



Workshops Report

Development of New Diagnostics

Molecular Testing and Biomedical Imaging

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**DG-RTD
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Report

Development of New Diagnostics

Molecular Testing and Biomedical Imaging

(for FP7 implementation)

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1. Executive summary

A European effort in biomedical imaging (or “bioimaging”, which includes functional and molecular imaging) and in molecular testing would be timely and lead to substantial advances in scientific research, clinical practice and healthcare. The development of novel technologies and deepening of our scientific understanding of the fundamental structures and processes underlying health, disease and therapy are essential in this endeavour. In order to make the most of the current opportunities in the adjoining fields of bioimaging and molecular testing, it is suggested to initiate or strengthen European efforts at multiple levels. European-wide efforts supported and coordinated through various European Commission (EC) funding schemes (such as Networks of Excellence (NoE), Small or medium-scale Focused Research Projects (FRP) and Large-scale Integrating Projects (IP)) are essential for maintaining competitiveness of European academic institutions and industries and should allow a leading role of Europe in the decades to come in a principal, rapidly expanding area of science and medicine that will increasingly benefit European citizens, research and industries.

We suggest supporting efforts in the development of:

- Novel clinical applications of current technologies, e.g. through the identification and validation of new molecular and imaging markers of disease;
- Novel reagents and probes for advanced diagnosis through biomedical imaging and molecular testing;
- Novel or improved molecular testing and bioimaging technologies, allowing new methods for identifying the causes or early effects of disease at the molecular level;
- Novel methods for image guidance of minimally invasive therapies;
- Knowledge and infrastructures for integrating highly diverse technologies and data sets from bioimaging and molecular testing into all-embracing fundamental models. This should result in mathematical representations of the physical structure and dynamics underlying health, disease and therapy.

In these endeavours it will be essential to push towards (further) standardisation and quantification, to ensure an expansion of the relevant disciplines through advanced education programmes and to ensure public awareness and support of a coordinated European approach through outreach programmes for the general public.

2. Introduction

This report is an outcome from workshops on “New diagnostics” that took place at the European Commission (DG-Research (DG-RTD), Health Directorate), in Brussels on 4-5 July 2005, 8 June 2006 and 17-18 November 2008, with independent experts from academia and industry. It is an update of the report of 2005.

The main aim of the workshops was to advise the Commission services on future research orientations on new diagnostics, which should be an important part of the area “*Detection diagnosis and monitoring*” in the domain “*Biotechnology, generic tools and technologies for human health*” of the 7th Framework Programme (FP7) (2007-2013) for research and technological development.

Emphasis was given to two major aspects: Molecular and genetic testing, and Biomedical Imaging. Even though infrastructures are an important part of the needs in these areas at European level, they were largely left out of the discussions, essentially because they mainly fit in another area of FP7 and they deserve a meeting in themselves.

2.1 Rationale

The last two decades of the 20th century witnessed enormous advances in our understanding of the processes of life at the molecular level, thanks to the outstanding achievements in molecular and cellular biology. This new information, including that gained on the factors determining the cause and progression of the most important diseases, has led to the development of innovative pharmaceutical and minimally invasive treatments.

Meanwhile, a number of *in vitro* techniques for molecular testing became available for monitoring molecular abnormalities that accompany the development of pathological states. The complementary field of molecular bioimaging emerged, that seeks to allow the *in vitro* and *in vivo* visualisation of biological processes at the cellular and molecular level. The combination of bioimaging and molecular tests allows the assessment, characterisation and quantification of gene and protein function, protein-protein interaction, profiling of signal transduction pathways and metabolism in the natural state, in animal models of human disease and in patients. By contributing to our fundamental knowledge of life, it allows us to understand the molecular and cellular pathology of specific diseases.

Medical treatment requires reliable diagnostic tools, and diagnostic tools are also important for drug discovery. The root causes of disease can often be traced down to molecular events and the first manifestations often occur at the molecular level, well before anatomic ones. The development of approaches that monitor and visualise molecules which are the “signature” of a given disease represents an outstanding breakthrough in the diagnostic modalities currently provided clinically. The development of advanced diagnostic tools will increase the availability of non- or minimally-invasive techniques and therapies in healthcare and will benefit health and quality of life of European citizens, and European research and industry.

The diversity of molecular diagnostic tools has rapidly expanded thanks to technological developments in both *in vitro* and *in vivo* techniques, such as flow cytometry, PCR (polymerase chain reaction), DNA sequencing, microarray, and structural and functional imaging. Further development increasingly requires the integration of multiple scientific disciplines, such as molecular and cellular biology, physiology, mathematics, physics, chemistry, nanotechnologies, microsystems, devices and information technologies, linking basic sciences and medical applications.

