of attenuations and the equivalent lengths the projection of the actual measurements on such a space. To give an example, it is common practice in medicine to evaluate the bone density (to study osteoporosis in aged people, for example) to use as basis functions the attenuation curves of bones and Plexiglas (absorption curve very close to that of water). Two radiographs of the patient are recorded at two different energies and for each pixel the equivalent lengths of Plexiglas and bone are calculated. Two images are obtained: the Plexiglas projection of the patient, where the grey value represents the equivalent thickness of Plexiglas crossed by x-rays and where no information about bones will be contained because bone is the orthogonal base. Vice-versa for the bone image, where only information about bone thickness will be present. If appropriately calibrated (as it is the case in medical imaging) this procedure is quantitative and thus gives an actual measurement of bone density.

The system of equations (h) is nevertheless non-linear and therefore complicated to be solved, so what is normally done is to model the solution in a polynomial form:
and then evaluate the polynomial coefficients by means of a calibration procedure. This consists in measuring the attenuation values for many different thicknesses of the two basis materials and then least squares interpolating the values with the two preceding polynomials. These can then be used to project any measured point $P(m_{E_1}, m_{E_2})$ in the two materials space and this procedure makes the technique quantitative. Graphically the calibration and decomposition procedures are represented in the following figure:

Figure 3: Dual energy calibration polynomials and decomposition of a measured point

More complicated schemes can be used, such as using higher grade polynomials or using x-rays beams of three or more energies. The advantage of material basis decomposition is that the projection of the physical measurements into a space narrower than the natural one $(m_{E_1}, m_{E_2})$ enhances the contrast exploiting the complementary information carried by the dual energy measurement. The limit is that when the two basis materials become too close, because of the experimental noise and because of the fact that polychromatic x-rays beams are usually used whose spectral separation is not perfect, the calibration procedure and the material basis decomposition become numerically unstable.

As previously stated, the projection of measurements of human patients in the bone/water material basis is commonly practiced, since the two basis material have absorption curves sufficiently different to allow a good separation of the two types of tissues, but the contrast is not enhanced enough to allow the distinction of different types of soft tissues (muscle from liver from brain, for example) between them. The aim of the XCT part of the project is actually to improve the contrast enhancement technique in order to overcome this limitation.

### X-ray Characteristics of Biological Tissues

The X-ray absorption properties of biological tissues were analysed and compared, taking into account the actual size of a mouse (2-3cm diameter, 8-10cm length) and its internal organs in order to identify the best couple of materials for dual energy decomposition.

Since no specific data related to mouse tissues were available to us, we took the mass attenuation data from ICRU44/NIST tables of human tissues. The spectra are reported in the figure below in the energy range 10-60 keV.

As it is evident from figure 4, all the tissues have very close spectra which get even closer at higher energies and become indistinguishable above about 50keV. Only bone tissues (both compact and cortical) show a significant difference. In the soft tissues set, two subsets with slightly different attenuations can be identified: a first one, including muscle, lung and brain tissues, a little bit more absorbing and with spectra which superimpose almost perfectly with that of water, and a second one, less absorbing, including adipose tissue, with an absorption curve very close to that of many plastics like Lexiglas, polyethylene or polypropylene. The only exception is breast tissue, whose absorption curve lies somehow half the way between the two sets, even though for the mouse it might be of minor importance. These considerations point out that a fine distinction between internal organs is not trivial,
even if high contrast images can be in principle obtained exploiting the differences between soft and adipose tissues. For their similarity to tissues, Plexiglas and water have therefore been chosen as representative materials both to build a tissue simulating phantom and as basis materials for dual energy decomposition. The problem is that the attenuation curves of these materials are so close that experimental noise makes it very difficult to obtain a good calibration and decomposition.

![Mass attenuation (in cm²/g) of biological tissues in the range 10-60 keV.](image)

**Figure 4:** Mass attenuation (in cm²/g) of biological tissues in the range 10-60 keV.

**ANALYSIS OF THE POSSIBLE ACQUISITION MODES**

As stated in paragraph 2, the acquisition sequence will be of type step and shoot, i.e. acquisition of a geometric projection after each rotation step of the gantry. What needs to be decided is weather to complete all the geometric dataset acquisition at the first energy and then acquire the full geometric data set for the second energy (modality 1) or to acquire two radiographs at the two energies at each rotation step of the gantry (modality 2).

![Two possible modalities of dual-energy acquisition protocol.](image)

**Figure 5:** Two possible modalities of dual-energy acquisition protocol.

Modality 1 is technically and practically easier to implement, gives the possibility of changing both the filters configuration and the tube settings between the two acquisitions, and allows faster scans since it introduces no dead times for energy configuration switching. The main drawback is the eventual motion of the subject and/or geometric misalignment between corresponding projections of the two sets, which is quite a critical parameter for the contrast enhancement protocol.

For Modality 2, the problem of misalignments between corresponding HE and LE measurements is less serious, but it requires more complex system and protocol. It requires synchronization between image acquisition and energy switching and it is not feasible to change the X-rays generator voltage at each step, so only filters configuration can be switched. This implies that energetic separation will necessarily be worse than for modality 1.
Given that motion artefacts (respiratory motion, heart beat, etc…) are a big and unavoidable issue for both modalities the first one seems to present many advantages over the second and is therefore preferable.

**OPTIMISATION OF THE DUAL ENERGY SPECTRA**

The problem of the choice of the two best energy configurations for dual energy decomposition is indeed multi-parametric and practically it can be solved only by means of software simulation and then validated with experiments. The general result that comes out from such simulations is that to improve the contrast, the two energies should be as separated as possible one from another and both as low as possible (the lower the energy, the bigger the difference in the attenuation coefficient between soft and adipose tissue). This requires lowering the operating voltage of the X-ray generator and using thicker filters to shape the energy spectra, consequently reducing the photon flux and increasing the detection noise. Ideally, it would be desirable to have a source capable of emitting x-rays at about 10kV and 30kV of anode voltages with a cathode current of tens of mA, which is not technically feasible. The solution needs therefore to be a compromise between energy separation and signal intensity.

The parameter to be optimized is the contrast in a dual energy XCT image between adipose tissue and other soft tissues. One method to evaluate such parameter is to calculate the values of X-rays attenuation produced by different thicknesses of the two base materials (water and Plexiglas) considering in the calculation the noise which can affect the data. Simply reporting these values on a plot of the HE attenuation as a function of the LE attenuation (without going further into the dual energy decomposition), two sheaves of straight lines are obtained which pass through the origin of the axes and whose angular width is related to the measurement noise: a direct estimation of the contrast is difficult from this data, but in first approximation the greater the mean angle between the two sheaves and the smaller their angular width, the better the contrast.

From the simulation data obtained up to now the best contrast between water and Plexiglas is obtained for the following configurations (data on figure 6):

- **Low Energy:**
  - Generator voltage: 40kV
  - Generator current: 6mA
  - Low pass filter: 50μm Tin

- **High Energy:**
  - Generator voltage: 70kV
  - Generator current: 2mA
  - Low pass filter: 100μm Lead

It’s important to remark that the data shown have been calculated on the basis of the physical parameters of the CEA-LETI prototype. For the final prototype, the optimization needs to be performed using its own physical data (shape of energy spectra, tube maximum power, detector pixel size) and the resulting set of parameters needs not to be exactly the same.

**Figure 6:** Left: LE and HE spectra for the optimal configuration. Right: LE and HE attenuation measurements and associated uncertainties, for increasing thicknesses of water and Plexiglas (0-6cm).
DUAL ENERGY DATA PROCESSING

Once the radiographs are acquired for the two energies, by Modality 1 or 2 as explained in §4.3, two main approaches can be envisaged for data processing to get an enhanced contrast volume:

- Dual-energy processing in the data acquisition domain: each pair of radiographs (LE, HE) are decomposed onto the material basis (or (density $\rho$, atomic number $Z$) frame), then two reconstructions are performed on the decomposed data, followed by a final combination,

- Dual-energy processing of the volumetric data: reconstruction of the two acquisition data set, LE and HE, then decomposition of the reconstructed volumes on material or ($\rho$, $Z$) basis and combination.

When possible, the first way is considered preferable, especially if based on an experimental material calibration, because it allows to take into account and correct the beam hardening effect (due to the poly-chromaticity of the spectra).

When trying to implement this approach, we met two problems:

- firstly, each pair of acquisition data (LE,HE) should perfectly match geometrically, which is not the case especially if selected acquisition protocol is Modality 1 (one complete rotation for each energy).

- secondly, numerical instability due to the badly conditioned decomposition (too closed materials) leads to almost non-exploitable results.

The second approach seems to be preferable in our case, all the more that beam-hardening effect is negligible according to our experiments, but this point is still under investigation at the moment.

REMAINING SUB-TASKS

Task 2.2, “Dual-energy development”, is supposed to end at month T0+15. The remaining works in order to reach the objective are as follows:

- Validation, based on additional experiments using phantoms and animals, of the choice of two energy spectra (voltage and filters) and of the acquisition modality (two successive rotations). Validation for the CEA-LETI laboratory prototype.

- When information is available and measurements using the source and detector of the final prototype is possible, refinement of the optimal configuration of energy spectra for the final prototype.

- Validation of the dual-energy processing on the reconstructed volumes, development the optimal decomposition in terms of contrast enhancement, specification of the material calibration.

- Evaluation of the level of scatter in the image for CEA-LETI prototype (first measurements give a scatter level of about 15%), investigation of the use of an anti-scattering grid, estimation of the requirement of a scatter correction method.
**TASK 2.6: USE OF X-RAY CONTRAST ENHANCEMENT METHODS**

Juan J. Vaquero, FIHGM, Alejandro Sisniega, FIHGM, Manuel Desco, FIHGM

The work package description specifies that partner FIHGM will contribute to develop several X-ray XCT contrast enhancement methods to achieve better soft tissue contrast resolution. This contrast improvement will allow the optimization of the CT-based a priory information that will be used on the FMT reconstruction methods.

To carry out this task FIHGM has resources equivalent to 18 person-months distributed on 24 months, with more emphasis on the second year. The deliverable is due on month 24th. During the project first year FIHGM has work on three different actions:

**USE OF X-RAY CONTRAST AGENTS**

The use of iodinated contrast agents, such as Iopamiro® or Fenestra® are being studied. Several animals have been scanned with different doses of contrast agent administered IP and IV. Measurements on the contrast enhancement between the original two images are being quantified. Since the two agents being tested have different biological decays (one is a systemic agent while the second one is a vascular agent), FIHGM is performing experiments to evaluate the spatial and temporal distribution variations of the agents in the animal body trying to define different time points that could be optimal for enhanced soft tissue contrast.

**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). This 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. The aforementioned method involves the acquisition of two different datasets with different radiation exposure levels, but the same spectral configuration. Then, both scans are appropriately combined taking into account the detector and sample properties. The so-called dual-exposure data have a better Contrast to Noise Ratio (CNR) and low contrast resolution, making the analysis of the different tissues easier and unmasking structures masked by quantification noise. Initial results are being evaluation during this quarter.

**DUAL-ENERGY X-RAY SOURCE**

Task 2.2 of this same workpackage focuses on the development of dual-energy methods to decompose the X-ray tomographic images in soft and hard tissues. In order to be able to produce such a scan, the system X-ray tube must have enough flexibility in terms of level of energy that could achieve without compromising other characteristics. Partner CEA-LETI is working on those dual-energy methods. FIHGM is currently improving their Cone-Beam CT device in terms of the X-ray beam energy variation to provide the proper X-ray radiation to perform these data acquisition according to CEA-LETI results. The system integrates a new, custom-made micro-focus X-ray source with a tungsten anode that reaches a peak energy of 110 kV. The maximum delivered power is 50 W, with a maximum anode current depending on the peak energy setting. The X-ray beam focus size depends on the output power requested, varying from a minimum value of 15 μm to a maximum of 80 μm when the X-ray source is operated at full power setting. The integration of this new, bulkier tube has been a time consuming task since shields and gantry have had to be modified. A new trapezoidal collimator made of a lead-bismuth alloy adapted to the X-ray source output beam has been designed and built. The X-ray spectral characteristics are selected by means of the X-ray peak energy setting and the beam filtration. The filter applied to the outgoing radiation can be selected by the user, using a filter wheel holding up to six different foils made of different materials and thicknesses. The use of the filter wheel as well as the broad X-ray peak energy selection choices provide a large range of X-ray spectral conformation, allowing the system to perform multi-energy data acquisition. The new
shielding elements to stop the X-ray radiation are under development and the system will be in operation in the following months. At the moment, we are testing several algorithms to process the dual-energy data using simulated datasets of different spectral configurations and sample materials are used.

SUMMARY

Task 2.2 FIHGM activities have been developed according to the original project plan without any mayor delay. In the following months FIHGM will be able to start to implement dual-energy protocols for X-ray tomography according to CEA-LETI proposals. Reports on X-ray agent contrast and double exposure contrast enhancement techniques will be presented on the deliver on the deliverable due on month 24th.
TECHNICAL APPENDIX WP3: THEORY FOR 360-DEGREE FMT

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: 1ST
PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
Work Package 3: Theory for 360-degree FMT

TECHNICAL APPENDIX

March 15, 2009

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This document is a technical appendix to the “Year one activity report”, that details scientific achievements of WP3 at month 12 of the project.

OBJECTIVE 3.1: TO IMPLEMENT DIRECT INVERSION BASED ON BOUNDARY REMOVAL METHOD FOR MEDIA WITH ARBITRARY BOUNDARIES

In diffuse media, tomography is based on using measurements from the boundary to obtain 3D spatial maps of optical properties or fluorophore concentration. Image formation consists of computing solutions of a propagation model (typically the diffusion equation) to predict photon propagation in tissues (forward problem) and use these to form the inverse problem, calculating the most probable distribution in the medium which satisfies the boundary measurements.

Many different approaches have been developed for solving the forward and inverse problems. The computation times required typically scale with a power-law to the data-set size and calculation requirements worsen when imaging tissues of arbitrary shapes, as the effects of the boundary need to be explicitly modeled by the forward model.

We have studied an approach which can significantly simplify and accelerate calculations in the tomographic problem. The method transforms surface measurements to measurements which would be obtained in the absence of the surface, i.e. if the diffusive volume were infinite and homogeneous. This approach has two important consequences: first, it allows the use of infinite Green functions to generate forward solutions, thus avoiding the use of complex numerical methods which solve for arbitrary geometries. Second, it enables the use of direct inversion methods by allowing data back-propagation from virtual detectors outside the volume to anywhere inside the volume and vice versa. Such transformation is not possible in the presence of arbitrary interfaces and it can allow for significant inversion acceleration, the computational complexity scaling as Nlog(N) instead of typically N^3, being N the number of measurements. We here present a derivation of this numerical transformation and study the performance achieved both in ideal and limiting experimental situations, i.e. in cases where there is varying spatial sampling or when the surface can only be partially accessed. Finally we present examples of the potential of this transformation by propagating measurements to an arbitrary point in space and then using this data to image the distribution of sources. Even though all the derivation is in the context of light diffusion, the solutions are more generally applicable in any situation of a dominant diffusive regime.

In conclusion, we have derived a method which effectively removes the contribution of the boundaries, in essence converting finite diffusive volumes into infinite diffusive volumes of the same absorption and scattering properties. The advantages of such an approach are many, since it opens a new way of treating measurements in diffusive media. In particular we have shown examples of how the data can be propagated anywhere in space and then inverted to retrieve the source distribution and strength with great accuracy, stressing the fact that this will allow the application of direct inversion methods in the presence of arbitrary geometries. A numerical study of the effect that detector size or different spatial sampling has on the retrieved data has been presented, strongly suggesting that the implementation of this method with experimental data in relevant experimental configurations is feasible. A detailed description of the method and its performance in arbitrary geometries is given in Deliverable 3.1.
OBJECTIVE 3.2: TO RESEARCH OPTIMAL DIRECT INVERSION APPROACH WITH SIMULATIONS AND EXPERIMENTAL DATA

Optical tomography has recently witnessed an increase of several orders of magnitude in the amount of data used for the reconstruction. This is primarily due to the use of large detector arrays, on the order of 103 elements or higher. When coupled with the large number of sources enabled by the use of non-contact measurements, this generates large data sets easily in the range of 105 source-detector pairs. These large data sets reduce the ill-posed nature of the inversion, but also present an inherently large computational burden. Using traditional real-space weight matrix and Algebraic Reconstruction Techniques (ART) for the inversion yields impractically long computational times, in some instances longer than 24 hours. There is therefore a need for a different approach that can use large data sets and still maintain low computational times.

One ideal approach to significantly reduce the number of measurements while maintaining the same amount of information is to work in Fourier Space. It is well known that diffuse light in the continuous regime (CW) presents only low frequency contributions to the spatial frequencies. This means that by using all real-space data and selecting only a few low-frequency components in Fourier space, we would still have the same amount of information while retaining low numbers of measurements. Using Fourier space to solve the inverse problem has been pursued in the past, and three different methods can be identified:

a. Backprojection: This approach was originally developed for X-ray imaging and was presented by Matson et al in the context of diffusive waves to recover the position of absorbing objects by using the detector measurements in Fourier space. This approach is extremely fast, however it does not yield quantitative results, presents very low resolution images and what is worse, is incapable of imaging two objects located at different depths at the same time. The methodology developed for this, however, has been the cornerstone for the other more accurate approaches explained below.

b. Complete Fourier Approach (Direct Inversion): Such an approach has been pursued by Schotland et al in their inversion approach, and termed Direct Inversion in the sense that an expression that related directly the reconstructed image and the data presented. In Schotland's et al approach, which we shall term from now on the Complete Fourier approach, both source and detectors are Fourier Transformed. This approach enables the use of very large source positions (103 or higher) while retaining low computation times and is therefore very useful when very large source position measurements, together with very large detectors are used. There is, however a disadvantage to this approach, in the sense that it fails when low number of sources are available. In cases in which the number of sources is in the order of 102, the Fourier transform of the sources is very poor and in consequence so is the obtained reconstruction. Another problem to this approach is that the reconstruction is performed in Fourier space, resulting in typical Fourier artifacts in real space when the data is not appropriate (by appropriate, we mean that Fourier data does not present aliasing due to incomplete measurements). This need has been partially accounted for by using matching fluid such as intralipid in order to conform arbitrary volumes to a slab.

The direct inversion method has been thoroughly developed in theory, reaching the direct inversion formulas to be used. These formulas will prove extremely fast when imaging using $2^N$ sources (suitable for Fourier transform) however we have found several important and severe drawbacks of this method:

Let's say we have a collection of fluorophores that are between $z = 0$ and $z = L$.

Let's also assume that we have a collection of detectors at $r_s \equiv (R_s, z = 0)$ and $r_d \equiv (R_d, z = L)$. In this case, the fluorescence measured due to the sources at $r_s$ and $r_d$ is:

$$U^{f}(r, r) = \int_{V} U^{inc}(r, r) G(r, r) F(r) d^3r$$

where $G$ is the Green function of the complete problem, i.e. including boundaries and the effect of the fluorophore. For simplicity, let's derive the inversion formulas for an infinite homogeneous diffusive
medium of optical properties $\mu_a$ for absorption and $\mu_s'$ for reduced scattering, and consider the Born approximation for $F$. In this case, Green's function is represented as:

$$g(r, r') = \frac{\exp(ik_0 |r - r'|)}{|r - r'|}$$  \hspace{1cm} (2)

where,

$$k_0 = \sqrt{-\frac{\mu_a + i\omega/c}{D}}$$  \hspace{1cm} (3)

is the diffuse wavenumber being $D$ the diffusion coefficient, defined for simplicity as $D = 1/3\mu_s'$, and $\omega$ the modulation frequency. From now on we shall consider we are working in the CW regime and thus $\omega = 0$, i.e:

$$k_0 = \sqrt{-\frac{\mu_a}{D}}$$  \hspace{1cm} (4)

The expression for light excitation at a point $r$ in space can be expressed in terms of the Green function as:

$$U_{\text{inc}}(r, r') = S_0 g(r, r')$$  \hspace{1cm} (5)

where $S_0$ takes into account the source strength. Introducing Eq. (5) into Eq. (1) we obtain the expression for the Born Approximation in real space:

$$U^B(r_s, r_d) = S_0 \int V F(r) g(r, r') d^3r$$  \hspace{1cm} (6)

Fourier transforming Eq. (6) for $R_s$ and then $R_d$, we obtain:

$$\tilde{U}^B(r_s, r_d) = S_0 \int V \tilde{W}(K_s, K_d; z_s, z_d; r) F(r) d^3r$$  \hspace{1cm} (7)

where,

$$\tilde{W}(K_s, K_d; z_s, z_d; r) = \tilde{g}(K_s, z_s; R, z) \tilde{g}(K_d, z_d; R, z)$$  \hspace{1cm} (8)

In Eq. (8) we have represented the Fourier transform of the Green function as:
\[
\tilde{g}(K_s, z_s; R, z) = \frac{2\pi i}{q_s(K_s)} \exp[iq_s(K_s)(z - z_s)] \exp(iLK_s \cdot R)
\]  

(9)

being \(q_s\) the projection of the z-component of the wave-vector for spatial frequency component \(K_s\), represented as:

\[
q_s(K_s) = \sqrt{k_0^2 - K_s^2}
\]  

(10)

Which results from:

\[
k_0^2 = q_i^2(K_s) + K_s^2
\]  

(11)

Note that in the CW case, \(q_s\) and \(q_d\) are perfect imaginary numbers, since introducing the \(\omega = 0\) expression for \(k_0\) gives:

\[
q_s(K_s) = \sqrt{\frac{\mu_s}{D} + K_s^2}, \quad K_s \in [-\infty, +\infty]
\]  

(12)

For the time being we will consider \(q_s\) imaginary, for generality. Introducing Eq. (9) into Eq. (8) and taking into account that \(g(r_s, r)\) is the reciprocal of \(g(r, r_s)\), we obtain:

\[
\tilde{\tilde{W}}(K_s, K_d; z_s, z_d; R, z) =
\frac{(2\pi i)^2}{q_s q_d} \exp[i(q_s(z - z_s) + q_d(z_d - z))] \exp[i(K_s - K_d)R]
\]  

(13)

Let’s assume from now on that \(z_s = 0\) and \(z_d = L\). Introducing Eq. (13) into Eq. (7) we obtain:

\[
\tilde{\tilde{U}}^0(K_s, K_d) =
S_0 \frac{(2\pi i)^2}{q_s q_d} x \int_z \exp[i((q_s - q_d)z + q_d L)] \int_R F(R, z) \exp[i(K_s - K_d)R] dR
\]  

(14)

Introducing the change of variable:

where we can identify in Eq. (14) the Fourier transform of \(F\) in \(K_s - K_d\):

\[
\tilde{F}^0(K_s - K_d, z) = \int_R F(R, z) \exp[i(K_s - K_d)R] dR
\]  

(15)
Using this expression, we can rewrite Eq. (14) as:

\[
\tilde{U}^{fl}(K_s, K_d) = S_0 \frac{(2\pi i)^2}{q_s q_d} \int_z dz \exp(i[(q_s - q_d)z + q_d L])\tilde{F}(K_s - K_d, z) \quad (16)
\]

Going back to Eq. (5), we may rewrite it at the detector plane \(z = L\) in Fourier space as:

\[
U_L^{(inc)}(K_s, K_d) = S_0 \frac{2\pi i}{q_s} \exp(iq_s L) \quad (17)
\]

Normalizing Eq. (14) by \(U_L^{(inc)}\) we obtain the Normalized Expression in Fourier space:

\[
\tilde{U}^{nb}(K_s, K_d) = \frac{\tilde{U}^{fl}(K_s, K_d)}{U_L^{(inc)}(K_s, K_d)} = \frac{2\pi i}{q_d} \int_z dz \exp(i[(q_s - q_d)(z - L)])\tilde{F}(K_s - K_d, z) \quad (18)
\]

which can be rewritten as:

\[
\tilde{U}^{nb}(K_s, K_d) = \frac{2\pi i}{q_d} \exp[-i(q_s - q_d)L] \int_z dz \exp[i(q_s - q_d)z]\tilde{F}(K_s - K_d, z) \quad (19)
\]

where, taking into account that \(q_s\) and \(q_d\) are perfect imaginary numbers, and using the change of notation \(\eta = -(q_s - q_d)\), \(\xi = -(q_s + q_d)/2\) we can rewrite the previous equation as:

\[
\tilde{U}^{nb}(K_s, K_d) = \frac{2\pi}{\eta - \xi / 2} \exp(\eta L)\tilde{F}(K_s - K_d, \eta) \quad (20)
\]

where, making use of the fact that data (fluorophore) is in \(z \in [0, \infty]\) we have written the Laplace transform on \(\eta\) as:

\[
\tilde{F}(K_s - K_d, \eta) = \int_{-\infty}^{\infty} \exp(-\eta z)\tilde{F}(K_s - K_d, z) dz \quad (21)
\]

Since each \([K_s, K_d]\) pair defines a \(\Delta K = K_s - K_d\) value and a \(\eta = -(q_s - q_d)\) value, we may rewrite our data \(U_{nb}\) in terms of \(\Delta K\) and \(\eta\) and therefore:
\[ \tilde{F}(\Delta K, \eta) = \frac{\eta - \xi}{2} \frac{\exp(-\eta L)}{2\pi} \tilde{U}^{\text{nb}}(\Delta K, \eta) \]  

Eq. (22) can be seen as a Direct Inversion formula for F.

