

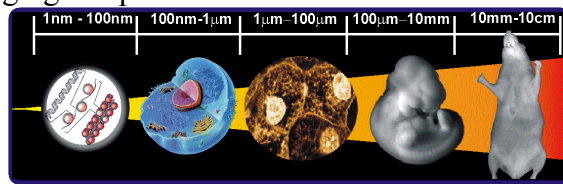
GOALS

The goal of the MOLECULAR IMAGING Integrated project is to generate and apply novel advanced technology for non-invasive imaging of biomolecular function in living systems ranging from single cells to whole animals.

Key areas for technological innovation include:

- i) Generation of new biosensors enabling novel ways of functional contrast,
- ii) Improving resolution of microscopic and tomographic imaging systems, and
- iii) Creating new multimodal imaging setups combining different contrast

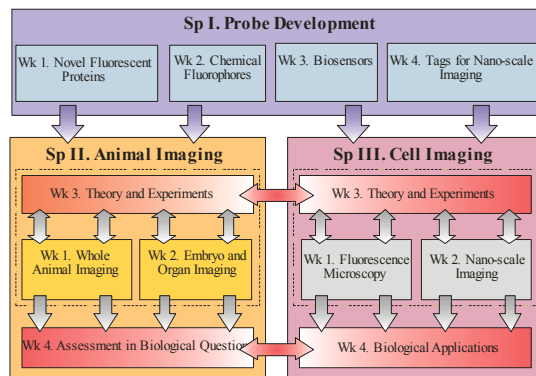
modes



THE PROJECT: DATA & STRUCTURE

Integrated Technologies for In-Vivo Molecular Imaging is an Integrated Project funded by FP6 EU contract LSHG-CT-2003-503259 and coordinated by FO.R.T.H for a total budget of 11 million euros. It involves 21 Partners from European research centers, academia and enterprises, with collaborators from Russia and the United States. The expected duration is of 60 Months, starting from the 1st of January 2004.

The Project is structured into three subprojects: Probe Development, Animal Imaging and Cell Imaging, each of which subdivided into workpackages. Interaction between workpackages and subprojects is key to the success of the project.



To achieve these goals we bring together a unique consortium of leading research groups in Europe combining:

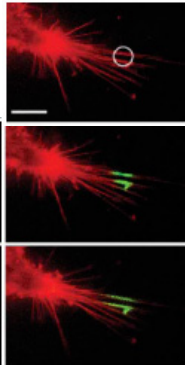
- engineers, experimental and theoretical physicists (who design new and improved, tomographic and microscopic imaging devices for in vivo-imaging);
- bioorganic chemists and molecular biologists (for the design of new chemical and genetically encoded molecular probes and biosensors);
- biologists examining fundamental questions at the cell, organ and whole animal level (providing the appropriate and relevant goals for the technological innovation).

It is hoped that our combined effort will provide exciting new opportunities for phenotyping functional (molecular) analysis in cells and animal models.

PROBE DEVELOPMENT

The main goal of the Probe Development subproject is the development of novel fluorescent probes and sensors with broad spectral range. Particular aims are:

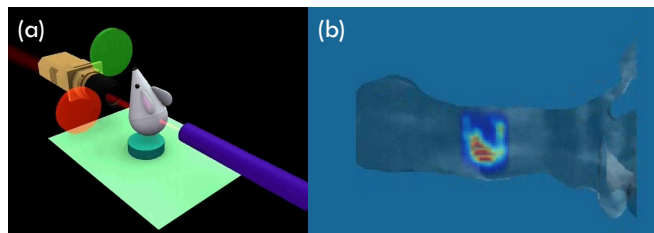
- Generation and optimization of far-red fluorescent proteins (FP) suitable for imaging in tissue.
- Generation and optimization of photoactivated Kindling Fluorescent Proteins (KFP).
- Generation of a novel set of red-fluorescent dyes and their incorporation in sensors.
- Modification of sensors to become membrane-permeant, bioactivatable derivatives.
- Development of new chemical multiphoton cages and improved biosensors.
- Development of advanced FRET pairs based on novel FPs, KFPs and chemical fluorophores.
- Use of gold-nanoparticles as tags and interferometric detection for subcellular imaging with nanometer resolution



Photoswitchable Cyan Fluorescent Protein tracking within Filopodia of HEK293 Cells. Signals in ECFP and FITC are shown in Red and Green pseudocolors respectively. The circle indicates the photoswitched region. Scale bar 10mm. Middle and Lower show images 0 and 4 minutes after photoactivation, respectively. [from Partner IBCH]

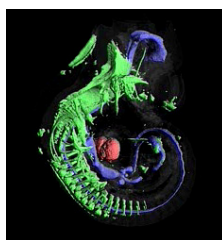
WHOLE ANIMAL IMAGING

The main goal of the Animal Imaging subproject is the development of tomographic technologies for non-invasive in-vivo imaging of embryos, organs and whole animals. This will be achieved by integrating advanced photonic technologies and improvements in tomographic approaches based on advances in the basic theory of light transport and fast and accurate inverse methods for 3D image generation and tissue characterization.