### 3. Practical Implementation of Inversion Formula

Once the formulas have been developed, the main formula that can be used for numerical inversion is Eq. (19):

\[ \tilde{U}^{\text{nb}}(K_s, K_d) = \frac{2\pi}{\sqrt{\mu_0 / D + K_d^2}} \int_0^L dz \exp \left[ \left( \sqrt{\mu_s / D + K_s^2} - \sqrt{\mu_0 / D + K_d^2} \right)(L - z) \right] F(K_s - K_d, z) \]  

Discretizing in z in order to solve for F we obtain:

\[ \tilde{U}^{\text{nb}}(K_s, K_d) = 2\pi \sum_{i=0}^{N_z} W(K_s, K_d, z_i) \tilde{F}(K_s - K_d, z) \Delta z \]  

where,

\[ W(K_s, K_d, z_i) = \frac{\exp \left[ \left( \sqrt{\mu_s / D + K_s^2} - \sqrt{\mu_0 / D + K_d^2} \right)(L - z_i) \right]}{\sqrt{\mu_0 / D + K_d^2}} \]  

Which written in Matrix form can be represented as:

\[ [\tilde{U}^{\text{nb}}]_{1 \times N_k} = [W]_{N_k \times N_z} \times [\tilde{F}(K_s - K_d, z)]_{1 \times M} \]  

where, \( M = N_s \times N_d \times N_z \) and \( N_k = N_s \times N_d \). It is very important to note here that the reconstruction is performed in the Fourier Domain, i.e. the resolution if F will depend on the K selected for both sources and detectors.

### OBJECTIVE 3.3: TO COMPARE THE DIRECT INVERSION PERFORMANCE WITH CONVENTIONAL, PREVIOUSLY DEVELOPED FMT INVERSION METHODS

As stated also above in Objective 3.2 there is a disadvantage to the direct inversion based on Fourier space, in the sense that it fails when low number of sources are available. In cases in which the number of sources is in the order of 102, the Fourier transform of the sources is very poor and in consequence so is the obtained reconstruction. Another problem to this approach is that the
reconstruction is performed in Fourier space, resulting in typical Fourier artifacts in real space when the data is not appropriate (by appropriate, we mean that Fourier data does not present aliasing due to incomplete measurements). This need has been partially accounted for by using matching fluid such as intralipid in order to conform arbitrary volumes to a slab.

These formulas will prove extremely fast when imaging using \(2^N\) sources (suitable for Fourier transform), however we have found several important and severe drawbacks of this method:

1) It can only be used with large numbers of sources. Only source grids in the order of 64x64 sources at least can be used to obtain relatively good data and are still prone to great Fourier artifacts. This is a great problem since all our FMT setups work with numbers of sources in the 100 range, and having more sources in unpractical, since the experiment times will increase unnecessarily.

2) The Direct inversion method gives the result of the fluorophore concentration in Fourier space. This means that once inverted the data it has to be Inverse-Fourier transformed in 3D. This yields significant Fourier artifacts (seen as 'waves' surrounding the main central points of data) that worsen as the number of sources or detectors gets smaller. This means that in practice even larger numbers of sources need to be used. Additionally, it is not possible to include physical parameters in the reconstruction as we could using ART in real space: we cannot give different sensitivity functions to different areas, we cannot weight the data depending on its noise etc.

Conclusion: After developing and implementing the main features of the direct inversion method, we have decided it is not the method to pursue for our FMT setups. Clearly we need a method that can solve fast the inversion needed but can work with small numbers of sources, in the order of <100 sources. We therefore opted to change this deliverable, DIRECT INVERSION method to a deliverable called 'ULTRA FAST INVERSION for FMT' that will be delivered on the next reporting period. On the meantime, the partners have access to an inversion method which is significantly faster that the currently existing ones, based on removing the boundary and then performing ART on the now infinite homogeneous data. This way, the flow of the project is not jeopardized until we obtain the ultra fast reconstruction method in 2010.

**OBJECTIVE 3.4: TO INCORPORATE ALGORITHMS FOR MULTI-SPECTRAL IMAGING**

**OxyFMT system**

The setup for fluorescence and absorption acquisitions is shown in Figure 1. The OxyFMT imager employs a series of laser sources used according to the measurement. For fluorescence excitation a diode pumped solid state laser emitting at 592nm (Soliton-GmbH, Germany) and an Argon ion laser (LaserPhysics, Reliant 1000m, West Jordan, USA) were used. For oxymetry measurements a solid state laser emitting at 635nm (Radius635, Coherent, Inc, USA) and a diode laser emitting at 786nm laser (PLP-10 high repetition picosecond light pulser operated in cw mode, Hamamatsu Corporation, Japan) were used. Laser light was directed inside the imaging chamber by means of flip mirrors. The imaging chamber contained an optical scanner (Scanlab, Scanhead, Germany) for accurate and customized raster scanning of subjects and a 16bit, 1024x1024 pixels CCD camera (DV 434, ANDOR Belfast, Northern Ireland) thermoelectrically cooled down to -70°C. Light collection was performed through a 50mm Macro f/2.8 objective (SIGMA Corporation, Tokyo, Japan) and different interference bandpass filters (Andover Corporation, USA) for isolating the fluorescence signal, depending on the targeted fluorophore, whilst no emission filters were used during absorption acquisition. The number and position of sources was identical in both FMT and OxyFMT acquisitions although this is not a requirement of the system.
Tomography Calculations

Fluorescence reconstruction

The tomographic measurements consisted of detecting the fluorescence and the excitation intensities by using bandpass filters that correspond to the fluorescence light emitted and the laser light used for excitation, respectively. The reconstruction algorithm utilized a normalized Born approximation that combines the two acquired intensities as shown in Eq. 1:

\[ U^{nB} = \frac{U_{fl}}{U_{exc}} = W_{fl} \cdot \text{fluo}(r) \quad (1) \]

Where \( U^{nB} \) is the normalized Born Approximation, \( U_{fl} \) and \( U_{exc} \) are the background corrected fluorescence and excitation measurements, respectively.

The recorded Born data were then inverted with an Algebraic Reconstruction Technique (ART) with positive restriction for the reconstruction of the 3D fluorescence concentration. Where \( W_{fl} \) is the weight matrix representing the probability of each photon visiting each voxel element of the discretized volume and \( \text{fluo}(r) \) is the unknown fluorescence concentration map. All calculations were performed using the same source – detector pattern as well as the same mesh points for the calculation of \( W_{fl} \).

Absorption reconstruction

In a similar way as above, we can reconstruct for tissue absorption. In this case the two measurements used for the normalization are \( U_{exc} \) which is the light propagated through the mouse and \( U_{inc} \) which is the light propagated through a homogeneous slab phantom consisting of an Intralipid and India ink solution with the same average optical properties of a mouse, (see insets of Fig. 1). This normalization is similar to the \( I/I_0 \) term in Beer’s law describing light attenuation and will be used as described in the next section to calculate the absorption coefficient of a specific absorber (in our case Hb and HbO). Note that the source pattern is identical to the one used for the fluorescence measurements.

\[ U^{abs} = \frac{U_{exc}}{U_{inc}} = W_{abs} \cdot \text{abs}(r) \quad (2) \]
Where $W_{abs}$ is the weight matrix representing the probability of each photon visiting each voxel element of the discretized volume and $abs(r)$ is the unknown absorption map. Again all calculations were performed using the same source – detector pattern as well as the same mesh points for the calculation of $W_{abs}$. The absorption map $abs(r)$ was calculated by inverting Eq. 2 using the same ART algorithm as above.

OxyFMT

Three dimensional oxymetry measurements require the independent calculation of Hb and HbO volumetric concentrations. This can be achieved by reconstructing the absorption map $abs(r)$ (see Supplementary Methods) for two wavelengths in the NIR region, where Hb and HbO can be considered the main absorbers of tissue. Having calculated the corresponding absorption map $abs(r)$ and using Beer’s law, the absorption coefficient of each absorber can be obtained as shown in Eq. 3:

$$\mu_a = -\log[abs] / dz$$

(3)

Where $\mu_a$ is the volumetric absorption coefficient at the specific wavelength, in this case the two laser sources emitting at 635nm and 786nm, and $dz$ is the thickness of the sample. In addition, the absorption coefficient can be expressed in terms of Hb and HbO concentrations as shown in Eq. 4:

$$\mu_a = \epsilon_{Hb} \cdot C_{Hb} + \epsilon_{HbO} \cdot C_{HbO}$$

(4)

where $\epsilon_{Hb}$ and $\epsilon_{HbO}$ are the extinction coefficients and $C_{Hb}$ and $C_{HbO}$ the concentrations of Hb and HbO respectively. Thus, calculating the unknown concentrations requires the solution of a simple 2x2 matrix:

$$\begin{bmatrix} \mu_{a,785} \\ \mu_{a,635} \end{bmatrix} = \begin{bmatrix} \epsilon_{Hb(785)} & \epsilon_{Hb(635)} \\ \epsilon_{HbO(785)} & \epsilon_{HbO(635)} \end{bmatrix} \begin{bmatrix} C_{Hb} \\ C_{HbO} \end{bmatrix}$$

(5)

Solving the system by inverting the square matrix results to the calculation of oxy- and deoxy-hemoglobin concentrations. Then calculating Total Blood Volume (BV) and Oxygen Saturation (OxySat) is performed using the following Equations 6 and 7.

$$BV = \left[ \frac{C_{Hb}}{1} \right] + \left[ \frac{C_{HbO}}{1} \right]$$

(6)

$$OxySat = \left( \frac{C_{HbO}}{C_{Hb}} \right) \times 100$$

(7)

Since all calculations have been performed in volumetric data BV and OxySat represent 3dimensional maps of total blood volume and oxygenation that can be visualized and co-registered with the FMT fluorescence maps.

Spectral Unmixing

The decomposition of the signals of different fluorochromes is based on a linear unmixing algorithm, which is applied to the reconstructed images obtained at each spectral region. The algorithm takes into consideration the relative strengths of the fluorophores at the spectral bands that the detection was made. These spectral strengths were calculated after measuring the fluorescence emission of the different fluorophores with a commercial fluorimeter (Varian, Cary Eclipse) and integrating the signal under the part of the curve that corresponds to the spectral band allowed by each filter. The uncoupled
images can be then calculated by solving the following linear nxm system, basically calculating the
unmixed values for every voxel in the mesh, representing the volume of interest:

\[
\begin{bmatrix}
\mathbf{I}_1 \\
\mathbf{I}_2 \\
\vdots \\
\mathbf{I}_n
\end{bmatrix}
= \begin{bmatrix}
\mathbf{S}_{G_1} & \mathbf{S}_{R_1} & \cdots & \mathbf{S}_{M_1} \\
\mathbf{S}_{G_2} & \mathbf{S}_{R_2} & \cdots & \mathbf{S}_{M_2} \\
\vdots & \vdots & \ddots & \vdots \\
\mathbf{S}_{G_n} & \mathbf{S}_{R_n} & \cdots & \mathbf{S}_{M_n}
\end{bmatrix}
\begin{bmatrix}
\mathbf{C}_G \\
\mathbf{C}_R \\
\vdots \\
\mathbf{C}_M
\end{bmatrix}
\]

(8)

were \(\mathbf{I}_1, \mathbf{I}_2, \ldots, \mathbf{I}_n\) are the reconstructed three dimensional images obtained from FMT, \(\mathbf{S}_{G_1}, \mathbf{S}_{G_2}, \ldots, \mathbf{S}_{G_n}\), \(\mathbf{S}_{R_1}, \mathbf{S}_{R_2}, \ldots, \mathbf{S}_{R_n}\) and \(\mathbf{S}_{M_1}, \mathbf{S}_{M_2}, \ldots, \mathbf{S}_{M_n}\) are the strengths of the fluorochromes in the spectral
regions measured as described above and \(\mathbf{C}_G, \mathbf{C}_R, \ldots, \mathbf{C}_M\) are the unknown unmixed images. For the
case of two fluorescent probes the system is as follows:

\[
\begin{bmatrix}
\mathbf{I}_1 \\
\mathbf{I}_2
\end{bmatrix}
= \begin{bmatrix}
\mathbf{S}_{G_1} & \mathbf{S}_{R_1} \\
\mathbf{S}_{G_2} & \mathbf{S}_{R_2}
\end{bmatrix}
\begin{bmatrix}
\mathbf{C}_G \\
\mathbf{C}_R
\end{bmatrix}
\]

(9)

were \(\mathbf{I}_1, \mathbf{I}_2\) are the reconstructed three dimensional images obtained from FMT, \(\mathbf{S}_{G_1}, \mathbf{S}_{G_2}, \mathbf{S}_{R_1}, \mathbf{S}_{R_2}\) and \(\mathbf{S}_{M_1}, \mathbf{S}_{M_2}\) are the strengths of the fluorochromes in the spectral regions measured as
described above and \(\mathbf{C}_G, \mathbf{C}_R\) are the unknown unmixed images.

The spectral unmixing algorithm has been tested for the detection of two overlapping fluorophores in
both phantom and in vivo experiments as well as experiments aiming to obtaining absorption maps. It
can be successfully applied in distinguishing between different fluorophores and/or absorbers and
accurately obtaining the concentrations of the targets as shown in Figure 2 for CFSE and ATTO590 at
varying concentrations. The completed algorithm will be fully incorporated in the reconstruction
process in the next reporting period along with all the tests and assessments for both fluorescence
and absorption reconstructions obtained from phantoms and in vivo experiments.
Figure 2: a) and b) Unmixed reconstructions of CFSE and ATTO590. c) and d) Unmixed reconstructions. e) and f) Reconstructions from unmixed raw data. The inset shows the obtained concentrations demonstrating the accuracy when unmixing is performed on the reconstructed data.

**OBJECTIVE 3.5: TO DEVELOP USER FRIENDLY SOFTWARE FOR INVERSION OF XCT-FMT DATA BASED ON DIRECT INVERSION APPROACHES**

**Automated software for FMT acquisition and analysis**

The main goal of the FMT software is to provide a user-friendly interface to take the measurement data and later perform the 3D reconstruction providing an image that can be analyzed with Open Source applications. There is a complete manual available to the partners with detailed information and guidance for all the functions and parameters. Here we present the only main features which are:

1. **Installation of the FMT software**
2. **Running an experiment for FMT acquisition.** Typical experimental measurements are:
   a. Excitation Image, one per source
   b. Emission Image, one per source
   c. Background for Excitation
   d. Background for Emission
   e. Whitelight (Reference) image
   Additionally, several other images can be taken and the steps above repeated for several combinations of lasers/filters. Other images that can be taken are:
   f. Fast Scan Excitation and Emission Images: These images consist on exposing the camera while the laser scans throughout the mouse. This is equivalent to epi-fluorescence imaging, with the additional advantage that by scanning the laser at a very slow speed, very large powers can be simulated (very high W/m2 as compared to a commercial Epi-fluorescence setup).
   g. A geometry Image: This has not yet been implemented, but will be implemented soon.

The software has been mainly developed in Labview and Matlab. Here are the specifics of each part:

1) **FMT Acquisition Software:**
   a. User Interface developed in Labview 7.1
   b. Long loops developed in C
   c. Tiff-reader Writer developed in C++
2) **FMT Analysis Software:**
   a. Main program developed in Matlab R14

Loops and core of program developed in C

**1) mini-FMT basics**

There are 3 main parts to the process:

A) **Calling mini_run.exe**: This can be done directly or through FMT_INVERT, as explained in Sec. 4.2. This will read the Reconstruction Defaults and you will either input the files to use directly or through a queue file. The main files it needs are:
   - Fluorescence *.tiff File
   - Excitation *.tiff File
   - Reference *.tiff File
   - Mask *.tiff File
B) Processing and Inverting the data: This will be a silent process while using FMT_INVERT or you will see what the code is doing through your command window. This is the long part and depending on how much data could take between a few minutes to a few hours.

C) Output of the reconstructed data: Once finalized the reconstruction, the following files will appear in the folder where you performed the reconstruction:

- **rec_info[#]_r.txt**: This file contains all the info of what the program did, and will display the errors, if any. *Please send this file whenever reporting an error!* They should be appearing in numerical order, unless you delete some of them (I would recommend deleting all, in this case)

- **mfmt_recon[#]_r.mat**: This file is a matlab generated file and can be read from any matlab which is version 7.0.1 (R14) or older. If you use a previous version of matlab, don’t you think it’s time to upgrade? In this mat file you have all the data that was used for the reconstruction. *Please send this file whenever reporting a problem!* Please note that if an error occurs there will be no *mat file, only the rec_info.txt file. The mat file is useful if you intend to use the data for more advanced imaging approaches, to do some multispectral deconvolution, etc. These approaches are not yet implemented in the current version (see Future Changes).

- **mfmt_recon[#]_r.nii**: This file has the reconstructed data saved as in NIFTI format, [http://nifti.nimh.nih.gov/](http://nifti.nimh.nih.gov/). It is based on the Analyze 7.5 format, but instead of having a *hdr and a *img files, it has one single *nii file. This has the reconstructed data, which has been interpolated for representation purposes and centered with respect to the whitelight and other reference images. This reconstruction may also be groomed and trimmed to account different artefacts due to the sources.

- **mfmt_recon[#]_r_orig.nii**: This file is like the one above, where no interpolation or centering has occurred. That is, it is the raw data directly as was output from the matlab reconstruction.

- **mfmt_recon[#]_r_mean_std.nii**: This file has 8 images, saved in double precision, that are very useful to analyze the raw data, and give additional information to the reconstruction. These files are:
  1. The White Light Image
  2. The Mean of the Excitation over all sources: mean(Uo)
  3. The Mean of the Fluorescence over all sources: mean(Ufl)
  4. The Variance of the Excitation over all sources: var(Uo)
  5. The Variance of the Fluorescence over all Sources: var(Ufl)
  6. The Covariance of the Excitation and the Fluorescence: cov(Uo,Ufl)
  7. The Mean of Fluorescence over Mean of Excitation: mean(Ufl)/mean(Uo), masked by the mask.
  8. The variance of the Fluorescence over the variance of the Excitation: var(Ufl)/var(Uo), masked by the mask.
  9. NOTE: These 8 files may be accessed separately by amide, MRICro and ImageJ.

- **mfmt_recon[#]_r_ref.nii**: This file contains the reference (whitelight) image stacked so that it has the same dimension than mfmt_recon[#]_r.nii. It is most useful in MRICro for rendering.

D) Visualization of Reconstructed data: Once the reconstruction has finalized, you will have to open your favorite program to view the data. You will notice some windows open if you have set ‘display=1’ in the queue or have run mini_run.exe directly. These will be explained below.

How these files are generated and how you can view them will be explained in detail in the following sections.

2) Installing mini-FMT

All the inversion routines are coded into mini_run.exe and the folders you must have by now installed at the directory C:\FMT\.

In order to run properly, mini_run.exe needs the following files:

- **FMT_Recon_Defaults.txt**: This file has all the necessary information for the reconstruction. You should only edit it if you know what you are doing. In principle, each user will have one depending on the application. Main differences are when the subject is
very close to the camera, for example, were the distances are in mm instead of cm. In that case the voxel size and detector sizes must be changed accordingly. An example has been placed in Appendix I.

- **ExposureTimeCalibration**: This file has the exposure time calibration. If it is not present, mini_run.exe will assume you don’t calibrate the exposure time (i.e. $T_{cal} = 1$). This file should be specific for each FMT setup.

- **FMT_Recon_Queue**: This file is not needed by mini_run.exe, but is placed here as an example. You will recover it from FMT_INVERT if you press ‘see example’. Here you see how to run several reconstructions one after the other. An example has been placed in Appendix II.

### 3) Installing external viewers

Once mini-FMT has been installed and you have been able to run a reconstruction you will need a program to view the results. Most of these have been included in the FMT_Installers\Viewers\ folder. There are several options:

1) **Matlab**: if you are familiar with matlab, you can load directly the mfmt_recon.mat file on matlab and use the old functions (fmt_render_proj(mFMT), fmt_image_2D_xy(mFMT), etc) to view the data. This is the advanced level. More on this later, but make sure you have installed matlab R14 or later.

2) **Amide**: Amide is nice opensource program which can be downloaded from: [http://amide.sourceforge.net/](http://amide.sourceforge.net/). You will find there all the information needed. It is good for volume rendering and has some useful tools such as gaussian fits of objects, but has very few updates and has some things lacking.

3) **MRicro**: Mrica is a good program for ROIs and statistics and is good for rendering only the reconstruction (for overlays, AMIDE is better). You can find it at: [http://www.sph.sc.edu/comd/rorden/mricro.html](http://www.sph.sc.edu/comd/rorden/mricro.html)

4) **ImageJ**: ImageJ offers great possibilities since many plugins exist for it. You can find imageJ here: [http://rsb.info.nih.gov/ij/](http://rsb.info.nih.gov/ij/). You will find there also the plugins and how to install them. The only thing you need to do is download them and place them in the plugins folder of imageJ (Program Files\ImageJ\plugins). In order to read the nii files, please use the File->Import->NIfTI Analyze option. The ones you need are:
   a. **Open and save Analyze format images**
   b. **NIfTI Input/Output**


For those using Mac

6) **OSIRIX**: There is a fantastic software named OSIRIX, which you can download at: [http://www.osirix-viewer.com/](http://www.osirix-viewer.com/). The only drawback is that (from what I understand) it does not offer compatibility with nii files). One possibility is to open the nii files with ImageJ and exporting them in Analyze format, which should be OSIRIX compatible.

7) Another possibility is ITK-Snap (see above), which also exists for MAC.
**OBJECTIVE 3.6: TO INVERT TRAINING DATA ACQUIRED FROM FMT-XCT SYSTEM FOR ALGORITHMIC FINALIZATION**

During the first reporting period and until the new inversion algorithms have been finalized we have used FMT experiments to try to compare different inversion methods. Further studies with the use of FMT-XCT data are expected during the second year of the project and will be presented in the next report together with the new deliverable “Ultra fast Inversion for FMT”. Never the less, a very large number of experimental measurements have been acquired at FORTH that can be used for optimization and finalization of algorithms. These measurements involve phantoms as well as in vivo experiments. In a study performed together with Partner 4UCL we have compared reconstructions obtained from the FMT inversion algorithm (running ART inversions) with the reconstructions obtained from the finite element based non linear inversion under the TOAST software developed by Partner 4UCL. The study presented here involves a phantom experiment where a capillary tube with CFSE at a depth of 6mm was imaged. In both cases identical conditions and parameters were used for the reconstructions. The results are presented in figure 2 for the FMT and TOAST reconstructions while a 3d contour at 2/3 of the maximum value has been applied for better visualization. Further similar studies involving more complex phantoms and in vivo experiments are currently investigated together with Partner 4UCL and will be presented in the next reporting period.

![Figure 2](image)

Figure 2: Reconstructions of a tube with CFSE placed in a slab phantom at 6mm depth obtained with the FMT and TOAST software of Partners 3FORTH and 4UCL respectively.
TECHNICAL APPENDIX WP4: FMT INVERSION WITH IMAGE PRIORS

GRANT AGREEMENT NUMBER: 201792

PROJECT ACRONYM: FMT-XCT

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

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FMT-XCT Year One Progress Report for
Work Package 4
Partner 4 : UCL

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1 Introduction

This document reports progress in year one on Workpackage 4 “FMT Inversion with Priors” within Framework 7 project 201792 “Hybrid Fluorescence Molecular Tomography (FMT) - X-ray Computer Tomography (XCT) method and system. Work in this period is divided into four Objectives

2 Objective 4.1 : To develop FMT inversion utilizing XCT image priors without strong anatomy functions correlations

The most widely used form of reconstruction in DOT is the regularised output least squares formulation

$$\| y^{\text{meas}} - F(x) \|_W^2 + \Psi(x) \longrightarrow \text{min},$$

(1)

where $y^{\text{meas}}$ is measured data, $F$ is the forward model, $x$ is the image being reconstructed, and $\|v\|_W^2 := \langle v, Wv \rangle$ is the weighted $L^2$-norm, $W$ being a symmetric positive definite weighting matrix. The term $\Psi(x)$ represents the prior, and in the case of multimodality imaging, is constructed in terms of an auxiliary image $x_{\text{ref}}$. In this objective we consider a number of different approaches for the use of the XCT data as the reference image for such priors in the reconstruction
1. Priors based on the level set structure of the XCT and FMT images
2. Priors based on the joint entropy between the XCT and FMT images

2.1 Level Set structural Priors

In the implementation of the structural priors, we consider a particular form of the functional $\Psi$

$$\Psi(x) = \alpha \int_{\Omega} \psi(|\nabla x|_D) \, dx, \ \alpha > 0. \quad (2)$$

where $\psi$ is an image to image mapping and

$$|\nabla x|_D := \sqrt{({\nabla x})^T D(x) \nabla x} \quad (3)$$

where $D$ is a symmetric tensor function and $\alpha$ is a regularization parameter.

Using standard variational techniques we obtain

$$\mathcal{L}(x) = -\nabla^T k D(x) \nabla \quad (4)$$

where we have defined the diffusivity

$$k := \frac{\psi'(|\nabla x|_D)}{|\nabla x|_D} \quad (5)$$

Choice of $D$ allows us to control the behaviour of the image update according to prior information about the image structure [5, 7]. In the following examples, we take the simple form

$$D = \gamma(x_{\text{ref}}) \mathbb{l} \quad (6)$$

where $x_{\text{ref}}$ is a reference image, and $\gamma$ is an edge-indicator function which we here take to be

$$\gamma(x_{\text{ref}}) = \exp \left\{ - \frac{|\nabla x_{\text{ref}}|}{T_{\text{ref}}} \right\} \quad (7)$$

where $T_{\text{ref}}$ is a threshold controlling the influence of the edges in $x_{\text{ref}}$.

Examples.

The result of applying this regularisation with the threshold parameter $T = \frac{1}{2} \max(|\nabla x_{\text{ref}}|)$, and regularisation parameter $\alpha = 10^{-3}$ is shown in figure 1. The regularisation weighting $\gamma$ is shown in figure 2. The interior of the target is flattened, this is clearer in the line plot shown in figure 3, which shows solutions at $\tau = 0$, $\tau = 10^{-6}$, $\tau = 10^{-3}$. 

2
Figure 1: Digimouse: Slices (z=0mm) through solutions with no prior-based regularisation (top), and regularisation \( \tau = 10^{-3} \) (bottom).
Figure 2: Digimouse: Slice ($z=0$mm) through regularisation edge weighting $\gamma$.

Figure 3: Digimouse: Solution on a line through the target at various values of $\tau$. 
2.2 Information Theoretic Priors

An alternative to the structural priors regularisation is to take an information theoretic measure of image similarity as the penalty term [12]. We make use of the Bayesian interpretation that all parameters are considered to be random variables (r.v). The mutual information between two such r.v., i.e. in this context the solution and the reference image, is given by a linear combination of entropic terms $H(\cdot)$:

$$MI(x,x_{ref}) = H(x) + H(x_{ref}) - H(x, x_{ref}) \quad (8)$$

More specifically, the terms $H(x)$ and $H(x_{ref})$ denote the marginal entropies for the corresponding distributions and the term $H(x, x_{ref})$ denotes the joint entropy of $x$ and $x_{ref}$. Mutual information and various normalized variants of Eq. 8 have found extensive use in medical imaging, as the driving force for multi-modal medical image registration problems [6, 11, 23], due to its inherent capacity of assessing the similarity between two images even when the gray values between inter-modality corresponding objects are incommensurately related. In the context of image reconstruction Somayajula[21, 22] proposed the application of a mutual information based regularization functional:

$$\Psi(x) = -MI(x, x_{ref}) \quad (9)$$

which has the attribute of being invariant to absolute intensity values. Entropy returns information regarding the shape of the probability density of the gray values which can be interpreted as the frequency which gray values appear on the image - irrespectively of their values or spatial distribution. Entropy may be interpreted as a measure of randomness; highly predictable random variables have less entropy than the ones which involve higher degree of randomness. An r.v. is more predictable if some outcomes are realized significantly more frequently than others. Joint entropy $H(x, x_{ref})$ is a measure of randomness characterizing the joint system of the two random variables. In an image reconstruction framework, we would like the reference image to act as a penalty function for the reconstructed image. This is a motivation for using mutual information rather than simply joint entropy. A large value of mutual information value involves a low joint entropy value $H(\hat{x}, x_{ref})$ and simultaneously high individual marginal entropies $H(\hat{x})$ and $H(x_{ref})$. As the prior image is usually constant throughout the reconstruction process $H(x_{ref})$ is constant.
We thus seek an estimate $\hat{x}$ which has low joint entropy with the prior image but also has maximum marginal entropy.

**Examples.**

Preliminary results which provide some evidence regarding the applicability of the mutual information functional as a form of regularization in optical tomography, have been obtained. The simulation corresponding to fig 4 involves a FEM mesh of $D = 3730$ nodes, $S = 32$ sources and $M = 32$ detectors. The reconstruction basis consists of $n_p = 50 \times 50$ bilinear pixels.

The marginal and joint probability density functions are computed via a univariate binned kernel density estimator which utilizes the fast Fourier transform [18, 20] and its multivariate extensions [17, 24], thus allowing the evaluation of the mutual information within reasonable time frames. The optimization of the objective function eq(1) is accomplished via an iterative gradient descent optimization. The first order derivative approximation of the MI prior, $\Psi(x)$, is obtained via an analytic expression. Two reference images have been employed in the process, corresponding to $\mu_a$ and $\mu_s'$ respectively. Both reference images (fig 4 middle row) present structural characteristics, identical with the ones of the target reconstruction. However, in order to simulate a multi-modal framework, we ensure that gray values populating the prior images, are incommensurately related to the ones of the corresponding target reconstructions. For implementation purposes, we scale the range of gray values to be similar to the expected reconstructed range.

3 **Objective 4.2 : To incorporate XCT image segmentation into the FMT code**

Segmentation is required for

1. construction of meshes for numerical modelling
2. construction of priors as required in Objective 4.1
3. post-reconstruction object labelling and analysis

We considered so far the first two of these requirements.
Figure 4: Reconstruction with mutual information. Top row: target images, middle row: reference images, bottom row: reconstructions.
3.1 Anisotropic Diffusion Based Segmentation

Reconstruction techniques in optical tomography for objects of complicated geometries rely on numerical solvers of partial differential equations (PDE), which requires a suitable computational mesh. We have developed methods for 3D mesh generation using 3D CT images provided by Partner 1; an example slice of a 3D CT image is shown on the left in Figure 5. These images have very high quality, but as usual they contain some noise. For segmentation we applied the Perona-Malik approach followed by thresholding. The Perona-Malik approach is based on solving the diffusion equation with varying diffusion coefficient. Thus, denoting the image intensity at a pixel by \( I \), we solve

\[
\frac{\partial I}{\partial t} = \nabla \cdot \kappa \nabla I,
\]

where the diffusion coefficient is computed according to

\[
\kappa = \frac{1}{1 + (\| \nabla I \| / K)^2},
\]

and the parameter \( K \) is chosen empirically. Very high \( \| \nabla I \| \) results in small \( \kappa \), which prevents diffusion of the image intensity across a region with high gradients. On the other hand, small \( \| \nabla I \| \) leads to \( \kappa \sim O(1) \) allowing the image to diffuse in this region and removing within region variation in image intensity \( I \). Segmentation is achieved by thresholding these anisotropically diffused images. A slice of a segmented and thresholded 3D image is shown on the right in Figure 5. In this case only 5 levels were chosen.

The original CT image contained two rods, cylinders, on which the object, a rat, was lying. These rods were removed in the segmented image.

3.2 Mesh Generation

The 3D segmented image was used for hexahedral mesh generation, including the Octal Tree data structure to facilitate mesh refinement. Starting initially from a parallelepiped root cell, we refine this cell up to some level. Refinement of a root cell creates 8 new cells as children. Recursive application of this process generates an octal tree. At each refinement step external cells are deleted, boundary cells are detected, and external and internal boundaries are discriminated. When the desired level of refinement is reached, boundary cells are deformed in order to approximate
boundary surfaces. We tried the following approach. Firstly, before mapping faces of boundary cells onto the surface of an object, we have to provide one-to-one mapping. It is clear that the Cartesian mesh does not have such property. However, edges of boundary cells having a common vertex can be used to define a direction, which is chosen as a vector sum in our case, along which this vertex is relocated. Vertices are relocated iteratively by small steps, which results in a smooth surface mesh having one-to-one mapping properties. Results of this approach are shown in Figure 6. Here for simplicity we assume that the object contains soft tissue and bones only. Figure 6 is generated by adaptation technique when boundary cells are refined more.

Generation of tetrahedral mesh from the hexahedral is simple enough: each cell is split into 6 tetrahedral elements. That was done for the reason of using the TOAST solver.

Boundary cell deformation is the most complicated step because it might create "ill-shaped" cells for the numerical PDE solver. Ill-shaped cells may result in an ill-posed sparse matrix for the finite elements forward solver. The described above approach in many cases works well enough but could not guarantee well-shaped cells for every case. Additional investigation of this approach will be the subject of the
Figure 6: (a) Rendered rat’s head. (b) Rat’s hexahedral mesh. Mesh is refined more along boundaries of the object. (c) Rendered rat’s skeleton. (d) Extracted skeleton mesh.
next report period.

It is clear, that octal tree data structure is very well suited for approaches using adaptive meshes. However, it is not straightforward to use mesh adaptation technique together with the Finite Elements Method (FEM). We therefore plan to investigate other forward problem solvers such as the Finite Volume Method or the Discontinuous Galerkin Method.

3.3 Boundary Element Method and hybrid Boundary-Finite Element Method

The Boundary Element Method (BEM) is based on discretisation only of the object surfaces and allows use of modified analytical methods such as the boundary removal method [14]. In order to include local variation we consider a hybrid BEM-FEM method.

The combination of BEM and FEM is performed on the system matrices, i.e. after the writing of their respective weak formulations, and during the set up of the numerical system. It is based on defining a rule at each interface between a volume integral-treated region and a surface integral-treated one:

\[ \kappa \mathbf{n} \cdot \nabla \phi^{FEM} = -J^{BEM} \]  

which is the flux definition for an outward directed surface normal on the one hand in BEM and on the other in FEM. In matrix terms, this equality is translated into a combined matrix system that is partially sparse (FEM part) and partially dense (BEM part).

Implementation was carried out using the existing Time-resolved Optical Absorption and Scattering Tomography [16] (TOAST) package, combined with the quadratic collocation BEM code [19] developed at UCL and which is in process of being added into TOAST.

A mouse model was created from the Digimouse [4] atlas, with the liver segmented and given different optical parameters \((\mu_s^{\text{body}} = 1.7 \text{mm}^{-1}, \mu_a^{\text{body}} = 0.01 \text{mm}^{-1}, n = 1.4, \mu_s^{\text{liver}} = 2 \text{mm}^{-1}, \mu_a^{\text{liver}} = 0.02 \text{mm}^{-1}, n = 1.4)\). The mouse body is treated as an homogeneous medium with BEM and the liver as an inhomogenous medium with FEM.
The meshes used are created from the Digimouse skin mesh (simplified with the Türk/Lindström memoryless surface mesh simplification algorithm [9] from the Computer Graphics Algorithms Library algorithm embedded in the ISO2MESH [13] MATLAB package). The liver surface mesh was treated as an input geometry and fully re-meshed with NETGEN [15]. The meshes are represented on Figure 7.

Results show the ability to model light transport inside complex geometries while concentrating the volume mesh-based analysis on the regions of interest.

The forward model still requires a reasonable amount of time (30 minutes - 1h) to give results, when we include the computation of the BEM matrix. We plan to ameliorate this technique by using known approximations, such as the Fast Multipole BEM [3] and the Adaptative Cross Approximation [8]. We note that only the FEM part of the matrix will be re-computed at each reconstruction step, which is a much faster process.

In the next period of the project we will use the combined BEM-FEM approach as a basis for image reconstruction.

4 Objective 4.3 : To calculate spatially varying optical attenuation in tissues in-vivo

Calculation of spatially varying optical attenuation is the inverse problem of “classical” diffuse optical tomography. Based on the steady state diffusion equation
it is well known that simultaneous unambiguous reconstruction of absorption and scattering images is not possible due to non-uniqueness [1]. Therefore we consider reconstruction only of the attenuation coefficient by a retransformation of the problem to an equivalent Helmholtz equation

\[ \nabla^2 U + \eta U = q \]  \hspace{1cm} (11)

where \( U = \kappa^{1/2} \Phi \) and

\[ \eta = \frac{\nabla^2 \kappa^{1/2}}{\kappa^{1/2}} + \frac{\mu_a}{\kappa} \]  \hspace{1cm} (12)

Using this representation we may reconstruct \( \eta \) from the projected excitation data. We note that in regions where \( \kappa \) is constant \( \eta \) is equivalent to the attenuation coefficient \( \mu_{\text{eff}} \). If \textit{a-priori} knowledge of \( \kappa \) is available we may instead reconstruct the absorption coefficient \( \mu_a \).

Towards this objective we used the Digimouse mesh as described in section 3.3 and simulated a forward model assuming projection of surface data from the mouse mesh to a camera detector. The data were collected at 8 simulated cameras positioned as if on a rotating gantry, at equal angular spacing, and some distance from the axis of the test volume, generating (128x128) pixel images. Projection matrices for each camera, from FEM mesh to image pixels, were precalculated. This was done
by using OpenGL to render an image of the surface. Rather than light intensity, this image contained the index of a FEM element at each pixel - i.e. the element that each pixel images. Projection matrices could then be computed using the element shape functions to weight each node (matrix column) for each pixel (matrix row). The reverse projection, from image to mesh, was computed as the row-normalised transpose. Figure 9 shows the resulting images for the Digimouse mesh.

This data can be used both for modelling and reconstruction of fluorescence data, and for reconstruction of the background optical properties. In the first period we concentrated on fluorescence only reconstruction. Reconstruction of fluorescent emission was computed, using the same FEM mesh. A Matrix-Free BiCGStab algorithm was run for a fixed 10 iterations for each reconstruction [25]. Figure 13 shows the reconstruction on Digimouse. The target (figure 12) is a 5mm radius sphere positioned at (5,0,0)mm. The fluorescent emission data from this reconstruction at each camera are shown in figure 11.

In the next period, simultaneous reconstruction of μ_a and fluorescence will be investigated.
Figure 10: Digimouse: Simulated fluorescence data from target fluorophore concentration radius 5mm, positioned at (5,0,0)mm. (Images individually log scaled)

Figure 11: Digimouse: Forward data from reconstructed fluorophore concentration, based on data above. Zero-order Tikhonov regularisation. (Images individually log scaled)
Figure 12: Digimouse: Target fluorophore concentration
Figure 13: Digimouse: Iso-surface through solution at 0.35, and slice through solution at z=0mm.
Figure 14: Overview of the approach. A reconstruction step is followed by an estimation step to identify class properties, followed by an approximation of the mixture model. The result of the method is a reconstructed image together with class labels per pixel and statistical properties of the classes.

5 Objective 4.4: To develop FMT inversion based on simultaneous XCT segmentation and classification

In this objective we aim to define a joint reconstruction segmentation algorithm

In most previous work, prior models (which for non-Bayesian approaches are considered as regularisation penalty terms) are based, either explicitly or implicitly, on Gaussian distributions. In contrast to these models, we consider the prior model as mixture of Gaussian distributions.

A summary of the idea is shown in Figure 14. We choose a predefined number of classes $n_c$. A Reconstruction step consists of a few iterations of a damped Gauss-Newton optimisation scheme for a regularised output least squares problem, where the regularisation scheme is first order Tikhonov with variable mean. The Estimation step is an EM method for class labels, means and variances. The whole cycle is iterated until convergence
Note that the EM step may be interpreted as a fuzzy K-means classification method [2, 10], but we provide a more complete general hierarchical Bayesian framework that allows more sophisticated approaches to be taken.

Preliminary results with this method produced pixel misclassification errors of less than 5% after two iterations.

Future work on this topic will involve testing on phantoms.

References


[22] S. Somayajula, A. Rangarajan, and R. M. Leahy. PET image reconstruction


TECHNICAL APPENDIX WP5: FMT-XCT INTEGRATION
DELIVERABLE 5.1: 360 DEGREE FMT PROTOTYPE
MILESTONE NO 3: 360 DEGREE FMT 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: 1ST
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Work Package 5: FMT-XCT integration

TECHNICAL APPENDIX
DELIVERABLE 5.1
MILESTONE NO 3

March 15th, 2009

Vasilis Ntziachristos
Ralf Schulz

360 degree FMT prototype

1. Imaging System
Within the first year of WP5, we implemented the prototype of a 360° free space FMT system suitable for integration onto an XCT gantry. The system was based on a custom-manufactured circular optical breadboard of 1m diameter (anodized aluminum of 1cm thickness), mounted vertically on top of a rotating motor (all delivered and manufactured by Newport Corporation, Irvine, CA, USA). On top of this gantry, a CCD camera and a scanned laser source were mounted orthogonally to the central rotation axis, as shown in Fig.1. A back-illuminated cooled CCD camera (Pixis 512B, Princeton Instruments, Trenton, NJ, USA) coupled to a 50mm macro lens (Carl Zeiss, Oberkochen, Germany) was selected for detection due to its high sensitivity. In front of the lens, a proprietary four-position filter wheel was mounted. The filter positions were occupied by different combinations of long pass glass filters (Schott, Mainz, Germany) and bandpass filters (Andover, Salem, NH, USA) to filter fluorescence light or the excitation wavelength. Beneath the filter wheel two electroluminescent plates are mounted to provide white light illumination of the animal when needed.
Opposite to the camera along its optical axis, two collimated source fibers are mounted which can be moved by an XY stage (Standa, Vilnius, Lithuania) through the field of view of the camera. The focus length of the collimator was chosen such that the focus would be close to the animal positioned in the rotation center.

Two diode laser sources at 670nm and 750nm (B&W Tek, Newark, DE, USA) with maximum optical power of 300mW are used for illumination through the two source fibers. The laser diode modules can be selectively switched on and off, and their optical output power can be controlled via an analog input. Switching and current control is performed using a universal digital/analog input/output box with 10bit resolution (RedLab with UBRE switchbox, Meilhaus Electronic, Puchheim, Germany). The switchbox is also used to switch on and off the white light illumination. All components for the FMT subsystem (camera, laser, D/A module, optical switch, stages) mounted on the gantry are controlled through a single USB2.0 connection, facilitating the later integration of this setup into an XCT system. Mechanical control of the instrument and data acquisition is performed using proprietary software written in LabView (National Instruments, Austin, TX, USA) and running on a standard PC (Pentium IV, 3GHz, 1GB memory).

The mouse bed had to be designed to minimally disturb the acquisition of optical signals. To prevent reflections and refractive effects, it was decided to place the animals onto two carbon rods (2mm) positioned 10mm apart, mounted on two miniature linear stages (Thorlabs, Newton, NJ, USA) to allow precise alignment of the animal to the rotation center of the gantry. While the carbon rods partially block the view, they are highly absorbing due to their black color, allowing the obstructed image regions automatically to be ignored in the reconstruction.
2. Calibration Procedures

Precise alignment and calibration procedures had to be established, ensuring measurements of optical quality. The following routines were coded in LabView for user-independent calibration of the developed system, which can be ported to the final FMT/XCT system foreseen within this project:

- Test of mechanical stability—with the laser source switched on at low power, a full rotation is performed with the gantry, recording the position of the laser source on the CCD. Changes in the geometrical arrangement between laser source and camera can be detected this way. All components were fixed and stabilized using additional mechanical conjectures until the maximum deviation in laser source position was less than +/- one pixel.

- Determination of rotation axis on CCD images.

- Calibration of laser stage movements with respect to change on the CCD image.

3. Data Acquisition

To start data acquisition, the user interactively defines a protocol, consisting of the angular positions at which optical imaging is to be performed, and a source pattern to be used at each angle. This source pattern is defined by the minimum and maximum axial positions and the distance between sources in axial and transversal direction. This source pattern is adapted to the imaged object at each angular projection as described below.

Optical projection data is then acquired by rotating the gantry around the animal. At each angular position that requires measurements, the rotation is stopped and FMT images are acquired. First a white light image of the animal is taken, using no filter. An automatic threshold is applied to the image to distinguish between animal and background. For each row of the image, the central line of the animal is determined. The desired source pattern is then centered to this line; sources that should fall outside the animal are ignored. For all other positions, the laser is moved to the according position, and transmission and emission images are acquired, subsequently, by using different filters. Additionally, for each transmission image, the laser power is set to an optimal value by controlling the voltage on the analog input of the laser module. This laser intensity is then kept constant for the acquisition of the according fluorescence image to facilitate the accurate normalization of imaging data. However, for optimal signal-to-noise, the fluorescence exposure time is adapted.

Input data is extracted from the images by first considering all pixels that (1) cover the animal, i.e., that are within the detected boundaries of the animal, and (2) reach above a certain intensity threshold in the excitation image. This way, pixels covering the rods of the animal holder are excluded, as well as very absorbing regions in the animal where no light could be detected. The area of the remaining pixels is then covered by detector points that keep a minimum distance to each other. From these points, actual measurement values are extracted as inputs to the reconstruction.

Subsequent to the FMT imaging, the animal holder together with the animal is removed from the FMT system and placed in the XCT system, in which X-ray tomographic data is acquired. XCT and FMT data is coregistered using a rigid transformation.
4. Preliminary Imaging Results

In a first series of experiments, an artificial brain lesion was created in a euthanized mouse by injecting a small bolus X-ray CT contrast agent and Alexa 750 fluorochrome into the brain. The animal was first placed into the FMT system to detect fluorescence. Subsequent XCT imaging revealed the location of the lesion.

With respect to data utilization, from 18 FMT projections with 2x7 sources each, a total of ~12,000 source-detector pairs were utilized. Voxel resolution for reconstruction was 1mm, yielding a total of 1700 voxels inside the mesh. For inversion, 100 least square (LSQR) iterations were used, regularizing the result using standard Tikhonov or Laplace methods, as described in the literature. Experimental measurements were acquired in approximately one hour with this prototype system. Constructing the mesh and weight matrix (forward problem) required ~10 min on a standard PC (Intel CoreDuo processor, 2GB RAM), using a hexahedral finite element grid with >60,000 first order elements. Weight matrix inversion was performed in ~1 min.

Results of this imaging session are presented in Fig. 2, showing X-ray CT data and the corresponding FMT slice obtained.

![Fig. 2. (a) XCT slice showing the artificial brain lesion due to the injected CT contrast agent. (b) Slice from the reconstructed FMT volume, overlaid on the corresponding XCT slice.](image)
TECHNICAL APPENDIX WP6: CANCER IMAGING WITH FOCUS ON BREAST CANCER

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: 1ST
PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
Work Package 6: Cancer imaging with focus on breast cancer

**TECHNICAL APPENDIX**

March 20, 2009

Bertrand Tavitian (CEA-LIME)

This document is a technical appendix to the “Year one periodic report”, that details scientific achievements of WP6 at month 12 of the project.

**OBJECTIVES**

While phantoms are extensively utilized in virtually every work-package, the use of animal models of cancer is essential in order to develop FMT-XCT for its intended application, i.e in-vivo imaging. The overall goal of this work package is therefore to provide appropriate animal models, fluorescence probes and validation tools in order:

6.1 To provide key fluorescence probes and quantify the sensitivity and contrast achieved in the animal models developed and as a function of tumor growth.

6.2 To develop animal models of breast cancer for studying FMT-XCT performance.

6.3 To develop animal models of other cancers 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.

6.5 To predict clinical utility.

**TASK 6.1 ANIMAL MODELS OF BREAST CANCER**

The following animal models have been established at CEA-LIME, and made available for in-vivo FMT-XCT imaging.

**a) mammary tumor xenografts**

The following tumours have been implanted subcutaneously in mice:

- MDAMB-231 human breast adenocarcinoma cells over-expressing MT4-MMP, a metalloproteinase which does not affect in vitro cell proliferation or invasion but strongly promotes primary tumor growth and associated lung metastases in RAG-1 immunodeficient mice 8 weeks post-injection (CEA-LIME).
Vascular leakage of breast cancer xenografts expressing MT4-MMP. The vascular permeability of subcutaneous xenografts expressing (MT4) or not (CTR) MT4-MMP was assessed by injecting intravenously a fluorescent probe AngioSense™ 680 (n=5). A, B : Representative distribution of fluorescent macromolecule remaining in the mice 7 hours (A) and 24 hours (B) after intravenous administration. Tumors are delineated by dotted circle. C: Quantification of fluorescence ratio tumour / surrounding abdominal tissue. Error bars represent SE. **P < 0.01.

- primary cell cultures from human tumors that either express or do not express erb-B2, the target of trastuzumab (Herceptin®), a monoclonal antibody used in breast cancer chemotherapy; these cells have been obtained by CEA-LIME through the Paris canceropole research collaboration and implanted in nude mice. See results in below.

b) mammary tumor transgenic mice models

- CEA-LIME has bred a transgenic strain of mice expressing the polyoma middle T oncoprotein (PyMT), under the control of the mouse mammary tumor virus long terminal repeat (MMTV LTR). These mice develop breast cancer with four distinct stages of tumor progression, from premalignant to malignant stages. Tumoral development is often, at late stages, associated with lung metastases. This model presents many similarities with breast cancer progression encountered in women and has been quantitatively documented using PET, SPECT and CT imaging.
TASK 6.2 ANIMAL MODELS OF BRAIN CANCER
Will be developed during year 2.

TASK 6.3 FLUORESCENT PROBES
This proposal does not aim in developing new fluorescence probes. However, successful completion, demonstration, training and dissemination of the proposed technology rely on the availability of fluorescent reporters to the consortium. To accomplish this we have taken certain steps to not only rely on commercially available fluorescent probes but enable the availability of highly performing fluorescent probes due to own developments:

Commercial probes
Angiosense 680 was tested in the PyMT model:

**Evaluation of the vascular permeability**

*intra venous injection of Superhance™680 (Visen Medical)*
Superhance binds to albumin and has a half-life in plasma of approximately 2 h

increased vascular permeability of the tumours during tumoral development.
Home-made probes:

a) Trastuzumab, (Herceptin®) is a monoclonal antibody that we labelled with AlexaFluor® 680 for use with the human erb-B2 in expression patterns of human erb-B2 in human mammary cancer cells as per models in Task 6.1.

Xenografts of primary human mammary carcinoma over-expressing Her-2

Both tumours over-express Her-2. The patient from which BK 151 was obtained was sensitive to Herceptin therapy, while the patient from which BK 111 was obtained was resistant. In collaboration with Marie France Poupon, Institut Curie, Paris

b) Aptamers: CEA-LIME has developed a new method to select aptamers against whole living cells against metastatic forms of cancer. Differential whole cell-SELEX can select aptamers specific for highly lung metastatic cells (versus low lung metastatic cells) [Iljan, Zueva, Tavitian, paper submitted 2009].

PLANNED WORK FOR SECOND YEAR:

1. develop rodent models of brain tumours
2. optimize the bio-distribution of these aptamers in vivo and resolved them using XCT-FMT.
3. Initiate In-vivo imaging and correlation. In vivo imaging of animal models will be performed originally with the CEALETI for XCT validation and in FORTH for stand-alone FMT validation using commercially available probes.
TECHNICAL APPENDIX WP8: FMT-XCT IMAGING ACCURACY VS. PET-XCT
DELIVERABLE NO 8.1

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: 1ST
PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
Work Package 8: FMT-XCT imaging accuracy vs. PET-XCT

This document is a technical appendix to the “Year one activity report”, that details scientific achievements of WP8 at month 12 of the project.

Work package 8 focuses on the validation of the FMT-XCT utility when compared with the gold-standard PET-XCT. Metrics form both systems will be recorded, compared and validated in terms of the utility of the FMT-XCT system proposed in this project. To be able to do this study it will be necessary to use a set of phantoms that will quantify relevant parameters that can quantify image accuracy in terms of sensitivity, resolution, and quantification accuracy. During the project first year partner FIHGM has worked on the definition and construction of such a phantoms.