Fluorescence Molecular Tomography (FMT) imaging of T-cell regulation. (a) FMT setup where the specimen rotates along an axis, an excitation source (Ar+ laser) is scanned over the surface and excitation and emission images are collected using appropriate filters. (b)

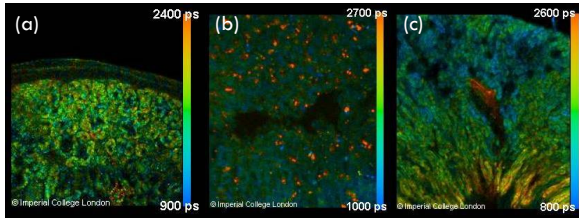
3D reconstruction of GFP concentration in the spleen for GFP-tagged T-cells in a F5 mouse. This figure indicates the potential that FMT has to image biological processes in-vivo. [from Partners FORTH and MRC-NIMR]



Optical Projection Tomography (OPT) reconstruction of an embryo that was fluorescently labeled with antibodies against neurofilament (green) and HNF3b (blue). [from Partner MRC-HGU]

CELLULAR/SUB-CELLULAR IMAGING

The main goal of the Cell Imaging subproject is the development and improvement of technologies for multidimensional high-resolution microscopy for cellular and sub-cellular imaging.



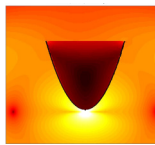
(a) and (b) show fluorescent lifetime images of fresh unstained tissue from liver and kidney (autofluorescence), respectively, excited at 800 nm in a two-photon scanning microscope. (c) shows a fluorescent lifetime image of fresh unstained tissue from colon (autofluorescence) excited at 400 nm in a scanning confocal microscope [from Partner IC]

Particular aims are:

- Improvement and development of optical microscopy techniques at the cellular and nanoscale level (Confocal and Multiphoton Microscopy, Multidimensional Fluorescence Imaging, Multimode Scanning Force Microscopy, Scanning Near-field Optical Microscopy, Super-resolution Microscopy and Total Internal Reflection Microscopy).
- To apply such imaging technologies to in vivo FRET studies
- To advance the understanding of photobleaching and apply photobleaching techniques (FRAP, FLIP) to characterize kinetics of macromolecules.
- To evaluate the new imaging technologies addressed to biological questions in specific model systems

THEORY DEVELOPMENT

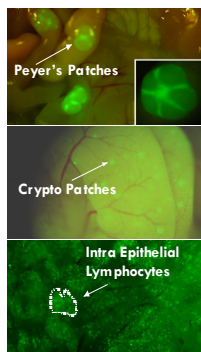
Theoretical methods are being applied to understand, improve and develop new imaging approaches. Fast Inverse and Forward methods are being developed to increase speed and fidelity of tomographically reconstructed images.



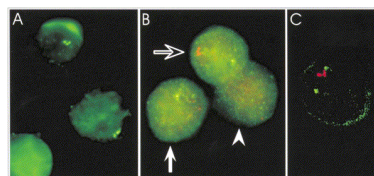
Single-molecule fluorescence close to an absorbing nanostructure. The emission rate is strongly modified by the local-field enhancement and the non-radiative coupling with the object. [From Partner ECP-CNRS]

BIOLOGICAL APPLICATIONS

Specific Biological questions are being posed in order to assess the utility of the newly developed technologies.



Visualization of the Intestinal Lymphatic system, from Partner MRC-NIMR



[Top] In Situ Hybridization of Fetal Liver Cells from a Transgenic Mouse Heterozygous for a Three-Copy Human *b*-Globin Locus (A) Hybridization with a 12 kb LCR probe (green). (B and C) Hybridization with probes for *a* (green) and *b* (red) introns detecting nuclear primary transcripts. Photographs by CCD camera (B) or by confocal microscopy with two focal planes superimposed (C). From Partner EMCR-I

PARTNERS

FORTH (Coordinator - E. N. Economou), Crete, GR
ULUND (S. Andersson-Engels), Lund, SE
UCL (S. Arridge), London, UK
UKM (C. Bremer), Munster, DE
ECP-CRSA (R. Carminati), Paris, FR
UAM (J. J. Saenz), Madrid, ES
IMM (M. Carmo-Fonseca), Lisbon, PT
IC (P. French), London, UK
UA (D. Gadella), Amsterdam, NL
MRC-NIMR (D. Kioussis), London, UK
MRC-HGU (J. Sharpe), Edinburgh, UK
EMBL (C. Schultz), Heidelberg, DE

ETH (V. Sandoghdar), Zurich, CH
IBCH (K. Lukyanov), Moscow, RU
UC3M (O. Dorn), Madrid, ES
EMCR-I (F. Grosveld), Rotterdam, NL
EMCR-II (C. Wyman), Rotterdam, NL
CNB-CSIC (C. Martinez / M. Mellado), Madrid, ES
ICMM-CSIC (M. Nieto-Vesperinas), Madrid, ES
LFSP-CSIC (N. Garcia), Madrid, ES
DIO-CSIC (M. Torres), Madrid, ES
KIP (C. Cremer), Heidelberg, DE
UHEI (H. Bujard / D. Bartsch), Heidelberg, DE
PHOTEK (J. Howorth), London, UK
PKLAS (P. Courtney), London, UK

Contact Person:

E. N. Economou

Molecular Imaging Coordinator

Foundation for Research and Technology-Hellas

FO.R.T.H.

P.O. Box 1527, 71110 Heraklion, Greece

email: economou@admin.forth.gr

Telephone: +30-2810-391565

FAX: +30-2810-391569