**TASK 8.1: HYBRID PHANTOMS**

This task is focused on designing and manufacturing physical phantoms that will be used for FMT-XCT system validation. The phantoms have to be hybrid in the sense that they should reveal the same imaging properties when used on a FMT-XCT system or on a PET-XCT system. This imposes a set of conditions that should be met simultaneously:

- The material used to build the phantom has to reflect the optical properties of the biological tissue, but it will also has to have attenuation and scatter properties of the biological tissue for the 511 keV characteristics radiation of the PET tracers.
- The spaces allocated for the different agent contrasts should be either disposable or easy to clean since not all the contrasts used (optical and PET) have the same decay constants.
- The phantoms should be hard, stable and easy to handle to be able to use them under different setups. They will be shipped among the different labs involved in the project.

The actions taken by FIHGM to achieve these goals have been:

1. Identify the preferred PET phantoms for small-animal imaging.
2. Study the physical properties of the ideal optical phantom.
3. Phantom proposal.

1. PET phantoms

   The most popular phantoms using in small-animal PET performance evaluations are the micro Derenzo and the NEMA NU-4 (NEMA Standards Publication NU-4 2008, *Performance Measurements of Small Animal Positron Emission Tomographs*, published by the National Electrical Manufacturers Association, 1300 N. 17th Street, Suite 1752, Rosslyn, VA 22209). Using both phantoms it is possible to characterize the most relevant features of a PET system in terms of usability. The NU-4 phantom is described in figure 1, taken form the above-mentioned document. The phantom is made of polymethylmethacrylate and consists of three parts: the main phantom body, composed of a fillable
cylindrical chamber with 30 mm diameter and 50 mm length, that contains 5 fillable rods with diameters of 1, 2, 3, 4, and 5 mm; a lid that attaches to the large uniform region end of the phantom supports two cold region chambers, and a lid on the opposite side of the phantom that is used to facilitate drying, and the removal of trapped air.

The micro Derenzo phantom is a small size version of the original one developed by Stephen Derenzo (LBL, CA). It has become a standard among the PET users to show the goodness of a system in terms of spatial resolution.

Figure 1: Image quality phantom. Coronal and transverse sections through the main phantom body (1) and (4), top cover (2) and (5), bottom cover (3) and (6). All dimensions are in mm with a 0.1 mm tolerance, except for the fillable rods with a 0.025 mm tolerance.

Figure 2: Picture of a micro Derenzo manufactured by DSC (Hillsborough, NC) and FDG PET image of the phantom acquired at FIHGM with the ARGUS PET-CT system.
These two phantoms are very well suited for PET imaging, but they can not be applied to FMT imaging since their optical properties and source distributions are not compatible with the real system that they are trying to emulate.

2. Optical phantoms

2.1 Physical parameters involved in light transport through biological media.

Scattering and absorption are the two dominant physical phenomena involved in light transport through tissues. The absorption coefficient $\mu_a$ (cm$^{-1}$) is the parameter that characterizes light absorption. It is defined as the distance at which the light intensity decreases by a factor of $e$.

The light scattering phenomena is described by the scattering coefficient $\mu_s$ (cm$^{-1}$), the anisotropy factor $g$, and the reduced scattering coefficient $\mu'_s$ (cm$^{-1}$). The scattering coefficient is defined as the reciprocal of the distance between two scattering events, namely the scattering mean free path. The anisotropy factor is the average cosine of the scattering angle, hence, it represent how much a photon is deviated from its original travel direction after each scattering event. In biological tissue $g \approx 0.8-0.9$, therefore, it is clearly a highly anisotropic process. However, over large distances (more than 5 scattering events), this anisotropic process turns into an isotropic scattering process with a higher scattering length, namely the transport mean free path. The reciprocal of this distance is the reduced scattering coefficient $\mu'_s$ (cm$^{-1}$), and it is related with $\mu_s$ by the expression

$$\mu'_s = \mu_s(1 - g)$$

Both $\mu_a$ and $\mu'_s$ depend on wavelength and on the type of tissue: organs with high blood content are good absorbers whereas in an organ with high lipid content the scattering is high. Many authors hypothesized that scattering is mainly due to the lipids of the cells membrane.

According to diffusion theory, the photon mean free path in biological tissue is around 1mm, imposing a physical limit to FMT resolution. However experimental set-ups that claim to have submillimeter resolution have been already developed.

2.2 Characterization of an optical phantom

For a large diffusive medium and a laser source working in the constant wave domain, and considering the diffusion approximation, the average intensity $U(r)$ (W*cm$^{-2}$) is described by the expression:

$$(\nabla^2 + k^2)U(r) = -\frac{S(r)}{D}$$

where $k=( -3 \mu_a \mu'_s )^{1/2}$, $D$ is the diffusion coefficient and $S(r)$ is the source distribution term.

Under this experimental conditions (large diffuse medium, constant wave source) $\mu_a$ and $\mu'_s$ are coupled, and only $k$ can be characterized. To separate $\mu_a$ and $\mu'_s$ time resolved methods must be applied.

2.3 Properties that must fulfill the phantom

Apart from mimicking the optical properties of the biological tissue, other considerations must be considered to design the phantom:

- The phantom is going to be built to test the performance of FMT and PET systems;
- FMT system will operate in a non-contact geometry allowing from 0 to 360 geometrical projections around the sample, and it will be coupled to a CT system. Under these circumstances the use of matching fluids is discouraged.
- The phantom will be sent to the different the laboratories across Europe participating on this project.

In consequence, properties that the phantom must fulfill are:
1) Stability: The materials chosen to make the phantom have to be physically and chemically stable, to ensure constant optical properties.

2) Design: The phantom has to be machined to allow the insertion of fluorescent probes and PET probes to compare both techniques in terms of resolution, quantification accuracy and sensibility.

3) Heterogeneity. Since the FMT system is coupled to a CT system, the phantom should mimic to a certain degree the optical heterogeneity of living animals. This heterogeneity should also appear on the CT images.

2.4 Available materials to make optical phantoms

Generally, optical phantoms are made of a bulk material (matrix) that matches the index of refraction of tissue (≈1.4). Scatter and absorbent materials are added at specific concentrations to tune the desired optical properties. The physical and chemical characteristics of the phantom will be determined by the choice of the matrix material.

To simulate absorption black indian ink or ProJet 900NP (Fujifilm) powder are used. Both dyes provide relatively flat absorption spectra in the whole visible region. To simulate scattering lipids, white oxide powders or polymer microspheres can be used.

2.4.1 Water based phantoms

Liquid phantoms are made of an aqueous suspension of scatter and absorber materials. Intrinsic scattering effects of water can be ignored, as well as the absorption, for wavelengths below 700 nm. The index of refraction is 1.34. These kind of phantoms allow to introduce flow or Brownian motion. Chemical environments similar to tissues can be also simulated (pH for example), to test realistically the behavior of the fluorescent probes. Biological components like blood (absorber) can also be added. Just adding a fluorophore to the mixture can simulate tissue autofluorescence.

Liquid phantoms are not suitable for transportation, they are not physically stable due to evaporation and in some cases it is not possible to achieve a homogeneous $\mu'_s$ due to the precipitation of the scatter material (TiO$_2$). The low degree of reproducibility of these phantoms doesn’t make them a good choice to make comparisons between systems performance.

These phantoms can be made fast and easily, making them very useful to perform preliminary tests of tomographic systems.

2.4.2 Gelatin based phantoms

If agar is added to an aqueous solution at the right temperature, the solution will become gelatin after cooling down. The index of refraction barely changes (n=1.35), and scattering and absorption properties remain the same. These kinds of phantoms share all the advantages of the liquid phantoms, except that flow and Brownian motion cannot be produced. Furthermore, homogeneity of the optical properties can be achieved, since the particles settle down after solidification. Layers of different optical properties can be made, and by using molds the phantom can be shaped as desired.

The main drawback is that water evaporation continues after solidification, so the phantom is stable for only a few hours; stability can be improved storing the phantom at low temperature, lasting for several days.

2.4.3 Resin based phantoms

The matrix of this kind of phantoms is made of polyester resin. This material is sold as a viscous liquid that can be mixed with scatter and absorbent materials. After stirring and degassing, a hardener is added and the mixture is poured into a mold. The mixture cures for a couple of days, turning into a solid and stable material. Titanium (TiO$_2$) can be added to introduce scattering, and since the mixture solidifies, no precipitation can occur. Commonly ProJet 900NP can also be used as the absorbent material. The resulting phantom can be easily machined, and many regions can be made with different optical properties. The fluorophore can be mixed with intralipid and India ink to match the optical properties of the matrix. This mixture could be inserted directly in a drilled hole, however if the diameter of the hole is small, capillarity can be an issue, making “filling and cleaning” a difficult task.

The main drawback of a phantom made with this matrix is that hydrophobic fluorophores (IR series) and hydrophilic fluorophores (Cy series), suspended in dichloromethane and methanol respectively, will not preserve their fluorescent intensity in time if they get in direct contact with this materials. To overcome this problem, the phantom has to be drilled to allow the insertion of glass capillary tubes that
contains fluorophore. This solution introduces a new diffusive/non-diffusive extra boundary that could significantly modify the accuracy of the solution of the forward problem.

Figure 3 depicts some resin phantoms made at FIHGM.

Figure 3: Phantoms made with resin loaded with scatter and absorber material. Different shapes and compositions have been tested. The material can be easily machined. Mouse shaped phantom carved by Anikitos Garofalakis at IESL-FORTH.

2.4.4 Silicon rubber based phantom

It has been proven that platinum catalyzed silicone is an appropriate bulk material for optical phantoms. The process for making a phantom with this material is very similar to the one for making resin phantoms. A mixture is added into a mold and it solidifies after adding a catalyst. In this case adding white or black silicone particles mimics scatter and absorption. This phantoms mimic the mechanical properties of stiff tissue and like resin phantom, several “optical areas” can be made. The biggest advantage of silicone phantom is that hydrophobic fluorophores mixed with this material retain its fluorescent properties in time, not needing to use capillaries or lipid solutions.

3. Scater materials

Available scatter materials can be divided in three main groups: lipids, white oxide powders and polymers microparticles.

3.1 Lipids

Lipid emulsions are very good choices to mimic scattering since they are biologically similar to cell membrane lipids. However, phantoms made with these solutions are cumbersome to reproduce since the lipid percentage and distribution can vary from one batch to another. Also, the stability of the emulsion last only for several days at room temperature. Milk is the simplest example of a “lipid emulsion”, and the differences in lipid content and homogeneity between milk samples cannot be easily determined, besides the fact that the stability of phantoms made out of milk last only for a few hours. Intralipid is the most popular commercially available lipid solution. It is made of soy oil and egg lipids and it has been used successfully with gelatin-based phantoms. The homogeneity between batches is thought to be excellent. The stability of the emulsion lasts for various days.

3.2 White oxide powders

Titanium dioxide and aluminum dioxide particles are strong scatterers that are the main components of white paint. Powders with different purities are commercially available, allowing making phantoms with a high degree of reproducibility. Liquid matrices are not suitable for these scatter materials since the particles precipitate with time changing the homogeneity properties of the phantom. This problem
can be overcome with gelatin and solid phantoms, however great care must be taken when mixing solutions containing these particles in order to achieve homogeneous distributions of the scattering powder. It is recommendable to apply sonic baths to a small fraction of solution before adding it to the bulk solution, afterwards, more than 30 minutes of automatic stirring is needed to achieve an homogeneous distribution.

3.3 Polymer microspheres

Technically speaking, these are the best scattering particles for phantom building purposes. The size and index of refraction can be known, so Mie’s theory can be applied to calculate the scattering coefficient accurately. Different commercial suppliers provide microspheres. Their main drawback is its high cost.

4. Phantom proposal: design and fluorophore distribution

Phantom A

The figure 4 shows and schematic representation of the proposed phantom A. It will be shaped as an actual adult mouse, using a silicone mold. Four cylinders inserted along the phantom will simulate absorption heterogeneities, whereas three spheres of 0.2 mm situated on the torso of the mouse will contain fluorophores at different concentrations.

![Figure 4: Picture of a preliminary phantom (upper, left panel). The red rectangle represent the axial slice shown on the right: the cylinders are represented in red, and the red spheres containing the fluorophore. Lower panel: transaxial view of the showing the inhomogeneity cylinder dimensions, and the spheres filled with fluorophore. All the measurements are in cm.](image)

Phantom B

It will be shaped like phantom A, changing the distribution of fluorophore as shown in figure 5.
Phantom materials and building process.

The fact that the phantom must travel around different labs suggest that it should be made of resin or silicone. At this point several alternatives are available for the fluorophore distribution proposed on the previous sections:

1. In a resin phantom pellets made of diffusive plastic filled with the desired fluorophore at selected positions could be inserted during the curing process.

2. As an alternative the same resin phantom can be drilled and plastic or glass tubes filled with fluorophore, intralipid and india ink at the appropriate concentrations can be inserted. In this case instead of fluorescent spheres, there will be fluorescent cylinders.

3. If a silicone phantom is going to be used, the fluorophore can be mixed with the silicone, and different parts can be assembled at the desired positions during the curing process.

**Task 8.3: FMT-XCT Image Performance Validation with PET-XCT Imaging**

Although this task is scheduled for the fourth year of the project, FIHGM is starting to assemble an FMT-XCT prototype that will facilitate the performance evaluation *in situ* before distributing the phantoms.

In this simple prototype all the FMT and CT components were attached to a rotating gantry as depicted in figure 6. On the FMT system, the light emerging from a diode laser is focused into the sample at the desired surface points by two mirrors moved by galvanometers.
Figure 6: FMT-XCT experimental setup. 1) Diode laser. 2) Scan Cube. 3) CCD Camera. 4) Motorized filter wheel. 5) X-ray source. 6) X-ray detector.

The CT system consists of a microfocus X-ray source and a flat-panel type digital detector, diagonally opposed on the rotating gantry. The system has cone-beam geometry with the appropriate FOV to acquire an average-sized mouse with a single bed position. A modified FDK algorithm is used to reconstruct the acquired volume.

Figure 7 shows a FDOT reconstruction slices of a capillary (inner diameter <1mm) filled with Alexa Fluor 750, inside an agar slab phantom [5] with $\mu_a = 0.3 \text{ cm}^{-1}$ and $\mu's = 10 \text{ cm}^{-1}$ of size 5x5x1.5 cm$^3$.

The data acquisition was made using one geometrical projection; 7x7 sources and 15x15 detectors were selected, equally spaced in a 2 x 3 cm$^2$ area. A cubic mesh of 20x20x10 elements was chosen for the tomographic reconstruction, using 15 ART iterations, which took less than 1 minute using a standard PC.

Figure 7: Upper left: gelatin based phantom loaded with Indian ink and titanium oxide, showing the capillary tube with the fluorophore. Upper right: Coronal slice of the FMT reconstructed image, the capillary can be clearly seen. The green line corresponds to the axial slice presented at the bottom left and the yellow line corresponds to the sagittal slice presented on the bottom right.

Figure 8 shows a rendered 3D image of a fused FMT image (in red color scale) with a XCT image. In red appears the FMT reconstructed image of a capillary (inner diameter <1mm) filled with Alexa Fluor.
750 witch was inserted in the esophagus of a euthanized one week year old rat. In gray color scale appears the bone structure of the subject given by the CT reconstruction conveniently thresholded.

Figure 8: Sagittal FMT (red) and XCT (gray) of a one week old rat with a capillary filled with Alexa Fluor 750 inserted in the esophagus. Animal chest is up.

Summary
Task 8.1 FIHGM activities have been developed according to the original project plan without any mayor delay. In the following months FIGM will make the first hybrid phantoms. On the completion of these prototypes validation, the phantoms will be circulated among the project partners to gather some feedback on their use and define modifications for the next interaction.
DELIVERABLE NO 1.1, MILESTONE NO 1: CONSORTIUM AGREEMENT

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: 1st

Period Covered: From March 01, 2008 to February 28, 2009
CONSORTIUM AGREEMENT

THIS CONSORTIUM AGREEMENT is based upon
REGULATION (EC) No 1906/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of
18 December 2006 laying down the rules for the participation of undertakings, research
centers and universities in actions under the Seventh Framework Programme and for the
dissemination of research results (2007-2013) hereinafter referred to as Rules for Participa-
tion and the EC Grant Agreement, adopted on 10 April 2007 hereinafter referred to as the
Grant Agreement and Annex II adopted on 10 April 2007 hereinafter referred to as Annex II of
the Grant Agreement and is made on 1 March 2008, hereinafter referred to as “Effective Date”
BETWEEN:
(1) HELMHOLTZ ZENTRUM MUENCHEN - DEUTSCHES FORSCHUNGSZENTRUM FUER
GESUNDHEIT UND UMWELT GMBH (HMGU or the Coordinator) and
(2) COMMISSARIAT A L'ENERGIE ATOMIQUE (CEA) and
(3) FOUNDATION FOR RESEARCH AND TECHNOLOGY HELLAS (FORTH) and
(4) UNIVERSITY COLLEGE LONDON (UCL) and
(5) FUNDACIÓN PARA LA INVESTIGACIÓN BIOMÉDICA DEL HOSPITAL GREGORIO
MARÁÑÓN (FIBHGM) and
(6) UNIVERSITAET ZUERICH (UZH) and
(7) VAMP VERFAHREN UND APPARATE DER MEDIZINISCHEN PHYSIK GMBH (VAMP)
hereinafter, jointly or individually, referred to as “Parties” or “Party” relating to the Project
entitled Hybrid Fluorescence Molecular Tomography and X-ray Computed Tomography sys-
tem and method, in short FMT-XCT and hereinafter referred to as “Project”

WHEREAS:
The Parties, having considerable experience in the field concerned, have submitted a Proposal
for the Project to the European Commission as part of the Seventh Framework Programme of
the European Community for Research, Technological Development and Demonstration Ac-
tivities under the funding scheme of “Collaborative Project”.
The Parties wish to specify or supplement binding commitments among themselves in addition
to the provisions of the Grant Agreement.

NOW, THEREFORE, IT IS HEREBY AGREED AS FOLLOWS:

Section 1: Definitions
1.1 Definitions
Words beginning with a capital letter shall have the meaning defined either herein or in the
Rules for Participation or in the Grant Agreement including its Annexes without the need to
replicate said terms herein.

1.2 Additional Definitions
“Consortium Plan”
Consortium Plan means the description of the work and the related agreed Consortium
Budget, including the payment schedule, as updated and approved by the Executive Commit-
tee.
“Consortium Budget”
Consortium Budget means the allocation of all the resources in cash or in kind for the activities as defined in Annex I of the Grant Agreement and in the Consortium Plan thereafter.

“Defaulting Party”
Defaulting Party means a Party which the Executive Committee has identified to be in breach of this Consortium Agreement and/or the Grant Agreement as specified in Article 4.2 of this Consortium Agreement.

“Needed”
Needed for the implementation of the Project means: Access Rights are Needed if, without the grant of such Access Rights, carrying out the tasks assigned to the recipient Party would be impossible, significantly delayed, or require significant additional financial or human resources. Needed for Use of own Foreground means: Access Rights are Needed if, without the grant of such Access Rights, the Use of own Foreground would be technically or legally impossible.

“Software”
Software means sequences of instructions to carry out a process in, or convertible into, a form executable by a computer and fixed in any tangible medium of expression.

Section 2: Purpose
The purpose of this Consortium Agreement is to specify with respect to the Project the relationship among the Parties, in particular concerning the organization of the work between the Parties, the management of the Project and the rights and obligations of the Parties concerning inter alia liability, Access Rights and dispute resolution.

Section 3: Entry into force, duration and termination
3.1 Entry into force
An entity becomes a Party to this Consortium Agreement upon signature of this Consortium Agreement by a duly authorized representative. This Consortium Agreement shall have effect from the Effective Date identified at the beginning of this Consortium Agreement.

A new Party enters the Consortium upon signature of the Accession document (Attachment 2) by the new Party and the Coordinator. Such accession shall have effect from the date identified in the Accession document.

3.2 Duration and termination
This Consortium Agreement shall continue in full force and effect until complete fulfillment of all obligations undertaken by the Parties under the Grant Agreement and under this Consortium Agreement. However, this Consortium Agreement may be terminated in accordance with the terms of this Consortium Agreement and Annex II of the Grant Agreement (Grant Agreement Article II.37. and II.38.).

3.3 Survival of rights and obligations
The provisions relating to Access Rights, Confidentiality, Liability, Applicable law and Settlement of disputes shall survive the expiration or termination of this Consortium Agreement as agreed in respective articles.

Termination shall not affect any rights or obligations of a Party leaving the Consortium incurred prior to the date of termination, unless otherwise agreed between the Executive
Committee and the leaving Party. This includes the obligation to provide all input, deliverables and documents for the period of its participation.

**Section 4: Responsibilities of Parties**

**4.1 General principles**

Each Party undertakes to take part in the efficient implementation of the Project, and to cooperate, perform and fulfill, promptly and on time, all of its obligations under the Grant Agreement and this Consortium Agreement as may be reasonably required from it and in a manner of good faith as prescribed by Belgian law.

Each Party undertakes to notify promptly, in accordance with the governance structure of the Project, any significant information, fact, problem or delay likely to affect the Project.

Each Party shall provide promptly all information reasonably required by a Consortium Body or by the Coordinator to carry out its tasks.

Each Party shall take reasonable measures to ensure the accuracy of any information or materials it supplies to the other Parties.

**4.2 Breach**

In the event the responsible Consortium Body identifies a breach by a Party of its obligations under this Consortium Agreement or the Grant Agreement, the Coordinator will give written notice requiring that such breach be remedied within 30 calendar days. If this does not occur, the Executive Committee may decide to declare the Party to be a Defaulting Party and to decide on the consequences thereof.

**4.3 Involvement of third parties**

A Party that enters into a subcontract or otherwise involves third parties (including but not limited to Affiliated Entities) in the Project remains solely responsible for carrying out its relevant part of the Project and for such third party’s compliance with the provisions of this Consortium Agreement and of the Grant Agreement. It has to ensure that the use of third parties does not affect the rights and obligations of the other Parties regarding Background and Foreground.

**Section 5: Liability towards each other**

**5.1 No warranties**

In respect of any information or materials supplied by one Party to another under the Project, no warranty or representation of any kind is made, given or implied as to the sufficiency or fitness for purpose nor as to the absence of any infringement of any proprietary rights of third parties. The recipient Party shall in all cases be entirely and solely liable for the use to which it puts such information and materials.

**5.2 Limitations of contractual liability**

No Party shall be responsible to any other Party for punitive damages, indirect or consequential loss or similar damage such as, but not limited to, loss of profit, loss of revenue or loss of contracts.

A Party’s aggregate liability towards the other Parties collectively shall be limited to once the Party’s share of the total costs of the Project.
The exclusions and limitations of liability stated above shall not apply in the case of damage caused by willful act or gross negligence.

The terms of this Consortium Agreement shall not be construed to amend or limit any non-contractual liability.

5.3 Damage caused to third parties
Each Party shall be solely liable for any loss, damage or injury to third parties resulting from the performance of the said Party’s obligations under this Consortium Agreement or from its use of Foreground or Background.

5.4 Force Majeure
No Party shall be considered to be in breach of this Consortium Agreement if such breach is caused by Force Majeure. Each Party will notify the competent Consortium Bodies of any Force Majeure as soon as possible. If the consequences of Force Majeure for the Project are not overcome within 6 weeks after such notification, the transfer of tasks - if any - shall be decided by the competent Consortium Bodies.

Section 6: Governance structure for Small Collaborative Projects

6.1 General structure
The Executive Committee is the decision-making body of the Consortium.

The Coordinator is the legal entity acting as the intermediary between the Parties and the European Commission. The Coordinator shall, in addition to its responsibilities as a Party, perform the tasks assigned to it as described in the Grant Agreement and this Consortium Agreement.

The Management Support Team assists the Executive Committee and the Coordinator.

6.2 Members
The Executive Committee shall consist of one representative of each party (hereinafter Members), whereas Party CEA shall be entitled to delegate two representatives - one for group LEMI and one for group LETI. Each Member shall be deemed to be duly authorized to deliberate, negotiate and decide on all matters listed in Article 6.3.6 of this Consortium Agreement.

The Coordinator shall chair all meetings of the Executive Committee, unless decided otherwise by the Executive Committee.

The Parties agree to abide by all decisions of the Executive Committee. This does not prevent the Parties to submit a dispute to resolution in accordance with the provisions of settlement of disputes in Article 11.8 of this Consortium Agreement.

6.3 Operational procedures for the Executive Committee

6.3.1 Representation in meetings
Any Member:
- should be present or represented at any meeting;
- may appoint a substitute or a proxy to attend and vote at any meeting;
- and shall participate in a cooperative manner in the meetings.

6.3.2 Preparation and organization of meetings
Convening meetings:
The Coordinator shall convene ordinary meetings of the Executive Committee at least once every six months and shall also convene extraordinary meetings at any time upon written request of any Member.

Notice of a meeting:
The Coordinator shall give notice in writing of a meeting to each Member as soon as possible and within at least 14 calendar days preceding an ordinary meeting and 7 calendar days preceding an extraordinary meeting. Any agenda item requiring a decision by the Members must be identified as such on the agenda.

Sending the agenda:
The Coordinator shall send each Member a written original agenda within at least 14 calendar days preceding the meeting.

Adding agenda items:
Any Member may add an item to the original agenda by written notification to all of the other Members within at least 7 calendar days preceding the meeting. During a meeting of the Executive Committee the Members present or represented can unanimously agree to add a new item to the original agenda.

Any decision may also be taken without a meeting by circulating to all Members a written document which is then signed by the defined majority of Members (see Article 6.3.3 of this Consortium Agreement).

Meetings of the Executive Committee can also be held by teleconference or other telecommunication means.

Decisions may only be executed once the relevant part of the Minutes is accepted according to Article 6.3.5 of this Consortium Agreement.

6.3.3 Voting rules and quorum
The Executive Committee not deliberate and decide validly unless a quorum two-thirds (2/3) of its Members are present or represented. Each Member shall have one vote. Defaulting Party Members may not vote. Decisions shall be taken by a majority of two-thirds (2/3) of the votes.

6.3.4 Veto rights
A Member which can show that its own work, time for performance, costs, liabilities, intellectual property rights or other legitimate interests would be severely affected by a decision of the Executive Committee may exercise a veto with respect to the corresponding decision or relevant part of the decision.

When the decision is foreseen on the original agenda, a member may veto such a decision during the meeting only. When a decision has been taken on a new item added to the agenda before or during the meeting, a member may veto such decision during the meeting and within 15 days after the Minutes of the meeting are sent.

In case of exercise of veto, the members shall make every effort to resolve the matter which occasioned the veto to the general satisfaction of all of all its members.

A Party may not veto decisions relating to its identification as a Defaulting Party. The Defaulting Party may not veto decisions relating to its participation and termination in the Consortium or the consequences of them.

A Party requesting to leave the Consortium may not veto decisions relating thereto.

6.3.5 Minutes of meetings
The Coordinator shall produce written Minutes of each meeting which shall be the formal record of all decisions taken. He shall send this draft to all of its members within 14 calendar days of the meeting.

The Minutes shall be considered as accepted if, within 15 calendar days from sending, no member has objected in writing to the Coordinator with respect to the accuracy of the draft of the Minutes.

The accepted Minutes shall be sent to all of the members of the Consortium Body and the Coordinator, who shall safeguard them. If requested the Coordinator shall provide authenticated duplicates to Parties.

6.3.6 Decisions of the Executive Committee

The Executive Committee shall be free to act on its own initiative to formulate proposals and take decisions in accordance with the procedures set out herein. The following decisions shall be taken by the Executive Committee:

- Content, finances and intellectual property rights
  - Proposals for changes to Annex I of the Grant Agreement to be agreed by the European Commission
  - Changes to the Consortium Plan (including the Consortium Budget)
  - Additions to Attachment 1 (Background excluded)

- Evolution of the Consortium
  - Entry of a new Party to the Consortium and approval of the settlement on the modalities and conditions of the accession of such a new Party
  - Withdrawal of a Party from the Consortium and the approval of the settlement on the modalities and conditions of the withdrawal
  - Declaration of a Party to be a Defaulting Party
  - Corrective measures to be required from a Defaulting Party
  - Termination of a Defaulting Party’s participation in the Consortium and measures relating thereto
  - Proposal to the European Commission for a change of the Coordinator
  - Suspension of all or part of the Project
  - Termination of the Project and/or the Consortium Agreement

- Appointments
  Agree on the members of the Management Support Team, upon a proposal by the Coordinator.

In the case of abolished tasks as a result of a decision of the Executive Committee Members shall rearrange the tasks of the Parties concerned. Such rearrangement shall take into consideration the legitimate commitments taken prior to the decisions, which cannot be cancelled.

6.4 Coordinator

The Coordinator shall be the intermediary between the Parties and the European Commission and shall perform all tasks assigned to it as described in the Grant Agreement and in this Consortium Agreement. In particular, the Coordinator shall be responsible for:

- monitoring compliance by the Parties with their obligations
- keeping the address list of members and other contact persons updated and available
- collecting, reviewing and submitting information on the progress of the Project and reports and other deliverables (including financial statements and related certifications) to the European Commission
preparing the meetings, proposing decisions and preparing the agenda of Executive Committee meetings, chairing the meetings, preparing the Minutes of the meetings and monitoring the implementation of decisions taken at meetings;
- transmitting documents and information connected with the Project, including copies of Accession documents and changes of contact information to the Parties;
- administering the Community financial contribution and fulfilling the financial tasks described in Article 7.3;
- providing, upon request, the Parties with official copies or originals of documents which are in the sole possession of the Coordinator when such copies or originals are necessary for the Parties to present claims.

If the Coordinator fails in its coordination tasks, the Executive Committee may propose to the European Commission to change the Coordinator.

The Coordinator shall not be entitled to act or to make legally binding declarations on behalf of any other Party.

The Coordinator shall not enlarge its role beyond the tasks specified in this Consortium Agreement and in the Grant Agreement.

6.5 Management Support Team

The Management Support Team shall be proposed by the Coordinator. It shall be appointed by the Executive Committee and shall assist and facilitate the work of the Executive Committee. The Management Support Team shall provide assistance to the Coordinator for executing the decisions of the Executive Committee. It shall be responsible for the day-to-day management of the Project.

Section 7: Financial provisions

7.1 General Principles

7.1.1 Distribution of the Financial Contribution

The financial contribution of the European Commission to the Project shall be distributed by the Coordinator according to:
- the Consortium Budget as included in the Consortium Plan;
- the approval of reports by the European Commission, and
- the provisions of payment in Article 7.3.

A Party shall be funded only for its tasks carried out in accordance with the Consortium Plan.

7.1.2 Justifying Costs

In accordance with its own usual accounting and management principles and practices, each Party shall be solely responsible for justifying its costs with respect to the Project towards the European Commission. Neither the Coordinator nor any of the other Parties shall be in any way liable or responsible for such justification of costs towards the European Commission.

7.1.3 Funding Principles

A Party which spends less than its allocated share of the Consortium Budget will be funded in accordance with its actual duly justified eligible costs only.

A Party that spends more than its allocated share of the Consortium Budget will be funded only in respect of duly justified eligible costs up to an amount not exceeding that share.

7.1.4 Financial Consequences for a leaving Party
A Party leaving the Consortium shall refund all advances paid to it except the amount of expended eligible costs accepted by the European Commission.

Furthermore a Defaulting Party shall, within the limits specified in Article 5.2 of this Consortium Agreement, bear any additional costs occurring to the other Parties in order to perform its and their tasks.

7.2 Budgeting

All resources made available for the Project shall be valued in accordance with the usual accounting and management principles and practices of the respective Parties and shall be budgeted.

7.2.1 Budgeted costs eligible for 100% reimbursement

These costs shall be budgeted in the Consortium Budget in the following order of priority:
- banking and transaction costs related to the handling of any financial resources made available for the Project by the Coordinator
- a reasonable costs of Parties related to
  - the delivery of certification of financial statements according to the Grant Agreement
  - the certification of the financial/administrative methodology, unless the methodology has already been used by the Beneficiary in a previous Grant Agreement and has not changed (Grant Agreement Article II.4.4 and II.14.1) and/or
  - the certification of the simplified method of calculation of a Party's full indirect eligible costs (Grant Agreement Article II.15.2.a), if any
- costs related to calls for new Beneficiaries
- costs related to updating this Agreement
- management costs of the Coordinator and the Management Support Team
- costs related to the tasks of the Executive Committee
- intellectual property protection costs
- costs for publications
- costs for the tasks of chairpersons
- any other costs eligible for 100% reimbursement

7.2.2 Budgeting of coordination costs

Costs of coordination of research which are not allowed as management cost according to Annex II of the Grant Agreement (Grant Agreement Article II.16.5) have to be budgeted separately.

7.3 Payments

Payments to Parties are the exclusive tasks of the Coordinator. In particular, the Coordinator shall:
- notify the Party concerned promptly of the date and composition of the amount transferred to its bank account, giving the relevant references
- perform diligently its tasks in the proper administration of any funds and in maintaining financial accounts
- undertake to keep the Community contribution to the Project separated from its normal business accounts, its own assets and property, except if the Coordinator is a Public Body or is not entitled to do so due to statutory legislation.

All payments shall be made without undue delay by the Coordinator after receipt of funds from the European Commission in accordance with the accepted decisions of the Executive Committee on the Consortium Budget, which includes the payment schedule.

Payments to Parties will be handled according to the following two kinds of modalities:
payments for past performance approved by the European Commission will be compared with the advance payment given to a Party for such past performance; the difference will be balanced directly with the Party concerned.

financing in respect of future work included in the Consortium Plan, which may be forwarded to Parties in separate installments in conformity with the decisions of the Executive Committee.

The Coordinator is entitled to withhold any advances either due to a Defaulting Party or to a Beneficiary not being a Party.

The Coordinator is entitled to recover any advances already paid to a Defaulting Party.

Section 8: Foreground

Regarding Foreground, Grant Agreement Article II.26. - Article II.29. shall apply with the following additions:

8.1 Joint ownership

In case of joint ownership of Foreground each of the joint owners shall be entitled to use the joint Foreground as it sees fit, and to grant non-exclusive licenses to third parties, without any right to sub-license, subject to the following conditions:
- at least 45 days prior notice must be given to the other joint owner(s); and
- fair and reasonable compensation must be provided to the other joint owner(s).

8.2 Transfer of Foreground

Each Party may transfer ownership of its own Foreground following the procedures of the Grant Agreement Article II 27. The transferring Party shall, however, notify the other Parties of such transfer and shall ensure that the rights of the other Parties will not be affected by such transfer.

The Parties recognize that in the framework of a merger or an acquisition of an important part of its assets, a Party may be subject to confidentiality obligations which prevent it from giving the full 45 days prior notice foreseen in Grant Agreement Article II 27.2.

8.3 Dissemination

8.3.1 Publication

Dissemination activities including but not restricted to publications and presentations shall be governed by Article II.30 of the Grant Agreement. The Party objecting a publication has to show that its legitimate interests will suffer disproportionately great harm and shall include a request for necessary modifications.

8.3.2 Publication of another Party’s Foreground or Background

For the avoidance of doubt, a Party may not publish Foreground or Background of another Party, even if such Foreground or Background is amalgamated with the Party’s Foreground, without the other Party’s prior written approval.

8.3.3 Cooperation obligations

The Parties undertake to cooperate to allow the timely submission, examination, publication and defense of any dissertation or thesis for a degree which includes their Foreground or Background. However, confidentiality and publication clauses have to be respected.
8.3.4 Use of names, logos or trademarks
Nothing in this Consortium Agreement shall be construed as conferring rights to use in advertising, publicity or otherwise the name of the Parties or any of their logos or trademarks without their prior written approval.

Section 9: Access Rights

9.1 Background covered
The Parties shall identify in the Attachment 1 the Background to which they are ready to grant Access Rights, subject to the provisions of this Consortium Agreement and the Grant Agreement. Such identification may be done by e.g. naming a specific department of a Party and/or by subject matter.

The owning Party may add further Background to Attachment 1 during the Project by written notice. However, only the General Assembly can permit a Party to withdraw any of its Background from Attachment 1.

The Parties agree that all Background not listed in Attachment 1 shall be explicitly excluded from Access Rights. They agree, however, to negotiate in good faith additions to Attachment 1 if a Party asks them to do so and those are needed.

9.2 General Principles
Each Party shall implement its tasks in accordance with the Consortium Plan and shall bear sole responsibility for ensuring that its acts within the Project do not knowingly infringe third party property rights.

As provided in the Grant Agreement Article II.32.3. Parties shall inform the Consortium as soon as possible of any limitation to the granting of Access Rights to Background or of any other restriction which might substantially affect the granting of Access Rights. If the Executive Committee considers that the restrictions have such impact, which is not foreseen in the Consortium Plan, it may decide to update the Consortium Plan accordingly.

Any Access Rights granted expressly exclude any rights to sublicense unless expressly stated otherwise.

Access Rights shall be free of any administrative transfer costs.

Access Rights are granted on a non-exclusive basis, if not otherwise agreed in writing by all the Parties according to the Grant Agreement Article II.32.7.

Foreground and Background shall be used only for the purposes for which Access Rights to it have been granted.

All Access Rights shall be granted upon written request.

The granting of Access Rights may be made conditional on the acceptance of specific conditions aimed at ensuring that these rights will be used only for the intended purpose and that appropriate confidentiality obligations are in place.

The requesting Party must show that the Access Rights are Needed.

9.3 Access Rights for implementation
Access Rights to Foreground and Background Needed for the execution of the own work of a Party under the Project shall be granted on a royalty-free basis, unless otherwise agreed in Attachment 1.
9.4 Access Rights for Use
Access Rights to Foreground if Needed for Use of a Party's own Foreground including for third-party research shall be granted on fair and reasonable conditions.
A third party shall not be granted direct Access to Foreground generated by other Parties unless those Parties explicitly agree to it.
Access rights for internal research activities shall be granted on a royalty-free basis.
Access Rights to Background if Needed for Use of a Party's own Foreground shall be granted on fair and reasonable conditions.

9.5 Access Rights for Affiliated Entities
Affiliated Entities have Access Rights under the conditions of the Grant Agreement Article II.34.3.
Such Access Rights to Affiliated Entities shall be granted on fair and reasonable conditions and upon written bilateral agreement.
Affiliated Entities which obtain Access Rights in return grant Access Rights to all Parties and fulfil all confidentiality and other obligations accepted by the Parties under the Grant Agreement or this Consortium Agreement as if such Affiliated Entities were Parties.
Access Rights may be refused to Affiliate Entities if such granting is contrary to the legitimate interests of the Party which owns the Background or the Foreground.
Access Rights granted to any Affiliated Entity are subject to the continuation of the Access Rights of the Party to which it is affiliated, and shall automatically terminate upon termination of the Access Rights granted to such Party.
Upon cessation of the status as an Affiliated Entity, any Access Rights granted to such former Affiliated Entity shall lapse.
Further arrangements with Affiliated Entities may be negotiated in separate agreements.

9.6 Additional Access Rights
For the avoidance of doubt any grant of Access Rights not covered by this Consortium Agreement shall be at the absolute discretion of the owning Party and subject to such terms and conditions as may be agreed between the owning and receiving Parties.

9.7 Access Rights for Parties entering or leaving the Consortium
9.7.1 New Parties entering the Consortium
All Foreground developed before the accession of the new Party shall be considered to be Background with regard to said new Party.

9.7.2 Parties leaving the Consortium
9.7.2.1 Access Rights granted to a leaving Party
9.7.2.1.1 Defaulting Party
Access Rights granted to a Defaulting Party and such Party's right to request Access Rights shall cease immediately upon receipt by the Defaulting Party of the formal notice of the decision of the Executive Committee to terminate its participation in the Consortium.

9.7.2.1.2 Non-defaulting Party
A Party leaving voluntarily and with the other Parties' consent shall have Access Rights to the Foreground developed until the date of the termination of its participation. The time limit for its right to request these Access Rights shall start on the same date.

9.7.2.2 Access Rights to be granted by any leaving Party

Any Party leaving the Project shall continue to grant Access Rights pursuant to the Grant Agreement and this Consortium Agreement as if it had remained a Party for the whole duration of the Project.

9.8 Specific Provisions for Access Rights to Software

For the avoidance of doubt, the general provisions for Access Rights provided for in this Section 9 are applicable also to Software.

Parties’ Access Rights to Software do not include any right to receive source code or object code ported to a certain hardware platform or any right to receive respective Software documentation in any particular form or detail, but only as available from the Party granting the Access Rights.

Section 10: Non-disclosure of information

All information in whatever form or mode of transmission, which is disclosed by a Party (the “Disclosing Party”) to any other Party (the “Recipient”) in connection with the Project during its implementation and which has been explicitly marked as “confidential”, or when disclosed orally, has been identified as confidential at the time of disclosure and has been confirmed and designated in writing within 15 days at the latest as confidential information by the Disclosing Party, is “Confidential Information”.

The Recipients hereby undertake in addition and without prejudice to any commitment of non-disclosure under the Grant Agreement, for a period of 5 years after the end of the Project:

- not to use Confidential Information otherwise than for the purpose for which it was disclosed;
- not to disclose Confidential Information to any third party without the prior written consent by the Disclosing Party;
- to ensure that internal distribution of Confidential Information by a Recipient shall take place on a strict need-to-know basis; and
- to return to the Disclosing Party on demand all Confidential Information which has been supplied to or acquired by the Recipients including all copies thereof and to delete all information stored in a machine readable form. If needed for the recording of ongoing obligations, the Recipients may however request to keep a copy for archival purposes only.

The Recipients shall be responsible for the fulfillment of the above obligations on the part of their employees and shall ensure that their employees remain so obliged, as far as legally possible, during and after the end of the Project and/or after the termination of employment.

The above shall not apply for disclosure or use of Confidential Information, if and in so far as the Recipient can show that:

- the Confidential Information becomes publicly available by means other than a breach of the Recipient’s confidentiality obligations;
- the Disclosing Party subsequently informs the Recipient that the Confidential Information is no longer confidential;
- the Confidential Information is communicated to the Recipient without any obligation of confidence by a third party who is in lawful possession thereof and under no obligation of confidence to the Disclosing Party;
- the disclosure or communication of the Confidential Information is foreseen by provisions of the Grant Agreement;
- the Confidential Information, at any time, was developed by the Recipient completely independently of any such disclosure by the Disclosing Party; or
- the Confidential Information was already known to the Recipient prior to disclosure.

The Recipient shall apply the same degree of care with regard to the Confidential Information disclosed within the scope of the Project as with its own confidential and/or proprietary information, but in no case less than reasonable care.

Each Party shall promptly advise the other Party in writing of any unauthorized disclosure, misappropriation or misuse by any person of Confidential Information as soon as practicable after it becomes aware of such unauthorized disclosure, misappropriation or misuse.

If any Party becomes aware that it will be required, or is likely to be required, to disclose Confidential Information in order to comply with applicable laws or regulations or with a court or administrative order, it shall, to the extent it is lawfully able to do so, prior to any such disclosure notify the Disclosing Party, and comply with the Disclosing Party’s reasonable instructions to protect the confidentiality of the information.

The confidentiality obligations under this Consortium Agreement and the Grant Agreement shall not prevent the communication of Confidential Information to the European Commission.

Section 11: Miscellaneous

11.1 Inconsistencies and severability

In case this Consortium Agreement is in conflict with the Grant Agreement, the terms of the latter shall prevail. In case of conflicts between the appendices and the body text of this Consortium Agreement, the latter shall prevail.

Should any provision of this Consortium Agreement become invalid, illegal or unenforceable, it shall not affect the validity of the remaining provisions of this Consortium Agreement. In such a case, the Parties concerned shall be entitled to request that a valid and practicable provision be negotiated which fulfils the purpose of the original provision.

11.2 No representation, partnership or agency

The Parties shall not be entitled to act or to make legally binding declarations on behalf of any other Party. Nothing in this Consortium Agreement shall be deemed to constitute a joint venture, agency, partnership, interest grouping or any other kind of formal business grouping or entity between the Parties.

11.3 Notices and other communication

Any notice to be given under this Consortium Agreement shall be in writing to the addresses and recipients as listed in the Grant Preparation Form A2.4. Any change of persons or contact details shall be notified immediately by the respective Party to the Coordinator in written form

Formal notices:
If it is required in this Consortium Agreement (Articles 9.7.2.1.1 and 11.4) that a formal notice, consent or approval shall be given, such notice shall be signed by an authorized representative of a Party and shall either be served personally or sent by mail with recorded delivery or telefax with receipt acknowledgement.

Other communication:
Other communication between the Parties may also be effected by other means such as e-mail with acknowledgement of receipt (e.g. Minutes).
11.4 Assignment and amendments
No rights or obligations of the Parties arising from this Consortium Agreement may be assigned or transferred, in whole or in part, to any third party without the other Parties’ prior formal approval.

Amendments and modifications to the text of this Consortium Agreement not explicitly listed in Article 6.3.6 require a separate agreement between all Parties.

11.5 Mandatory statutory law
Nothing in this Consortium Agreement shall be deemed to require a Party to breach any mandatory statutory law under which the Party is operating.

11.6 Language
This Consortium Agreement is drawn up in English, which language shall govern all documents, notices, meetings and processes relative thereto.

11.7 Applicable law
This Consortium Agreement and all clauses in the Grant Agreement affecting the rights and obligations between the Parties shall be construed in accordance with and governed by the laws of Belgium.

11.8 Settlement of disputes
All disputes arising out of or in connection with this Consortium Agreement, which cannot be solved amicably, shall be finally settled under the Rules of Arbitration of the International Chamber of Commerce by one or more arbitrators appointed in accordance with the said Rules. The place of arbitration shall be Brussels if not otherwise agreed by the conflicting Parties. The award of the arbitration will be final and binding upon the Parties.

Nothing in this Consortium Agreement shall limit the Parties' right to seek injunctive relief or to enforce an arbitration award in any applicable competent court of law.

Section 12: Signatures
AS WITNESS:
The Parties have caused this Consortium Agreement to be duly signed by the undersigned authorized representatives on the day and year first above written.
FMT-XCT Consortium Agreement

HELMHOLTZ ZENTRUM MUENCHEN - DEUTSCHES FORSCHUNGSZENTRUM FUER GESUNDHEIT UND UMWELT GMBH (HMGU)

Signature(s)  

Name(s)        PD Dr. Christian Langebartels  
Title(s)       Head of Scientific-Technical Dept.  

Gerolf Schmidl  
Head of Finance Dept.

Stamp  
HelmholtzZentrum münchen  
Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)  
Ingolstädter Landstr. 1 · 85764 Neuherberg

30 04. 2008
COMMISSARIAT A L'ENERGIE ATOMIQUE (CEA)

Signature(s)

Name(s)                  Title(s)                                      
Prof. Pierre Legrain    Head of CEA Life Science Division          
Dr. Malgorzata Tkatchenko Head of CEA Biomedical Imaging Institute

Stamp
FOUNDATION FOR RESEARCH AND TECHNOLOGY HELLAS (FORTH)

Signature(s)  A. A. Payatakes
Name(s)        Prof. Alkiviades Payatakes
Title(s)       Chairman of the Board of Directors
Stamp          S/5/08
UNIVERSITY COLLEGE LONDON (UCL)

Signature(s)  

Name(s)      Michael Browne  Mark Burgess  
Title(s)      Assistant Director Research Europe  Assistant Director Research Services

Stamp

University College London  
Gower Street  
London WC1E 6BT
FUNDACIÓN PARA LA INVESTIGACIÓN BIOMÉDICA DEL HOSPITAL GREGORIO MARAÑÓN (FIBHGM)

Signature(s)

Name(s)  Dr. Albino Navarro Izquierdo
Title(s)  Managing Director

Stamp: GERENCIA
FMT-XCT Consortium Agreement (draft, March 11, 2008)

UNIVERSITAET ZUERICH (UZH)

Signature(s)  
Name(s)  Prof. Heini Murer  
Title(s)  Vice President  
Prof. Hans Weder  
University Management

Stamp
VAMP VERFAHREN UND APPARATE DER MEDIZINISCHEN PHYSIK GMBH (VAMP)

Signature(s)

Dr. Holger Brüenner
Managing Director/CEO

Robert Hebel
Managing Director/CEO

Stamp

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[please indicate]

UNIVERSITY COLLEGE LONDON (UCL)
[please indicate]

FUNDACIÓN PARA LA INVESTIGACIÓN BIOMÉDICA DEL HOSPITAL GREGORIO MARAÑÓN (FIBHGM)
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of a new Party to

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[OFFICIAL NAME OF THE NEW PARTY AS IDENTIFIED IN THE GRANT AGREEMENT]

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DELIVERABLE NO 1.2: MINUTES KICK OFF CONSORTIUM MEETING

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: 1ST
PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
Kick-off meeting for the FMT-XCT project
“Hybrid Fluorescence Molecular Tomography and X-ray Computer
Tomography system and method”
EU-FP7 grant agreement no. 201792

Helmholtz Zentrum München, 14 May 2008, 12:00h – 18:30h

Minutes

Participants for

1. HMGU Vasilis Ntziachristos (project co-ordinator)
   Jürgen Ertel
   Karl-Hans Englmeier
   Ralf Schulz
   Marta Zientkowska
   Daniel Razansky
   Susanne Stern
   Saskia Björn
   John Baeten
   Thomas Jetzelfellner

2. CEA Bertrand Tavitian (CEA Orsay)
   Jean-Marc Dinten (CEA Grenoble)

3. FORTH Jorge Ripoll
   Giannis Zacharakis
   Rosy Favicchio

4. UCL Simon Arridge
   Vadim Soloviev
   Athanasios Zacharopoulos

5. FIBGHM Juan José Vaquero

6. UZH Thomas Müggler
   Florian Stuker

7. VAMP Holger Brünner

Chair of the meeting: Vasilis Ntziachristos
Minutes: Susanne Stern
Agenda:

1. 12:00 - 13:00 Welcome  
   Jürgen Ertel: administration and finances  
   Vasilis Ntziachristos: program overview, work packages, milestones and deliverables

2. 13:00 – 13:30 Jean-Marc Dinten (CEA Grenoble): Dual-energy CT & radiation protection of camera's etc

3. 13:30 – 14:00 Holger Brünner (VAMP): X-ray Gantry and dual-energy CT

4. 14:00 – 14:30 Jorge Ripoll (FORTH): Fast inversion methods

5. 14:30 – 15:00 Simon Arridge (UCL): Inversion & Use of image priors

6. 15:00 – 15:30 Coffee Break

7. 15:30 – 16:00 Juan José Vaquero (FIBGHM): Correlative studies with PET

8. 16:00 – 16:30 Bertrand Tavitian (CEA Orsay): Probes and models for imaging

9. 16:30 -- 17:00 Thomas Müggler (for Markus Rudin, UZH): Correlative Imaging and models

10. 17:00 – 18:30 Discussion and plan of action of year 1

The agenda had been sent to the partners via e-mail on 17 April (preliminary version, due to flight schedules of the participants the order of presentations was slightly adjusted)

The .pdf of Jürgen Ertel’s presentation with info on project administration was sent out to the partners on 21 May

1. **HMGU – IBMI and general information**

Vasilis Ntziachristos welcomes all participants of the meeting.

Jürgen Ertel (JE) of the department for Program Planning and Management gives an overview of the administrative and management aspects. He states that the financial issue is very important and presents a plan for the distribution of the pre-financing made by the Commission which is accepted by all partners (signatures by S. Arridge, J. J. Vaquero, J. Ripoll, H. Brünner, B. Tavitian, T. Müggler).

He estimates that the grant agreement countersigned by the EU will take about one more week to be returned. HMGU has not received the Form As yet. When they are sent out they shall be returned as soon as possible. *By the present day the grant agreement was returned by the EU and sent out to the partners on 05 June. The Form As still need to be returned by the partners.*

The project consists of 9 work packages, WP1 = Management, WP2 – WP9 = scientific. The management structure is explained, JE mentions that all partners are members of the executive committee.

Regarding financial issues JE states that the activity “other” is new and was formerly a part of RTD. “Other” includes activities like dissemination, exploitation and training. If subcontracts are needed they shall be drawn up as fast as possible.

JE presents the reporting and payment schedule. Explicitly mentioned are the following items:
- There will be one pre-financing which is going to be transmitted in 2 shares. The 1st instalment should last for at least 12 months. The money is distributed to the partners on a pro-rata basis.
- When the reports are due the partner has 60 days for submitting the report. After that the EU-commission has 105 days to approve the report followed by an interim payment. If problems arise concerning a report the 105 days start again.
- 5% of the total funding are paid into a guarantee fund.
- At the end of the 3rd interim period the partners will have received 85% of the total funding (10% retention due to the EC regulation of maximum 90% payment for interim reporting including pre-financing + 5% for Guarantee Fonds), the rest will be paid at the end of month 54.
- Public bodies or legal entities whose participation is guaranteed by a Member State or Associated Country and higher and secondary education establishments will receive back all money from the guarantee funds, there might be deductions for industrial companies.

Finally JE states that audit certificates are mandatory as soon as the EU contribution exceeds 375,000€ (all partners except for VAMP and UZH). The format of the audits has to follow Annex VII, Form D

Questions:

Simon Arridge asks for a web-page type of approach and Vasilis Ntziachristos agrees that IBMI at HMGU will set up a website that contains information on FMT-XCT.

Regarding the financial guidelines the question of how to best count working hours is also discussed (collaborators working on different projects, holidays, etc.).

Vasilis Ntziachristos (VN) gives an overview of all tasks and the guidelines according to Annex 1

The aim is to build a system that is rotating around the object / animal that is going to be examined.

The work packages and their leaders are:

- **WP1** management
- **WP2** science, leader CEA-LETI (Grenoble)
- **WP3** science, leader FORTH
- **WP4** science, leader UCL (co-operation with HMGU, Karl-Hans Englmeier)
- **WP5** science, leader HMGU (combination of by then existing parts into a functioning system)
- **WP6** science, leader CEA-LIME (Orsay)
- **WP7** science, leader UZH
- **WP8** science, leader FIBGHM (comparison with an established method – PET-XCT, FMT-XCT will not involve ionizing radiation, is cheaper, accelerators are not needed)

VN describes in brief the work packages with their objectives and deliverables.

Training will be organized by HMGU, the next meeting in Grenoble is fixed, the locations of the following ones are still to be discussed.

2. **CEA-LETI (Grenoble), presentation of the part-project**
Jean-Marc Dinten (JMD) introduces LETI as a part of CEA. His group works on the systems integration of micro and nanotechnologies and he gives background information about dual energy imaging (DE).

Topics like management of x-ray scattering, experimental setups, calibration, previous research results, 3D reconstruction - partly with motion compensation (lung) are discussed.

JMD explains CEA-LETI’s tasks (i.e. development of a DE-XCT lab bench and its specification for the FMT-XCT prototype) and presents the time schedule and deliverables of the part-project.

Specific scattering problems (e.g. behind bones) and their management are mentioned.

3. **VAMP, presentation of the part-project**
Holger Brünner (HB) introduces his company, fields of knowledge and products. VAMP is a spin-off from the Universität Erlangen. There is still a strong connection with the university, however VAMP’s main interest is transferring scientific results into commercial products.

Important items for HB are
- the cost factor of the slip ring design
- does the prototype need to be fully radiation shielded
- dose considerations in micro CT, lethal dose for mice is about 1Gy.
- high resolution and low dose are not possible, one has to find a compromise

HB describes the tasks of VAMP within the work packages of the project.

Questions:
Ralf Schulz asks if the slip ring design will be too expensive. HB states that it has the advantage of higher speed and dynamics. The system first accelerates and then the imaging starts. Imaging takes about 4 sec. with existing systems, the overall scan time is about 2 min. depending on the detector.

4. **FORTH, presentation of the part-project**
FORTH as the leader of WP3 will closely work together with the UCL group. Jorge Ripoll (JR) introduces his in-vivo imaging group which works with objects sized 1µm-10mm (Drosophila, fish) as well as with mice (area 10mm-10cm).

JR states that problems in FMT are e.g. auto fluorescence, that it is sometimes unknown how wrong an image is and the finding of fast inversion methods.

JR also describes plans for direct inversion methods to be investigated as part of WP3.

User friendly software (in Labview) can be provided.

5. **UCL, presentation of the part-project**
The group of Simon Arridge (SA) will provide mathematical and computational techniques. SA’s group is part of the Centre for Medical Image Computing at UCL. He gives an overview of previous and related research, mathematical approaches of the inverse problem, experimental setups and parameter reconstruction.
SA states the principle challenges of his part-project. His results will finally be available in a Matlab based code.

6. Break

7. **FIBGHM, presentation of the part-project**
   The group of Juan José Vaquero (JJV) does preclinical studies on clinical workstations. He gives an overview of his institution and the existing equipment and tools.

   He describes the role of FIBGHM in the FMT-XCT project, especially with creating hybrid FMT-PET-XCT-phantoms. The research shall not only be a simple comparison between FMT-XCT and PET-XCT but also provide information on the utility of FMT-XCT.

   FIBGHM has developed epoxy phantoms that can simulate different tissues of small animals. JJV states that absolute quantification is really difficult.

8. **CEA-LIME (Orsay), presentation of the part-project**
   Bertrand Tavitian (BT) at the Institute for Biomedical Imaging focuses on cancer imaging (primarily breast cancer) to evaluate treatment efficiency. Their experimental imaging platform contains Micro-PETs, SPECT-CT, etc.

   BT informs about previous and current research at the institute both with imaging of animals and cell cultures and various imaging methods. Introduction of specific mouse models, methods of therapy evaluation and imaging probes.

   There are fluorescent probes and antibodies that are commercially and non-commercially available. CEA-LIME will investigate different labeling methods as part of WP6.

9. **UZH, presentation of the part-project**
   Thomas Müggler (TM), senior scientist in the institute of Markus Rudin, first introduces the Animal Imaging Center as part of a network of several academic and clinical institutes also with connections to industrial partners.

   He informs about UZH’s technical equipment and previous research (free-space FMT, tumor models, development of a comprehensive model of Angiogenesis, research about a Hypoxia inducible factor).

   TM outlines UZH’s role in the collaboration and their objectives. The next steps will be to develop biological tools and instrumentation.

   Question by Ralf Schulz regarding the experimental setup

10. **Discussion and plan of action of year 1**
    The deliverables, especially for the coming 12 months until the next mandatory meeting, are discussed.

    Months 1 – 12 are planned for the XCT-design. Month 18 will be the deadline for a final technical specification. Parameters and components are discussed, mostly between the
most involved partners JMD and HB who are to find a consensus on the suitable
components. JMD will draft a certification for HB.

The XCT shall be primarily designed for mice, but also that rats can fit in.
The tube is expected to become the major problem. There are technical limitations for
special tubes. A supplier for tubes is located near Berlin. Initial geometrical parameters
are discussed.

Effects from rotation are expected and will be further investigated.

The CT shall meet the commercial standard of 80µ resolution.

According to HB’s experience a scan time of 2-4 min. for a full scan can be reached.

Binitio as a suitable contrast agent is proposed by Marta Zientkowska as it gives a
better contrast for optical imaging.

SA and JR will collaborate on the development of inversion algorithms. SA’s solution
will incorporate priors. After 3 months the research results should give some
preliminary idea about how the development is going.

Rotating data will be available soon.

The prototype at HMGU is planned to be ready in month 29, first as a rotating system
without FMT-XCT.

HMGU has already started working on a prototype.

Molecular probes will be developed / improved by CEA-LIME. BT asks to be a bit
cautious with expecting the new probes to be available within 12 months as the
development of complex molecules takes time and he has to hire somebody for the
project. Probes “to play with” are however already available.

By month 9 UZH is expected to have a SAF model and FIBGHM will be working on
constructing phantoms.

Munich, 10 June 2008

Vasilis Ntziachristos
DELIVERABLE no 1.4: PROGRAM CONSORTIUM MEETING

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: 1st
PERIOD COVERED: FROM March 01, 2008 TO February 28, 2009
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

Munich, Klinikum rechts der Isar, Pavillon, April 24, 2009, 11 a.m – 18 p.m.

Participants for

1. HMGU (project co-ordinator)
   Vasilis Ntziachristos
   Ralf Schulz
   Veronika Erben
   Eric Söhngen
   Marta Zientkowska
   Angelique Ale
   Saskia Björn
   Thomas Jetzfellner
   Marcus Freyer
   Tatjana Wunderlich
   Irina Uhlig

2. CEA
   Leti: Marco Bramilla
   Lime: Anikitos Garofalakis

3. FORTH
   Giannis Zacharakis

4. UCL
   Simon Arridge
   Vadim Soloviev
   Tim Rudge,
   Josias Elisee

5. FIHGM
   Juan José Vaquero
   Juan Aguirre

6. UZH
   Florian Stuker
   Katerina Dikaiou

7. VAMP
   Holger Brünner

Chair of the meeting: Vasilis Ntziachristos

Minutes: Veronika Erben
Initially, Vasilis Ntziachristos welcomes all participants and introduces Veronika Erben, who talks about financial and administrative issues related to the project.

Veronika Erben, as the project manager, gives a short overview of the project, followed by notes related to the reporting and finishes with the list of deliverables and milestones for reporting period one and two. The most important points are:

- Since all documents (scientific and financial) required for the year one report arrived in time, the second rate of prefinancing will be transferred to all partners, presumably in May 2009 (exception VAMP: second rate prefinancing was already transferred). As soon as the European Commission (EC) transfers the first interim payment (=sum of costs claimed in Form Cs) to the Helmholtz Zentrum München (HMGU) the money will be transferred to all partners.
- The next consortium meeting (month 24=Feb. 2010) is planned in Herakleon, Crete. This meeting will be co-localized together with a workshop on free space FMT (mo24).
- A training session on XCT is planned as described in Annex1 (WP2 and WP9) and will be an opportunity of training in dual energy XCT. It should be housed by CEA-LETI at Grenoble in second part of June 2009.
- If there is a delay in deliverables, milestones or similar (compared to Annex 1) this is not problematic as long as it is stated in the report (clarified with Dr. Ingemansson, the responsible project officer at the European Commission).
- Time sheets are obligatory.
- A platform for data exchange between all partners will be established (already initialized).
- After submission of the first report to the European Commission and approval by the project officer Dr. Ingemannson, all partners/beneficiaries will receive the report.

Marco Brambilla (CEA-LETI) and Holger Bruenner (VAMP) together present the results of the first year within WP2, the XCT development.

- X-ray CT design (task 2.1): The XCT design (deliverable 2.1) was finalized in November 2008 and distributed to the consortium. This report fulfils most of the objectives of the task 2.7 « optimal XCT design », this was decided because of the (long) delivery time of the system key components.
- X-ray CT dual energy development (task 2.2): In order to help dual energy development and validation a on-bench CT at LETI has been built.
- The XCT prototype is now under development at VAMP. First parts have been constructed and others ordered. Transfer to HMGU is planned for the end of the year (mo 22= Dec. 2009).

Juan José Vaquero (FIHGM) introduces the contribution of FIHGM to WP2 (XCT Development), in particular the use of X-ray contrast agents, double exposure techniques and a dual energy X-ray source. Juan Aguirre (FIHGM) presents the studies performed required for manufacturing physical phantoms that will be used for FMT-XCT system validation (WP8).

Consortium decision on phantom design:

- Cylinder (bulk material resin) with different size insertions (open capillaries).
- There will be some initial phantoms circulated among the partner. According to the feedback of the partners, phantoms will be modified.
- With regard to standardization, imaging results obtained in different labs will be compared.
Giannis Zacharakis (FORTH) presents the progress of WP3 (Theory for 360 degree FMT) during year one:

- An inversion method for FMT based on boundary removal and back-propagating the data has been developed and is available to all partners (Objective 3.1).
- A direct inversion method based on reconstruction in Fourier space has been developed and is also available to all partners (Objective 3.2).
- The direct inversion has been tested and compared with the standard methods and proved to be significantly faster when a large number of sources has been used. However, for small number of sources as in the case of FMT acquisitions, it does not give satisfactory results (Objective 3.3).
- A multispectral algorithm has been developed and tested for simultaneous detection of multiple fluorophores and absorbers. It will be incorporated in the FMT software in the next reporting period according to the time schedule (Objective 3.4).
- A user-friendly software has been developed under Matlab and Labview environments, enabling users to analyze experimental data and visualize results in a fast and efficient way (Objective 3.5).
- A large number of experimental measurements have been acquired that are available to all partners for optimization and finalization of algorithms. These measurements involve phantoms as well as in vivo experiments (Objective 3.6).
- A decision has been made to start a new Deliverable “Ultra – fast Inversion for FMT” that will be delivered on the next reporting period.

Simon Arridge (UCL) introduces the department of Computer Science at the University College London and describes the progress towards objectives within WP4 (FMT inversion with image priors).

- Structured priors orientating the reconstructed FMT images to have level sets parallel to those of XCT image and theoretic priors orientating the reconstructed FMT images to have maximum joint entropy with the XCT image were developed. Initial tests on simulated 3D images of mouse from a realistic atlas were performed (Objective 4.1).
- Segmentation of XCT based on anisotropic diffusion (Peronal-Malik algorithm) and hexahedral adaptive mesh generation from XCT images were developed. Mesh reduction methods using public-domain software ISO2MESH were incorporated and Boundary Element (BEM) and hybrid Boundary-Finite Element (BEM-FEM) methods were developed (Objective 4.2).
- A Non-linear reconstruction method for attenuation making use of Louiville transformation from diffusion to Schrodinger equation was developed (Objective 4.3).
- A combined reconstruction/segmentation method combining Gauss-Newton image reconstruction with fuzzy-kmeans image classification and a fully hierarchical Bayesian framework was developed (Objective 4.4).
- A workshop “Image reconstruction algorithms and the use of priors” is planned for September 2009 in London.

Ralf Schultz (HMGU) describes the progress within WP5 (FMT-XCT integration), which focuses on the development of a 360 degree hybrid FMT-XCT imaging system.

- According to schedule a 360-degree functional FMT prototype was developed.
- The XCT module, developed within WP2, will be integrated into a hybrid imaging system at the end of the year (mo 22= Dec. 2009).
- A FMT-XCT prototype, based on a commercial XCT (General Electric (GE) Locus Preclinical µCT Scanner) system in which imaging at two wavelengths is supported was developed.
- First in vivo studies are already on-going ahead of schedule.
Anikitos Garofalakis (CEA-LIME) describes the animal models and fluorescent probes, which have been established at CEA-LIME within WP6 (Cancer imaging with focus on breast cancer):

- **mammary tumor xenografts:**
  - MDAMB-231 human breast adenocarcinoma cells over-expressing MT4-MMP, a metalloproteinase which does not affect in vitro cell proliferation or invasion but strongly promotes primary tumor growth and associated lung metastases in RAG-1 immunodeficient mice 8 weeks post-injection.
  - primary cell cultures from human tumors that either express or do not express erb-B2, the target of trastuzumab (Herceptin®), a monoclonal antibody used in breast cancer chemotherapy.

- **mammary tumor transgenic mice models:**
  - CEA-LIME has bred a transgenic strain of mice expressing the polyoma middle T oncoprotein (PyMT), under the control of the mouse mammary tumor virus long terminal repeat (MMTV LTR). These mice develop breast cancer with four distinct stages of tumor progression, from premalignant to malignant stages.

- **Probes :**
  - commercial probes (Angiosense 680, Superhance 680)
  - home-made probes (Trastuzumab, Aptamers)

One breeding accident with the PyMT mice has delayed partly the programme with these animals.

Florian Stuker (UZH) presents the work of Steffi Lehmann, illustrating the feasibility of hypoxia-inducible factor (HIF) stabilization and activity in tumor cells, performed within WP7 (Imaging cancer therapy for enabling intervention), and subsequently describes the development of a new MRI-FMT system.

- Multiple reporter assays for measurement of HIF pathway activity were developed.
- HIF1α activity by expressing firefly luciferase under the control of the HIF-responsive element HRE identified as most promising readout for fluorescence imaging.
- Tumor cells (C51 colon carcinoma) have been transfected using the HRE constructs and injected subcutaneously in nude mice. In vivo imaging has been performed.
- Initial FMT experiments with protease imaging in tumor models were performed.
- The development of the MRI-FMT system is based on:
  - Setup design without fibers,
  - Free beam illumination and point grid scan,
  - Detector operation inside the magnet,
  - Investigation of the interaction between the modalities.

Munich, 05 May 2009

Vasilis Ntziachristos
DElIveraBLe no 2.1, Milestone 2: xCT Design

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: 1ST
period covered: FROM March 01, 2008 to February 28, 2009
FMT-XCT
Work Package 2 : « XCT Design »
Deliverable 2.1

Date: 2nd November 2008
Ref (chrono): DTBS/STD/LDET 08-82

Participants to the tasks:

CEA-LETI: J.M. Dinten, A. Da Silva, V. Rebuffel
VAMP: H. Bruenner, O. Ott, M. Karolczak

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<th>J.M. Dinten/A. Da Silva/V. Rebuffel</th>
<th>Function</th>
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<tr>
<td>Verified and approved by:</td>
<td>V. Rebuffel</td>
<td>Scientific Project Manager WP2</td>
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List of distribution

FMT-XCT Consortium:
- HMGU
- V. Ntziachristos
- CEA LETI
- V. Rebuffel
- CEA SHFJ –LIME
- B. Tavitian
- FORTH
- G. Zacharakis
- UCL
- S. Arridge
- FIBGHM
- JJ. Vaquero Lopez
- UZH
- M. Rudin
- VAMP
- H. Brunner

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Historical

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<td>J.-M. Dinten / LETI, 2008-05-28</td>
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<td>Added Req. for interfacing, confirmed and amended X-ray specs.</td>
<td>H. Bruenner / VAMP, 2008-06-19</td>
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<td>H. Bruenner / VAMP, 2008-07-11</td>
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<td>A. Da Silva / LETI, 2008-07-29</td>
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<td>H. Bruenner / VAMP, 2008-09-08</td>
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Summary

The objective of this document is to provide the FMT-XCT consortium with a specification of the X-Ray acquisition chain and of the global gantry of the FMT-XCT design. In a first section this document specifies the terms FMT-XCT prototype and XCT laboratory bench, and precises how the XCT design specifications will be taken into account for the XCT the laboratory bench. In the second part, the geometry, the X-ray detector, the X-Ray source, the CT acquisition protocol are specified. The third section provides additional details on mechanical, electrical and software requirements. Section 4 recalls the responsibilities of each group and in section 5 are recalled the lists of requirements.

Keywords

Work package 2; XCT development; Specifications.
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1 Introduction

Objective of the document

The main purpose of the present document is to provide the consortium with a pre-description of the X-Ray acquisition chain of the FMT-XCT prototype. To this end, this report is written to evolve all along the duration of the project in order to end with an optimal design for the micro X-ray CT system that can facilitate optics. The options for the design retained in this report follow the main characteristics and specifications discussed at the kick-off meeting.

The final objective of this report is to define the optimal specifications regarding the X-ray imaging chain of the final FMT-XCT prototype that is to be integrated by VAMP (WP5). For the methodology developments (WP2), a homemade XCT laboratory bench will be used as a dedicated intermediate step handled by CEA-LETI.

Main characteristics of final FMT-XCT prototype as described in WP5

“The proposed system will employ a microfocus X-ray tube, and a solid state digital X-ray image sensor for the image acquisition; implementing cone-beam geometry. A computer will operate the X-ray source, an X-ray stopping shutter (to minimize X-ray dose per study) and the frame grabber to acquire the projections. Administered dose will be within the European and LETI guidelines for small animal XCT as applied to similar commercial micro-CT systems, ensured by VAMP. The system will be tightly enclosed into a 4mm lead sheet chamber to minimize stay radiation.”

Fig.1 depicts a simplified drawing of the FMT-XCT prototype.

Description of CEA-LETI XCT laboratory bench dedicated to WP2 developments

In parallel to the development of the FMT-XCT prototype, CEA-LETI will work on a laboratory XCT platform, highly versatile/flexible for development and improvement of the dual energy method. This system intends to be representative of the final prototype image quality, in order to achieve dual-energy CT developments, but does not pretend to fulfil all the requirements of the final prototype especially regarding the geometry, the velocity of data acquisitions... It has nevertheless to take into account that all the methodology of data acquisitions and the processing will have to be transferred towards the final FMT-XCT prototype.

The whole XCT laboratory bench system is placed inside a dedicated shielded XRay room, with a command monitor located outside the room. The different devices of the laboratory bench are placed according to a horizontal axis and the tomographic axis is vertical (Fig. 2 and 3). The imaging chamber is placed on a rotating stage, allowing a complete examination of the animal along 360°, while detector and generator are kept motionless. At each rotation step, the system acquires a radiography (or 2 radiographies at 2 different energies if the dual-energy method of acquisition is considered). Its X-Ray generator is a monoblock X-Ray source, manufactured by Gilardoni (Italy) with a CW power of 500W. The kV ranges from 40 to140 allowing Xray emission with an energy spectrum ranging from 10 to 140 keV. The flux of the radiation emitted by the tungsten anode is tuned by the electrode current supply ranging from 1 to 7 mA. The Xray detector is a C7942CK flat panel distributed by Hamamatsu (Japan).
This flat panel is composed with a matrix of 2240x2344 pixels of size 50 μm. The sensibility ranges from 20 kV to 80 kV. This system of acquisition is coupled with a Feldkamp algorithm, classically adopted for cone beam geometry, used for the cone-beam CT reconstructions.

Hereafter are listed the elements regarding the design of the X-Ray acquisition chain and the gantry for the FMT-XCT prototype.
2 Design of X-Ray acquisition chain for the FMT-XCT prototype

2.1 FMT-XCT prototype: Schematics

The elements of the figures will be explained in the following sections.

Fig.4: Design proposal

Fig.5: Block diagram of the components:
2.2 Geometric design

As the FMT-XCT prototype will integrate on the same gantry the XCT and the FMT functionalities, the following geometrical characteristics have been determined in regards of the geometrical gantry of the FMT gantry presently developed at IBMI.

The present gantry of IBMI is circular and with a diameter of 1m. However, given the detector which shall be used and the availability of air-cooled x-ray sources with appropriate output power, a smaller distance is desirable to avoid loss of dynamics in the x-ray projections due to insufficient exposure and efficiency of the detector. Hence, it was agreed on a distance between the X-Ray source and the X-ray detector of 400mm. It shall be determined during actual development, if and how the optical parts can be fitted in a way, so that they a) stay out of the x-ray beam path, b) the x-ray chain parts do not disturb the optics FOV, c) the scattered x-ray energy to the CCD camera is minimized as low as possible. An angle of 90° between x-ray and optical imaging chain would be common, as depicted in the figure above. Other configurations shall be investigated. Timing between X-rays acquisitions and optical acquisitions will also condition possible geometric design.

In order to carry out the tomographic acquisitions by rotation of the set generator/detector, the examined object will be situated at equal distance between the source and the detector, i.e:

**Distance (X-Ray source – Object ) = Distance (Object – X-Ray detector) = 200mm**

This geometry leads to a magnification factor of 2.

![Magnification](image)

As the system is expected to be used for mouse and rat examination, the maximum field of view to be considered is a cylinder of diameter 60 mm.

In order to limit irradiation to the needed field of view of the considered object an adaptable collimator will be put at the output of the X-ray source. Additionally a shutter will be employed.

The global geometry implemented for the X-Ray acquisition chain will be a conical geometry, obtained with a 2D X-Ray detector and an adapted collimation of the X-Ray source.

The expected voxel size of the reconstructed object should be the state of the art value for small animal CT systems, i.e 0.04 to 0.05 mm. According to Shannon sampling criterion, this corresponds to a resolution of 0.08 to 0.1 mm.

2.3 Logical / electrical design considerations

The base design is comprised of two logical control units. The external control PC runs the GUI for all functions of the whole prototype system. Also, it runs the control software for the optical setup as well as the frame grabber for the FP detector. The gantry itself, along with all safety systems, motion controllers and auxiliary systems, shall be controlled by an PLC unit (cyclic numeric control). Whenever possible, the peripheral components such as the controllers for the motors, HV, ... shall be interfaced with CAN bus, depending on the components used, other interfaces can be easily added to the PLC module. Bus operation as well as several different interfaces may lead to timing issues, which
have to be determined beforehand. Especially the timing with mixed mode operation (X-ray control by PLC, detector readout by external control PC) has to be investigated closely. VAMP will provide simulations and system analysis beforehand. Alternatively a PC/104 system could be used, which could also control image acquisition.

### 2.4 X-Ray detector characteristics

In order to carry out conical geometry, a **digital flat panel** X-Ray detector will be considered.

Due to the magnification factor of 2 and the expected resolution on the examined object, a **pixel size on the detector between 0.08 and 0.1 mm** will have to be chosen.

To respect an object field of view of 60mm, the detector should present a size of about **120x120 mm²**

According to these specifications following flat panel X-Ray detectors proposed by Hamamatsu might be considered:

<table>
<thead>
<tr>
<th>Detector type</th>
<th>Pixel size (mm)</th>
<th>Area (mm x mm)</th>
<th>Frame rate (frame/s)</th>
<th>Energy range (kV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9311</td>
<td>0.100</td>
<td>124.8 x 115.2</td>
<td>30</td>
<td>20 - 80</td>
</tr>
<tr>
<td>C7942*</td>
<td>0.050</td>
<td>120 x 120</td>
<td>2</td>
<td>20 - 100</td>
</tr>
<tr>
<td>C7943</td>
<td>0.100</td>
<td>124.8 x 124.8</td>
<td>7</td>
<td>20 - 100</td>
</tr>
</tbody>
</table>

* C7942 is the detector that CEA-LETI will use for the development of the dual-energy CT methodology on the laboratory bench.

According to manufacturer specifications C9311 has 50 to 60 times higher sensitivity than the C7942, that is saturates at radiation dose 50-60 times lower. As a consequence statistical noise level rises significantly (> 5x). This makes this detector inappropriate for the planned application (despite its frame rate).

Concerning the C7942, compared to 7943 this detector exhibits 4x lower charge capacity (electrons per pixel), so even in a binning mode the maximum signal to noise ratio is at least twice lower than 7943.

Consequently, the 7943 is preferred.

In order to deal with the X-Ray scattering that will be generated by the conical geometry, the **positioning of an adapted anti-scatter grid** in the system will be planned.

The exact interface specification will be provided by VAMP, the responsibility for the actual development of the actual reading and control software should be taken by the Integrator. Vamp will provide sufficient interfaces to make the control easy.

A software for the monitoring of the detector readings and control (Hamamatsu C7942/43) of LETI’s laboratory bench will be developed at LETI and LETI will make it available to VAMP (IBMI?) as a model for implementing the monitoring of the detector chosen for the final prototype.

### 2.5 X-rays generator characteristics

Due to the geometrical constraints and to the expected resolution on the observed object a **focal spot of about 0.080 to 0.100 mm** has to be chosen. If possible, smaller sizes should be preferred, if the available/required x-ray power allows for that.

As this generator will be included in a tomographic gantry with a rotation of the acquisition chain around the observed animal, the X-ray source should be either a **monoblock system** or a **tube cooled by air**. This limits the available power to approximatively 100 W. On the other hand, a minimum of 50 Watts is required for adequate exposure of the detector.
For small animal dual energy examination the range of energy of the X-ray tube should be between 35/40kVp to 80/100kVp.

According to these specifications the following X-ray tubes and generators might be considered:

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Type</th>
<th>Spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamamatsu, Japan</td>
<td>L9631, air cooling</td>
<td>40-110 kV, 0.8 mA, 50 W,</td>
</tr>
<tr>
<td></td>
<td>integrated HV generator</td>
<td>15-80 µm focus,</td>
</tr>
<tr>
<td>Oxford Instruments, USA</td>
<td>UltraBright, forced air cooling</td>
<td>20-90 kV, 2.0 mA, 80 W,</td>
</tr>
<tr>
<td></td>
<td>integrated HV generator</td>
<td>13-40 µm focus</td>
</tr>
<tr>
<td>RTW, Germany</td>
<td>MCBM 65 B – 50, , air cooling</td>
<td>20-65 kV, 1.1 mA, 50 W,</td>
</tr>
<tr>
<td>Spellman, USA</td>
<td>XRM external HV generator</td>
<td>50 µm focus</td>
</tr>
</tbody>
</table>

Best suited seems to be the Oxford system, so development will start with this tube.

In addition, ample provisions for inserting different filters into the beam path (e.g. aluminium pre-filtering) have to be provided. Optionally, form filtering should be considered (at least for comparison purposes). It has to be taken into account that pre-filtration costs intensity, therefore leads to longer exposure times. For the purpose of taking dual spectrum images on the same position immediately after each other, plus eventually a dark field image, an automated filter wheel will be provided. The filters can be selected beforehand and changed during operation. Although this leads to an extended scan time, this approach will probably be chosen for flexibility, and the prototype should make it possible.

### 2.6 Characteristics for CT

In order to keep a moderate complexity of the prototype the tomographic system will be without slip ring. The gantry will therefore have to be operated in non-continuous rotation mode. Connection to the rotor will be made by appropriate cable carrier system. The possible rotation angle will be of the order of 380° to allow for data completeness under all circumstances.

If LE and HE data acquisitions are performed sequentially, the rotation will be done within a bidirectional mode, e.g. with one rotation in clockwise orientation and the following rotation counterclockwise. In that case the misalignment parameter set will have to be determined separately for the two directions of rotation.

If LE and HE data acquisitions are performed alternatively, then a simple rotation mode is required.

The base acquisition mode could be step-and-shoot, meaning the gantry will rotate for a (small) angular increment, then stop for acquisition of x-ray projection, opening the shutter for the desired exposure time, reading the detector, closing the shutter and moving further. Every n (n determined by software control) projections, the system will not move but halt for the acquisition of optical projections. There the control PC of the optical system will send appropriate messages to the gantry control. Only after the optical acquisition is finished, the gantry will resume normal operation pattern. It is expected this pattern will reduce motion artefacts, but makes for needing an appropriate shutter mechanism.

Other acquisition patterns include separate rotations for XCT and FMT acquisition, e.g. one clockwise rotation without shutter operation for XCT, turning off x-ray power, one counterclockwise rotation for FMT.

As the maximum size of the objects will be of 60mm and the expected resolution of about 0.08 to 0.1mm, the corresponding discretization of the object will be about 1200 to 1500 sampling points, leading to a number of acquisition angles of about 1200 to 1500. This will lead to data volumes of about 3.5 GB per set (7 GB per dual energy set).

Acquisition time for each projection should be between 0.2s (1200 acquisitions in 4mn) and 0.08s (1500 acquisitions in 2mn), corresponding to 5 to 12 frames/s. For dual energy CT, this acquisition time has to be multiplied by two to take into account that at low energy and high energy images have to be acquired and stored. Also the acquisition mode plays an important role; step-and-shoot requires for the shutter to open and close, and the gantry to reach the next position between projections. The
expected scan time will therefore be of minimum 4 minutes per single energy XCTscan. Longer scan times are to be expected, if the tube/detector combination needs a higher flux to reach ample saturation. Otherwise, loss of dynamic will occur.

2.7 System shielding

The system shall be enclosed in a housing suitable for blocking x-ray radiation to the ambient. This can be achieved by using 4 mm of lead (Pb) which is placed on the inside of an appropriate designed metal housing. Safety interlocks are to be employed. The overall design of radiation protection gear will be guided by the standards described in section 4.4.2.

For safety reasons as well as in order to minimize dose load for the animal, the x-ray radiation shall be interrupted for times without acquisition. To achieve this, a shutter unit in addition to the collimators must be provided. Since shutter operation is time-consuming, switchable HV generators would be preferred, although there is actually no generator known to us, which provides this feature.

2.8 Software

The system shall be controlled by a GUI based software, which integrates the different interfaces to the gantry control, the optical and x-ray detectors and the illumination control. The tender specification for the interfaces will be derived during development of the gantry. Also, use cases, workflow and parameterization will be developed successively. VAMP will provide definitions of the interfaces, the Integration into the GUI will be carried out by VAMP and the optics group. Of course LETI should bring its expertise acquired on LETI’s laboratory bench development.
### 2.9 Summary of the XCT x-ray imaging chain design

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance X-Ray source to X-Ray detector</td>
<td>400 mm</td>
</tr>
<tr>
<td>Distance X-Ray source to object</td>
<td>200 mm</td>
</tr>
<tr>
<td>Distance object to X-Ray detector</td>
<td>200 mm</td>
</tr>
<tr>
<td>Maximum diameter of the examined object</td>
<td>60 mm</td>
</tr>
<tr>
<td>Resolution of reconstructed object</td>
<td>0.08 to 0.1 mm</td>
</tr>
<tr>
<td>X-Ray source collimation</td>
<td>Cross collimation</td>
</tr>
<tr>
<td>Source filtering</td>
<td>Filter wheel with at least 3 Positions, one empty.</td>
</tr>
<tr>
<td>Detector pixel size</td>
<td>0.08 to 0.1 mm</td>
</tr>
<tr>
<td>Detector area</td>
<td>≥ 120 x 120 mm²</td>
</tr>
<tr>
<td>Frame rate</td>
<td>≥ 5 frames/s</td>
</tr>
<tr>
<td>Anti-scatter grid</td>
<td>Provisions integrated; Grid tbd</td>
</tr>
<tr>
<td>Shutter</td>
<td>Integrated, System tbd</td>
</tr>
<tr>
<td>X-ray tube focal spot</td>
<td>≤ 0.08 to 0.1 mm</td>
</tr>
<tr>
<td>X-Ray source power</td>
<td>&lt; 100 W</td>
</tr>
<tr>
<td>X-Ray source type</td>
<td>Forced air cooling</td>
</tr>
<tr>
<td>X-Ray source energy range</td>
<td>35/40kVp to 80/100kVp</td>
</tr>
<tr>
<td>Scan time</td>
<td>Min 4 minutes*</td>
</tr>
<tr>
<td>Number of acquisitions angles</td>
<td>≥ 1000</td>
</tr>
<tr>
<td>Data volume per set</td>
<td>Approx. 3-4 GB</td>
</tr>
</tbody>
</table>

*Note: Step-and-shoot mode with filter interchange may significantly increase scanning times. Step and shoot times depend on the speed of the interchanging of the filters, the shutter speed… which have to be examined during development.
3 Requirements

3.1 Mechanical and basic design requirements

The gantry will be comprised of two main parts, the strator and the rotor.

The strator will be made from aluminium profiles to provide stable and vibration-free support for the rotor. Also, it holds all components, which do not have to go on the rotor, like auxiliary power supplies, mains power and electrical wiring. From the strator to the rotor, there will be a means of cable management to ensure free motion of the rotor for the specified angular movement.

The rotor will be mounted to the strator by means of a ball bearing to ensure accurate radial round-out. This limits the free diameter in the center to approximatively 200 mm max. All parts will be finished in black by powder coating, varnish or anodizing to minimize stray light. The components of the optical and X-ray imaging chains will be mounted on adjustable units (2d) to allow for fine adjustment of the relative positions.

The rotational motion will be realized by a belt-drive system. The angular position of the rotor will be monitored by suitable angular sensors and end switches to avoid range errors and allow for referencing.

The mouse bed will be mounted on a linear motion unit which can be controlled by the PLC on behalf of the GUI.

Base mechanical constraints:

Rotor:
- Position accuracy absolute / step-to-step open loop better than +/-10’ (+/-6’ expected), closed loop better than +/-0,015° with balanced system

Linear table:
- Position accuracy better than 0.1mm
- Max speed of movement better than 1cm/s

The system will be enclosed in appropriate housing, providing light and radiation shielding. A monitored opening in the cover will allow for access to the mouse.

3.2 Electrical requirements

The details of the electrical requirements have to be developed in the process. Base requirements include:

- Electrical power req.: 230V / 16 A
- safety interlock system according to RöV
- Small voltage supplies for components on-gantry, less than 48 V
- Connections GUI – Gantry:
  - to PLC: Ethernet, CAN or similar system
  - to detector: RS 485, 12 bit digital Framegrabber connection, 36p for status and command signals
  - to ShutterController: TTL logical signal
  - to optical chain: to be defined by the optics group.
3.3 **Signals**
- GUI → PLC: Position commands, program commands, status requests, status signals
- GUI → Detector: Trigger_Exposure, BinningMode
- GUI → ShutterController: Trigger_OpenShutter
  (logical AND with PLC → ShutterController Enable_ShutterOpen)
- Detector → GUI: Image_Data
- PLC → GUI: Position acknowledge, command acknowledge, status signals

3.4 **Software requirements**

*Programming of the control software / GUI*

The GUI will have to be programmed by the integrator. VAMP will provide the information necessary to realize conversation between the GUI PC and the gantry control PLC.

To be defined along with the development:
- Workflow
- Use cases (intended use / step-by-step definition of events and reactions)
- Error handling
- Communications protocol
- Program architecture

For this task, VAMOP and IBMI will draft and discuss a separate specification sheet.
## 4 Responsibilities

### 4.1 VAMP

VAMP will provide

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>consisting of</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray chain</td>
<td>x-ray-tube</td>
<td>Oxford</td>
</tr>
<tr>
<td></td>
<td>Fast shutter system</td>
<td>For cancelling radiation while in step-and-shoot acq. mode</td>
</tr>
<tr>
<td></td>
<td>Cross collimators</td>
<td>Standard VAMP part</td>
</tr>
<tr>
<td></td>
<td>Detector</td>
<td>As defined above, C7943</td>
</tr>
<tr>
<td></td>
<td>Detector drivers (?)</td>
<td>If neccessary</td>
</tr>
<tr>
<td></td>
<td>Fixtures for antiscatter grid</td>
<td>Detector side</td>
</tr>
<tr>
<td></td>
<td>Fixtures for prefiltrers</td>
<td>Tube side</td>
</tr>
<tr>
<td></td>
<td>Filter wheel</td>
<td>DE acquisition</td>
</tr>
<tr>
<td>Linear table</td>
<td>Linear motion table</td>
<td>z-translation</td>
</tr>
<tr>
<td></td>
<td>Linear motion controller</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tray fixture</td>
<td></td>
</tr>
<tr>
<td>Gantry</td>
<td>Base frame (strator)</td>
<td>Including bearing and all mechanical parts</td>
</tr>
<tr>
<td></td>
<td>Base plate (rotor)</td>
<td>including all fixtures for x-ray- and optical component. Fixtures for opt. comp tbd</td>
</tr>
<tr>
<td></td>
<td>Gantry drive</td>
<td>(motor &amp; belt drive &amp; gear)</td>
</tr>
<tr>
<td></td>
<td>Reference marks and Position sensors for rotary motion</td>
<td></td>
</tr>
<tr>
<td>Optical chain</td>
<td>Fixtures for optical equipment</td>
<td>To be defined by optical group</td>
</tr>
<tr>
<td></td>
<td>Electrical supply for o. e.</td>
<td>As above</td>
</tr>
<tr>
<td>Control</td>
<td>System controller (plc)</td>
<td>PLC system, CAN bus interface or similar (tbd)</td>
</tr>
<tr>
<td></td>
<td>Motion controller rotor drive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Motion controller linear drive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interlock logic driver</td>
<td></td>
</tr>
<tr>
<td>Housing</td>
<td>Lead-shielded housing (box)</td>
<td>accession doors, design etc tbd</td>
</tr>
<tr>
<td></td>
<td>Safety interlock switches</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radiation indicator</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Power supplies for all components</td>
<td>Exact spec tbd</td>
</tr>
<tr>
<td></td>
<td>Electrical interfaces</td>
<td>According to standards</td>
</tr>
<tr>
<td></td>
<td>Cable management</td>
<td></td>
</tr>
<tr>
<td>Software</td>
<td>Control and safety software for</td>
<td>Frontend has to be programmed by final integrator (ie the one who programs the optical</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Project</th>
<th>N° Chrono</th>
<th>Date</th>
<th>Author</th>
<th>Issue</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMT-XCT</td>
<td>DTBS/STD/LDET 08-82</td>
<td>2008-11-02</td>
<td>J.M. Dinten/ A. DaSilva/ V. Rebuffel</td>
<td>1</td>
<td>15/22</td>
</tr>
</tbody>
</table>
PLC part also – integrated GUI)
SW interfaces to windows tbd, maybe DLL, other communication protocol possible
(GUI software) In cooperation with IBM

<table>
<thead>
<tr>
<th>Documentation</th>
<th>Full description of assembly and function for controlling the gantry system, including how to program the Windows-frontend</th>
</tr>
</thead>
<tbody>
<tr>
<td>User manual</td>
<td>X-Ray and optical risks can only be addressed after completion of final system</td>
</tr>
<tr>
<td>Risk analysis for all mechanical and electrical parts. The system will be build according to, but not formally be tested for compliance with the RöV and EMC! If desired, the tests can be carried out after completion of the system. This has to be done by an external laboratory / notified body. Costs not included in proposal, since as a prototype, the system can be operated without. But no CE mark, since the gantry is part of a superordinated system and cf. the remark above.</td>
<td></td>
</tr>
<tr>
<td>Declaration of conformity</td>
<td></td>
</tr>
</tbody>
</table>

### 4.2 LETI

LETI will provide

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>consisting of</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE Acquisition protocol</td>
<td>Specification of Energy spectra to be used (Voltage and filters), and of acquisition protocol</td>
<td>Including recommendation on alternate / sequential LE and HE acquisition, requirement of dark field image</td>
</tr>
<tr>
<td>DE Calibration protocol</td>
<td>Definition of calibration objects (material, thickness), protocol</td>
<td></td>
</tr>
<tr>
<td>Data processing method, software</td>
<td>Processing method of the two acquisition data sets, corresponding software</td>
<td>Reconstruction after / or before DE processing, decomposition method resulting in material specific images or (rho, Z) images, specific processing to enhance the contrast while reconstruction process</td>
</tr>
<tr>
<td>Scatter correction software</td>
<td>Analysis of requirement of scatter correction, and if so, scatter correction method and software</td>
<td></td>
</tr>
<tr>
<td>Global system</td>
<td>Optimization of all the parameters of the system</td>
<td>Including resolution vs number of angles</td>
</tr>
<tr>
<td>Global system</td>
<td>Optimization of delivered dose</td>
<td></td>
</tr>
<tr>
<td>FMT-XCT prototype</td>
<td>Specifications</td>
<td></td>
</tr>
<tr>
<td>FMT-XCT prototype, detector</td>
<td>Spec and prototype of detector control and readout</td>
<td></td>
</tr>
<tr>
<td>Global method evaluation</td>
<td>DE-CT experiments and report</td>
<td>Including comparison with contrast agent method</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FMT-XCT</th>
<th>DTBS/STD/LDET 08-82</th>
<th>2008-11-02</th>
<th>J.M. Dinten/ A. DaSilva/ V. Rebuffel</th>
<th>1</th>
<th>16/22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project</td>
<td>N° Chrono</td>
<td>Date</td>
<td>Author</td>
<td>Issue</td>
<td>page</td>
</tr>
</tbody>
</table>


4.3 **Optics group @ IBMI**

The optics group will provide:

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>consisting of</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imaging chain</td>
<td>Camera and lighting for FMT</td>
<td>To be defined in other work packages</td>
</tr>
<tr>
<td></td>
<td>Specification (mech/elec) for above</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td></td>
<td>FMT recon</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td></td>
<td>FMT protocols</td>
<td>&quot;-&quot;</td>
</tr>
<tr>
<td>Software</td>
<td>GUI software</td>
<td>In cooperation with VAMP, IDE and OS to be defined, separate specification needed.</td>
</tr>
</tbody>
</table>

4.4 **Documents**

4.4.1 **Communication during design and manufacturing**

During design, VAMP will provide working drafts of the construction on request by LETI. Responsible for communication (team leaders):

VAMP: H. Bruenner

LETI: V. Rebuffel

Communications between other members of the respective teams as necessary, team leaders shall be informed, eg. by cc: mail.

Each member of the team will hold records on activities as appropriate (no formal spec needed)

**Milestones (preliminary dates):**

1. Final decision on the x-ray components VAMP/LETI 09/08
2. Approval of design proposal by VAMP VAMP 11/08
3. Approval of design by LETI and the optics group LETI 01/09 after redesign
4. Draft and Approval of GUI design IBMI/VAMP 01/09
5. manufacturing and putting into operation of the system VAMP 6/09
6. finalizing of system including GUI VAMP/IBMI 06/09

⚠️ Has to be checked against project plan!

4.4.2 **Standards to be applied:**

- Röntgenverordnung (RöV) 2006 declaration of conformity with the basic requirements for x-ray radiation shielding
- ISO 12100-1 2006 Machine safety, Part 1: General
- ISO 14971 2007 Risk management
- EN 61000-6-2 2006 EMC: active
EN 61000-6-4  2002  EMC: passive
EN 61010-1  2004 (2008) Safety requirements for electrical equipment for measurement, control, and laboratory use
EN 60204-1  2007 Safety of machinery - Electrical equipment of machines.

To be extended as appropriate.
5 List of requirements

The following lists summarize the requirements for the fully functional prototype from an user’s point of view. The requirements listed shall be part of the pass/fail tests and be translated into performance specifications according to the following naming conventions and definitions apply:

(The system) **shall** (have/be/do…)  soft requirement; feature which is to be realized if possible within reason, i.e. within the given financial or timely constraints.

(The system) **shall not** (have…)  Opposite of above, feature which shall be avoided if possible

(The system) **must** (have…)  Hard requirement, feature that has to be realized under any circumstances

(The system) **must not** (have…)  Opposite of above, knock-out-criteria, unacceptable feature / behaviour / property

(The system) **may / might / can / could <not>** (have…)  Possible realization of specific feature or possible use case

**Abbreviations and definitions :**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLC</td>
<td>Programmable logic control. Used as generalization for the controller, regardless of the actual implementation (PC architecture / PLC / PC/104…)</td>
</tr>
<tr>
<td>Rotor</td>
<td>Moving part of the gantry, platform to mount the imaging chains onto</td>
</tr>
<tr>
<td>Strator</td>
<td>Immobile part of the gantry, holding the rotor and supply components</td>
</tr>
<tr>
<td>UI</td>
<td>User interface, usually graphically realized by a Windows application (syn.: GUI) but here also used as general term for any kind of interfacing of the user with the system; meaning also switches, signal lamps or sounds, other means of interaction.</td>
</tr>
<tr>
<td>Gantry</td>
<td>Synonym: System, Prototype. Identifies the assembly which holds the x-ray and optical parts and allows controlled movement of the parts in a way suitable for XCT and FMT.</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view; transversal geometrical area in the isocenter of the gantry which is to be examined</td>
</tr>
</tbody>
</table>
5.1 General requirements

<table>
<thead>
<tr>
<th>ID</th>
<th>Requirement</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-001</td>
<td>The system must be designed as vertically rotating CT-like gantry</td>
<td></td>
</tr>
<tr>
<td>G-002</td>
<td>The system must fulfil the requirements defined by the optical group</td>
<td>To be done; this req. govern space and weight, electrical and geometrical specifications for the optical assembly</td>
</tr>
<tr>
<td>G-003</td>
<td>The system must be capable of acquiring XCT and FMT projections at defined angular positions</td>
<td></td>
</tr>
<tr>
<td>G-004</td>
<td>The system must have a linear table for moving the mouse bed</td>
<td></td>
</tr>
<tr>
<td>G-005</td>
<td>The magnification of the x-ray chain shall be equal, that is 2:1</td>
<td></td>
</tr>
<tr>
<td>G-006</td>
<td>The distance between source and detector of the x-ray chain shall be 400 mm</td>
<td></td>
</tr>
<tr>
<td>G-007</td>
<td>The diameter of the FOV must be no less than 60 mm</td>
<td></td>
</tr>
<tr>
<td>G-008</td>
<td>The axial length of the FOV shall be 60 cm</td>
<td></td>
</tr>
<tr>
<td>G-009</td>
<td>The optical camera shall be protected from the x-ray-radiation</td>
<td></td>
</tr>
<tr>
<td>G-010</td>
<td>The resolution of the XCT system shall be on a magnitude of 0.1 mm</td>
<td>0.08 – 0.1 mm</td>
</tr>
<tr>
<td>G-011</td>
<td>The projections must be acquired by an external PC bearing a frame grabber card and linked / synchronized to the PLC</td>
<td></td>
</tr>
<tr>
<td>G-012</td>
<td>The system could be capable to be operated unattended</td>
<td>FMT scans last several hours, automation would be nice</td>
</tr>
<tr>
<td>G-013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2 Functional requirements

<table>
<thead>
<tr>
<th>ID</th>
<th>Requirement</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-001</td>
<td>The system shall be capable of acquiring a complete XCT dataset in 4 minutes for one energy scan in continuous mode (ie no step-and-shoot)</td>
<td></td>
</tr>
<tr>
<td>F-002</td>
<td>The angle of rotation must be no less than 380°</td>
<td>Cf. Req. F-005</td>
</tr>
<tr>
<td>F-003</td>
<td>The xray source shall have the characteristics described in 2.5</td>
<td>Cf. 2.5</td>
</tr>
<tr>
<td>F-004</td>
<td>The x-ray detector shall have the characteristics described in 2.4</td>
<td>Cf. 2.4</td>
</tr>
<tr>
<td>F-005</td>
<td>The system can be designed without slip-ring</td>
<td>Ample cable management provided</td>
</tr>
</tbody>
</table>
F-006 The rotor must be mounted to the stator by ball bearing
Radial round-out better than… tbd

F-007 The gantry shall have a clear aperture of 200 mm

F-008 The animal bed shall be mounted on the side of the rotor opposed to the mounting of x-ray and optical components

F-009 The animal bed shall have a linear travel of no less than 100 mm
To be defined!

F-010 The fixture for the animal bed must be interchangeable

F-011 The drive for angular motion shall be designed as belt drive gear unit, alternatively direct gear drive

F-012 The position of the rotor shall be monitored
Accuracy cf. F-014

F-013 The minimum time for a complete rotation (380°) shall not be longer than 10 s
Fast gear for referencing and course positioning

F-014 The angular resolution (accuracy and repeatability) of the rotation must be greater than 0.25° (15'), it shall be better as or equal to ~0.08° (5')
Expected in open loop mode: +/- 6', in closed loop approx +/- 0.015° with balanced system, cf. 3.1

F-015 The x-rays must be blockable by a shutter
Shutter system integrated

F-016 The x-ray source must be equipped with provisions for mounting prefilter materials

F-017 The detector shall be equipped with fittings for mounting antiscatter grids

F-018 To minimize dose, collimation shall be integrated
Controllable collimators employed

F-019 The system must work under standard environmental conditions
Laboratory use according to ISO 61010-1

F-020 Automatic source filtering
Controlled by software, synchronized with x-raxy controller

5.3 Nonfunctional requirements

<table>
<thead>
<tr>
<th>ID</th>
<th>Requirement</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-001</td>
<td>The system shall be controlled by external PC GUI (command interface)</td>
<td></td>
</tr>
<tr>
<td>N-002</td>
<td>The system shall be controlled by internal PLC or similar (internal control)</td>
<td></td>
</tr>
<tr>
<td>N-003</td>
<td>The system must be shielded to prevent emission of x-ray radiation (&quot;Vollschutzgerät&quot;)</td>
<td></td>
</tr>
<tr>
<td>N-004</td>
<td>The system should be usable without special training</td>
<td></td>
</tr>
<tr>
<td>N-005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 5.4 Safety and legal requirements

<table>
<thead>
<tr>
<th>ID</th>
<th>Requirement</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-001</td>
<td>The radiation protection of the system must be in accordance with the legal requirements at the final site.</td>
<td>Final place of operation is Munich → German RöV applies.</td>
</tr>
<tr>
<td>S-002</td>
<td>The radiation protection measures must not be easily defeatable. Every opening designed to give access to the experimental area must be secured in a way, that either special tooling or keys is required or the radiation emission is blocked instantly on opening the housing.</td>
<td></td>
</tr>
<tr>
<td>S-003</td>
<td>The system must have safety switches (internal and external) to prevent damage to system, user, experimental animal or environment.</td>
<td></td>
</tr>
<tr>
<td>S-004</td>
<td>The system shall be designed and manufactured according to the standards EN 12100-1, EN 60204-1, EN 61000-x and EN 61010-1.</td>
<td></td>
</tr>
<tr>
<td>S-005</td>
<td>The system must meet conditions defined in ISO 61010-1.</td>
<td></td>
</tr>
<tr>
<td>S-006</td>
<td>The shutter system must be failsafe and protected against overheating.</td>
<td></td>
</tr>
<tr>
<td>S-007</td>
<td>The system shall be documented in a standard way.</td>
<td>User documentation according to standards mentioned above.</td>
</tr>
<tr>
<td>S-008</td>
<td>The system may not carry a CE mark.</td>
<td></td>
</tr>
<tr>
<td>S-009</td>
<td>The system shall undergo risk management measures. The results shall be documented.</td>
<td>ISO 14971</td>
</tr>
<tr>
<td>S-010</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DELIVERABLE NO 3.1: DIRECT INVERSION ALGORITHM

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: 1st
PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
Work Package 3: Theory for 360-degree FMT

DELIVERABLE 3.1

March 15, 2009
Jorge Ripoll, FORTH
Giannis Zacharakis, FORTH

D3.1 A direct inversion algorithm for fluorescence implementing boundary removal principles (Month 9)

In diffuse media, tomography is based on using measurements from the boundary to obtain 3D spatial maps of optical properties or fluorophore concentration. Image formation consists of computing solutions of a propagation model (typically the diffusion equation) to predict photon propagation in tissues (forward problem) and use these to form the inverse problem, calculating the most probable distribution in the medium which satisfies the boundary measurements. Many different approaches have been developed for solving the forward and inverse problems. The computation times required typically scale with a power-law to the data-set size and calculation requirements worsen when imaging tissues of arbitrary shapes, as the effects of the boundary need to be explicitly modeled by the forward model.

We have studied an approach which can significantly simplify and accelerate calculations in the tomographic problem. The method transforms surface measurements to measurements which would be obtained in the absence of the surface, i.e. if the diffusive volume were infinite and homogeneous. This approach has two important consequences: first, it allows the use of infinite Green functions to generate forward solutions, thus avoiding the use of complex numerical methods which solve for arbitrary geometries. Second, it enables the use of direct inversion methods by allowing data back-propagation from virtual detectors outside the volume to anywhere inside the volume and vice versa. Such transformation is not possible in the presence of arbitrary interfaces and it can allow for significant inversion acceleration, the computational complexity scaling as Nlog(N) instead of typically N^3, being N the number of measurements. We here present a derivation of this numerical transformation and study the performance achieved both in ideal and limiting experimental situations, i.e. in cases where there is varying spatial sampling or when the surface can only be partially accessed. Finally we present examples of the potential of this transformation by propagating measurements to an arbitrary point in space and then using this data to image the distribution of sources. Even though all the derivation is in the context of light diffusion, the solutions are more generally applicable in any situation of a dominant diffusive regime.

Let us consider the geometry depicted in Fig. 1(a) where we have a homogeneous diffusive volume $V$ with diffusion coefficient $D$, absorption coefficient $\mu_a$ and index of refraction $n_r$. 
In terms of the reduced scattering coefficient $s'$, $D$ is usually defined as $1 / s'$ in the presence of low absorption. We shall denote the surface that encloses volume $V$ as $S$. Let us assume that we have a source distribution $\Phi(r)$ inside the medium ($r \in V$) whose intensity is modulated at a frequency $\omega$. In this case the average intensity $U$ represents a diffuse photon density wave and obeys the Helmholtz equation, $\nabla^2 U(r) + \kappa^2 U(r) = -\Phi(r) / D$, $r \in V$ with a complex wave-number $\kappa$ given by $\kappa = ( - \mu / D + i \omega n_{\alpha} / c D )^{1/2}$, being $c$ the speed of light in vacuum. Taking into account rigorously the effect of the interface $S$, the average intensity $U$ inside volume $V$ is found through Green's theorem as:

$$
\kappa \int_{\partial S} \left[ \Phi(r) g \left( \frac{r \cdot \hat{n}}{s} \right) - g \left( \frac{r \cdot \hat{n}}{s} \right) \frac{\partial U(r)}{\partial \hat{n}} \right] dS,
$$

where $\hat{n}$ represents the surface normal pointing outwards and,

$$
U^{(inc)}(r) = \frac{1}{4\pi D} \int_{\partial S} \Phi(r') g \left( \frac{r \cdot \hat{n}}{s} \right) dV,
$$

is the average intensity that is obtained in the absence of the surface. In Eqs. (1) and (2) $g$ is Green’s function for an infinite homogeneous medium. The boundary condition between the diffusive and non-diffusive medium is given by $U(r) |_{S} = C_{nd} \cdot J(r) |_{S} = C_{nd} J_n(r) |_{S}$, where $J$ is the flux vector, $J_n$ is the total flux traversing the interface and $C_{nd}$ takes into account the refractive index mismatch between both media ($C_{nd}$~5 for the cases presented here). Substituting Fick’s law, $J = -D \nabla U$, into the boundary condition and applying it to Eq. (1) we obtain:

$$
U(r) = U^{(inc)}(r) \int_{\partial S} \left[ C_{nd} D \frac{\partial g \left( \frac{r \cdot \hat{n}}{s} \right)}{\partial \hat{n}} + g \left( \frac{r \cdot \hat{n}}{s} \right) \right] J_n(r) dS', \quad r \in V
$$

In order to solve the integral of Eq. (3) one usually either solves for the surface flux $J_n$ or for the average intensity $U$ at the boundary. This is typically achieved by using accurate algorithms such as the Boundary Element Method or approximations to it such as the Kirchhoff Approximation. Note that all the Green functions involved are infinite Green’s functions.

If we consider an experimental setup which enables us to detect the light that emerges from all points of the surface $S$, it is possible to experimentally measure the total distribution of emerging flux $J_n$. In this case $J_n$ can be directly substituted by the experimental measurement. High spatial sampling of the total outward flux on the boundary has become possible by using non-contact approaches recently developed, by projecting onto the surface the values measured at a CCD detector. In the case that $J_n$ is known we can directly obtain $U^{(inc)}$, i.e. the average intensity.
created by the source distribution in the absence of the interface. This effectively means that volume $V$ has become infinite filling all space with a diffusive medium of constant properties $D$, $\mu_a$ and $n_a$ [see Fig. 1(b)], while retaining the position of the sources and detectors. The measured infinite-case average intensity $U_{\text{meas}}^{\text{(inc)}}$ at each detector position $r_i$ for a total of $N$ detectors can be found as:

$$U_{\text{meas}}^{\text{(inc)}}(r_i) = C_{\text{cd}} J_{\text{inc}}^{\text{meas}}(r_i) - \frac{1}{4\pi D} \sum_{j=1}^{N} \left[ \frac{\partial}{\partial n_j} \left( g(\kappa |r_j - r_i|) \right) + g(\kappa |r_j - r_i|) \right] J_n(r_j) dA_j, \quad r_i \in S$$  

where we have represented each surface measurement of area $dA_j$ at $r_j$ as $J_{\text{inc}}^{\text{meas}}(r_j)$. We have introduced this notation in order to distinguish the measured quantities from $J_n(r_j)$ and $dS$ which give the rigorous solution in the case where the boundary is taken into account. This allows for partial coverage of the surface and/or detector areas that would otherwise compromise the accuracy. Note that care must be taken for $r_j=r_n$, the so-called self-induction term in electromagnetic theory, in which case the solution depends on the geometry of the area $dA_j$.

There has been extensive work devoted to the meaning and expression of this term. In order for the obtained values for $U_{\text{meas}}^{\text{(inc)}}$ in Eq. 4 to be accurate the following conditions need to be met: a) the (average) optical properties of volume $V$ need to be known a priori, b) the surface geometry must be known accurately (typical errors allowed are in the order of one transport mean free path), and c) we must have all values of the surface outward flux measured in order for the series in Eq. 4 to be equivalent to a surface integral. In practice it is possible to obtain accurately the surface geometry and it is possible to collect the outward flux in all positions, especially by using the new systems that offer 360° complete projection data acquisition. Even though Eq. 4 assumes an optically homogeneous volume of interest, it has been recently shown that the use of normalized measurements greatly reduces the effect of optical heterogeneity and its use is justified for tissue imaging.

In the following we study the accuracy of Eq. 4 for limiting cases where the number of surface measurements is reduced or the surface $S$ is known but only partially accessible. To study these limiting cases we performed a numerical simulation assuming a diffusive cylinder of radius 1 cm and index of refraction of water ($n_a = 1.333$) surrounded by air ($n_{\text{out}} = 1$). For simplicity we studied the 2D system, without loss of generality. The simulation consisted of 4 cw delta sources ($\omega = 0$) of equal strength inside this volume at locations $(x,z) = (-0.1,0.5), (0.1,0.5), (0.5,0.2)$ and $(-0.5,0.5)$ cm as shown in the inset of Fig. 2. Eq. (3) was then solved numerically by using the Boundary Element Method without any approximation.

**FIGURE 2**: Error in retrieving the infinite-case measurements versus the detector area ($dA$) for several values of the absorption ($\mu_a$) and scattering coefficients ($\mu_s'$). In all cases a cylinder of radius $R=1 cm$ with index of refraction $n_a=1.333$ surrounded by air ($n_{\text{out}}=1$) was considered (see text for source positions).
The corresponding infinite-case values were retrieved using Eq. 4 for a given detector area \( dA \). The error was measured in % as \( \left( 1 - \frac{U^{inc}_{\text{meas}}}{U^{inc}_{\text{meas}}} \right) \times 100 \), where \( \langle \cdot \rangle \) denotes average over all detector positions, \( U^{inc} \) being given by Eq. (2). Plots of the error as a function of different detector areas (i.e. different spatial sampling) are shown in Fig.2 for several absorption and reduced scattering coefficients within realistic in-vivo values. In this case we assumed the spatial sampling of detectors of area \( dA \) gives full coverage of surface \( S \). As can be inferred from Fig. 2, the effect of detector area is negligible, yielding errors that are less than 1% for detector widths as large as \( \sim1 \text{mm} \). Since typical experimental noise values are usually above 1%, the finite size of the detectors is not expected to be a limiting factor.

Let us now look at the case when it is possible to recover the outward flux \( J_n \) from only a part of the surface. In this study we have used the same geometry and source distribution as in Fig. 2, assuming two possible configurations, i.e. when the detectors span all the upper-half of the cylinder and are thus near the sources, and when the detectors span the lower-half. Results are shown in Fig 3, where both configurations are depicted in the insets.

**FIGURE 3:** Error in retrieving the infinite-case measurements versus the angular coverage of the detectors when these span the top-half (curves to the right) or the bottom-half (curves to the left). Two cases are presented, \( \mu_a = 0.02 \text{cm}^{-1} \) (solid line) and \( \mu_a = 0.2 \text{cm}^{-1} \) (dotted line). In all cases \( \mu_s' = 10 \text{cm}^{-1} \), for the geometry presented in Fig. 2.

We have studied the error in retrieving the infinite-case values versus the angular coverage: 0 degrees represents full coverage for the upper-half measurements (to the right of Fig. 3) and no coverage for the lower-half, whereas 180 degrees represents full coverage for the lower-half measurements (to the left of Fig. 3) and no coverage for the upper-half. In Fig. 3 we see that if we are near enough the sources (the upper-half spanned by the detectors in this case), we obtain results under 10% error even when covering only half of the surface (\( \theta = 90^\circ \) in the figure). This however is not the case if we choose this coverage in the lower-half where the error is over 30% and in the low-absorption case above 50%. Since one in principle does not know where the sources are \textit{a priori}, Fig. 3 evinces the fact that full-angular measurements are needed in order to obtain accurate images. As expected, Fig 3 further demonstrates that higher absorption values yield lower errors, since the effects of the boundaries diminish.

To showcase some of the important features of Eq. 4 we discuss here its relation with direct inversion methods. Since Eq. 4 effectively operates with infinite homogeneous media [see Fig. 1(b)], we may perform several classical transformations on our data. We have opted for a simple and visual approach that can be also applied to direct inversion methods, consisting on propagating the infinite-case data to arbitrary points in space outside volume \( V \). To that end we shall use the first Rayleigh-Sommerfeld integral formula which states that if the field (in this case the average intensity) is known at a virtual plane \( z=z_0 \), the field at \( z>z_0 \) is given by:
\[ U(R, z) = \frac{1}{2\pi} \int_{(x,y)} U(R', z' = z_o) \frac{\partial g(R - R', z - z')}{\partial z'} \, dx' \, dy', \forall z > z_o \]  

(5)

where we have written \( r \) as \( (R(x,y),z) \) to point out that this formula holds only for a flat surface \( z \), the integration being performed on the \( xy \) plane. A simple approximation to the contribution of an arbitrary surface \( S \) is to decompose the surface into its planar components \( dA(r) \) and assume that in each of these planes \( U \) is zero for all \( r \neq r_i \). In this approximation the average intensity \( U \) inside the surface integral of Eq. 5 can be substituted by \( U(r)\delta(r - r_i) \), and we may project the data by adding all the surface components by applying Eq. 5 to all \( N \) surface measurements [see Eq. 4] as:

\[ U_{inc}^{prop}(r) \approx \frac{1}{2\pi} \sum_{i=1}^{N} \left[ U_{inc,meas}(r_i) \frac{\partial g(k|r - r_i|)}{\partial \hat{n}_i} H((r - r_i) \cdot \hat{n}_i > 0) \right] dA_i \]

(6)

where \( H \) is the Heavyside step function which has been included so that only those points that are above the surface defined by the normal \( \hat{n}_i \) are considered for each surface area \( dA_i \). This is done to ensure that, at least locally, we do not measure \( U_{inc}^{prop} \) inside volume \( V \). In case \( S \) is a planar surface, Eq. 6 reduces to the discretized version of Eq. 5. In all other cases, Eq. 6 is an approximation to the real solution, being termed the Rayleigh Hypothesis in electromagnetic theory. Note that Eq. 6 can only be used when \( S \) is an interface between two diffusive media, which stresses the importance of the boundary removal formula, Eq. 4.

In order to test the example Eq. 6 we have performed numerical simulations equivalent to those in Figs. 2 and 3, for the same source distribution but two different geometries: a cylinder with constant radius \( R=1 \) cm and a cylinder with a sinusoidally-varying radius \( R = 1 \pm 0.1 \) cm with a period of \( \pi/6 \). In all cases we have assumed detector areas of \( dA \approx 0.05 \) cm. Results for two different absorption coefficients are shown in Fig. 4, where we plot the ratio of \( U_{inc}^{prop}/U_{inc}^{prop} \) using Eqs. 6 and 2.

**FIGURE 4:** Propagated value of the boundary-removed data normalized by the direct infinite-case measurement for two different geometries: a cylinder of constant radius \( R=1 \) cm and a cylinder with sinusoidally-varying radius \( R = 1 \pm 0.1 \) cm with period \( \pi/6 \). Results for two absorption values are presented, \( \mu_a=0.02 \) cm\(^{-1}\) (a) and (c) and \( \mu_a=0.2 \) cm\(^{-1}\) (b) and (d). In all cases \( \mu_s'=100 \) cm\(^{-1}\), \( n_i=1.333 \), \( n_o=1 \) and \( dA \approx 0.05 \) cm. The error included in each plot represents the error in retrieving the boundary-removed data at the surface.
In this figure several features need to be outlined: first, we can see that near the surface the values are close to unity. This is expected, since the closer we are to the surface the more locally-plane it is and the more Eq. 5 holds. Secondly and most remarkably, even though some of the propagated values may differ 30% from the direct source distribution measurements, this error is quite homogeneous throughout space yielding basically a constant difference. This difference will be considerably diminished and practically cancelled out when using normalized approaches. Finally, the propagated intensity is always less than the direct source-distribution measurements, except in the convex areas of Figs. 4(c) and (d) near surface S. In these cases we are adding contributions that actually do go inside volume V, violating Eq. 5.

The analysis presented above can be used to significantly accelerate inversion schemes by using direct inversion algorithms that yield inversion times of the order of seconds at current computational speeds. To demonstrate the tremendous impact of this approach we showcase the retrieval of the source distribution by forward-propagating the data from the surface S to a collection of virtual detectors outside volume V and then use these measurements to retrieve the source distribution and strength inside V. To that end, we have chosen the configuration and optical properties presented in Fig. 4(d) and have projected the data to a total of 168 detectors, whose spatial arrangement is shown in Fig 5 as bullets.

In order to enable retrieval of the source positions we have used 20 frequencies logarithmically spaced between $\omega = 10^9$ and $10^{12}$ rad/s (note that the inverse problem is not well defined using only the $\omega = 0$ case). The source distribution was retrieved by inverting Eq. (2) using the Algebraic Reconstruction Technique with 1000 iterations and positive restriction for a total of 1795 mesh points. Results are shown in Fig 5 where it can be seen that even in this extreme case (rough surface and forward-propagating data with an approximate expression) the positions and strengths (all sources are equivalent) are retrieved with remarkable accuracy. Note that the small artifacts that appear in the lower section are due to the forward propagating formula, Eq.6, and disappear when using the measurements at the boundary. Whenever using directly the surface values we found that the boundary-removed and the infinite values yielded images which differed less that 10% in absolute terms and presented undistinguishable distributions.

![Figure 5](image.png)

**FIGURE 5:** Inversion results for 1000 ART iterations with positive restriction for the boundary-removed measurements propagated to 168 detectors (shown as bullets). The optical properties and geometry used to generate the forward data are those shown in Fig. 4(d), for $\omega$ logarithmically spaced between $10^9$ and $10^{12}$ rad/s.

In conclusion, we have derived a method which effectively removes the contribution of the boundaries, in essence converting finite diffusive volumes into infinite diffusive volumes of the same absorption and scattering properties. The advantages of such an approach are many, since it opens a new way of treating measurements in diffusive media. In particular we have shown examples of how the data can be propagated anywhere in space and then inverted to retrieve the source distribution and strength with great accuracy, stressing the fact that this will allow the application of direct inversion methods in the presence of arbitrary geometries. A numerical study of the effect that detector size or different spatial sampling has on the retrieved data has been presented, strongly suggesting that the implementation of this method with experimental data in relevant experimental configurations is feasible.
DELIVERABLE NO 3.2: EXPERIMENTAL TRAINING SET MEASUREMENTS

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: 1st

PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
D3.2. Experimental measurements to serve as training set for algorithmic optimization (Month 12)

A large number of experimental measurements have been acquired with FORTH’s stand alone FMT system and are available to all partners for testing, optimization and finalization of algorithms. These measurements involve phantoms as well as in vivo experiments. In the followings some typical experiments are described, but it has to be noted that experiments can be designed according to specific needs and the Partners are welcomed to suggest experiments that could be useful for their algorithmic evaluation.

**Fluorescence quantification**

A series of slab phantoms were prepared using Intralipid and ink solution and glass capillary tubes with varying concentration of fluorophores to assess linearity of system and theory. Experiments were performed in both reflection and transmission geometry. Reconstructions and analysis are presented in Figures 1 and 2 for CFSE and ATTO590 respectively.

![Figure 1: Quantification experiment for CFSE performed in reflection and transmission with varying concentration of fluorophores. The inset shows the linearity of the method.](image-url)
Figure 2: Quantification experiment for ATTO590 performed in reflection and transmission with varying concentration of fluorophores. The inset shows the linearity of the method.

Resolution experiment
A series of experiments have been performed to assess the resolution of the system and method involving slab phantoms consisting of Intralipid and ink solution and glass capillary tubes with same concentration of fluorophores but varying distances and varying depths. Experiments were performed in both reflection and transmission geometry. Reconstructions are presented in Figures 2 for CFSE at 3mm depth and two distances.

Figure 3: Resolution assessment using tubes with same concentration of fluorophore and varying distances.

Multispectral experiments
A very large number of experiments have been also performed involving multispectral fluorescence detection. In these experiments similar phantoms as above having two different fluorophores have been used and the fluorescence of the overlapping signals has been analyzed.
using a linear unmixing algorithm as described in Objective 2.2.3.4 in this year’s Report. The
results from a typical such experiment are presented in Figure 4 where a slab phantom containing
two capillary tubes with varying concentration of CFSE and ATTO590 has been measured in two
spectral regions corresponding to the two fluorophores. The unmixing algorithm has been applied
to the reconstructed signals to get the separated fluorescence from the two fluorophores and
recover the correct concentrations. Similar experiments have been performed involving surgical
subcutaneous insertion of tubes with the fluorophores in control mice to assess the performance
of the algorithm in an inhomogeneous background. Furthermore, in vivo experiments with
transgenic mice expressing different fluorescing proteins have been also performed for a more
realistic investigation in biologically relevant applications. In such an experiment GFP and DsRed
expressing cells have been injected in Rag-/- mice and their migration has been followed in the
cervical lymph nodes.

With a similar approach and based on the OxyFMT method described in Objective 2.2.3.4 in this
year’s Report, a large number of experiments have been performed targeting tumor growth and
oxygen supply measurements. In these experiments Katushka expressing HeLa cells have been
grown subcutaneously in Rag-/- mice and fluorescence and absorption have been measured in a
longitudinal study for several days after cell injection.

**Figure 4:** Typical multispectral experiment with a phantom containing two different fluorophores
to be separated by the unmixing algorithm.

**In vivo experiments**

Furthermore, a series of in vivo experiments have been performed specifically for use with
optimizing parameters and procedures. These experiments are used for quantification, sensitivity
and detection accuracy assessment. One such study performed explicitly for algorithm
optimization together with Partner 2 CEA–LIME during a visit of Dr. Aniktos Garofalakis to
FORTH aimed at investigating the limits of detection of the FMT system and FMT inversion in
transmission geometry. A series of capillary tubes with varying concentrations of AlexaFluor 647
(0.18μM – 60.5μM) were inserted subcutaneously in the torso area in mice in both camera and
laser side as shown in Figures 5 and 6. The 633nm DPSS laser was used for excitation of
AlexaFluor 647 and a 700nm bandpass filter was used for detection of fluorescence. This
experiment will be used by FORTH and CEA-LIME for comparing systems and tomography
algorithms since the same study has been performed by CEA-LIME in their FMT system. The
results of this comparison will be presented in the next reporting period. In Figures 5 and 6 the
results of the reconstruction using FORTH’s FMT inversion with ART for the two cases are
presented. In all cases the fluorescence of the tubes is clearly reconstructed down to 0.2μM and
0.9μM for the camera and laser sides respectively. The higher detection limit when the tube is
deeper is expected, however the reconstructions are free of artifacts and at the correct depth. The results of the analysis are shown in Figure 7 where the reconstructed concentrations have been plotted against the actual ones and fitted with a linear and a quadratic fit demonstrating a deviation from linearity when imaging very superficial targets at high concentrations.

Figure 5: Reconstruction of a tube with AlexaFluor647 inserted subcutaneously on a mouse at the camera side and at varying concentrations.

Figure 6: Reconstruction of a tube with AlexaFluor647 inserted subcutaneously on a mouse at the laser side and at varying concentrations.
Figure 7: Results from the reconstructions of the s.c. AlexaFluor647 tube for the camera and laser side. Note the deviation from linearity when imaging superficial targets at high concentrations.
DELIVERABLE NO 7.1: HIF TRANSFECTED CANCER CELLS

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: 1st

PERIOD COVERED: FROM March 01, 2008 to February 28, 2009
**Work Package 7: Imaging cancer therapy**

**DELIVERABLE 7.1**

**March 15, 2009**

Markus Rudin, UZH

**D 7.1: Preparation of HIF transfected cancer cells**

**Hypoxia signaling**

Normoxia: under normoxic conditions HIF1α, which is constitutively expressed, is degraded by proteosomal degradation through an oxygen dependent enzymatic process (via prolyl-hydroxylase).

Hypoxia: Enzymatic degradation is not taking place due to lack of oxygen. Stabilized HIF1α dimerizes with HIF1α and is translocated into nucleus, where it acts as transcription factor, inducing genes under the control of a hypoxia responsive element (HRE).

[S. Lehmann et al., submitted]

**regulation of HIF1α-mCherry fusion protein in cells**

Reporter constructs for luminescence imaging

![Diagram showing regulation of HIF1α-mCherry fusion protein in cells.](image)

Cellular imaging of transfected mouse embryonic fibroblasts

1) hypoxia: inhibition of degradation machinery stabilizes HIF reporter construct
2) stabilized product is found in cellular nucleus (transcription factor)
3) HIF down-stream genes are induced by HIF1α-reporter construct

[S. Lehmann et al., submitted]