Diagnostic tools that monitor, visualise or measure molecular events associated with disease are envisaged to become increasingly important for predicting disease (e.g. assessing predisposition for its development and preventive strategies); screening for disease (e.g. in specific at-risk subjects); early diagnosis; classification of the disease in relation to prognosis, grading and staging at initial presentation; monitoring disease progression (also for prevention of recurrence and complications); companion diagnostic (e.g. accompanying drugs); selecting and guiding treatment, and assessing therapeutic efficiency, all aiming at facilitating “personalised/individualised medicine”. For this purpose newly developed methods have to be translated from cellular level to animal models, to human organisms and finally to population level.

Targeted therapies (e.g. transcription inhibitors, kinase inhibitors, monoclonal antibodies) need careful selection of targets for each disease category, followed by monitoring of treatment efficacy, particularly for early detection of resistance to treatment. Consequently, development of new forms of targeted therapies should always be accompanied by the development of reliable techniques for sensitive monitoring, preferably in the form of “point-of-care” diagnostics. Visualisation of the specific distribution and local concentration of such molecules *in vivo* is also very important for the development and clinical application of these targeted therapies.

Efficient diagnostics in medicine should fulfil several criteria:

- quick;
- quantifiable;
- highly sensitive and specific, through focusing on the relevant target (cell, molecule, tissue...);
- minimally invasive;

- broadly applicable (preferably to various subgroups within one disease category, and ideally to all);
- possibilities for high throughput (particularly for screening purposes);
- possibilities for automation (important for “point-of-care” diagnostics);
- validated and evidence-based;
- robust and “simple”, allowing routine application;
- low costs (in terms of laboratory facilities, equipment, reagents, personnel).

Choice of techniques

Speed, sensitivity and specificity are particularly important for the efficient management of patient care, but methods should also be minimally invasive and quantitative. To increase efficiency and reduce health care costs it is preferable to use a limited number of different (but complementary) techniques that allow fast, easy and quantitative integration of the obtained results. Standardisation, validation, and quality assurance need special attention for the implementation in routine practice. These considerations should guide the development of new diagnostics using molecular tests and biomedical imaging.

Requirements in microbiology

For diagnosing infections, the following particular needs and constraints need to be addressed:

- need to simplify and shorten diagnostic procedure in microbiology;
- need for “generic” diagnostics, e.g. to diagnose a family of viruses with the same clinical relevance using a single screening test;
- variability of microorganisms (mainly viruses) hampers precise identification and produces continuous diagnostic problems: constant improvement of diagnostic tests is needed;
- frequent escape from antimicrobial treatment: early diagnosis of resistance is needed;
- need for “field” tests to be used for rapid bed-side analyses, e.g. for fast large-scale analyses before admittance to hospital in the early stages of an outbreak;
- special requirements for prevention of infection of medical-diagnostic personnel during outbreaks, e.g. with avian flu, SARS (Severe Acute Respiratory Syndrome), viruses causing hemorrhagic fevers;
- accumulation of infections over time, particularly in immuno-suppressed patients or patients with chronic diseases.

Bioimaging and molecular testing have complementary applications in diagnosis and monitoring of disease. Molecular tests are highly specific. They offer low-threshold and routine tests on samples taken from patients. In a few well-established, very straightforward cases molecular diagnosis does not require a prescription (e.g. pregnancy tests) or may be performed routinely by patients themselves (e.g. in monitoring diabetes). There is a major need for such straightforward tests. For instance, a fast diagnostic near-patient test to discriminate between a myocardial infarction and angina pectoris without tissue damage would save a number of the patients. Equipment for such tests needs to be robust and should be suitable for non-professional use (e.g. by first-aid workers or ambulance personnel). In contrast, bioimaging aims to achieve a more comprehensive view of the type and nature of (cellular and molecular) structures or processes affected by disease within their biological context, and often requires an expensive and elaborate infrastructure. Such imaging, or multi-modal imaging, is also essential – for example – in guiding interventions like biopsies, surgery or minimally-invasive therapies (e.g. with MRI, endoscopy and optical microscopy).

Bioimaging and molecular testing share the need for specific quantifiable (*bio*)*markers* and *probes*.

Markers are molecules or structures the presence of which may indicate processes associated with disease. Several types of markers can be defined in diagnostics:

- predictive markers (mainly inherited markers);
- screening markers (screening in specific at-risk groups for presence of potential disease);
- diagnostic markers;
- classification or staging markers;
- markers for disease monitoring (including therapy monitoring)

- surrogate markers for evaluation of the disease process.

Probes are molecules that bind to specific targets, including (bio)markers, and that may be detected *in vivo* (through molecular bioimaging technology) or *in vitro*. Labelling with radioactive, fluorescent or other substances is often needed for their detection. Recent developments in nanotechnology may lead to the design of even more powerful probes, allowing improved detection *in vivo* through bioimaging, or more powerful on-line *in vitro* testing through advanced lab-on-a-chip designs.

In the case of protein analysis, construction of affinity probes cannot be based on simple rules of complementarity, unlike the situation for DNA probes. Indeed, high-affinity protein binding reagents can only be found by screening large libraries of affinity reagents, either using the immune systems of animals like rabbits and mice, or through *in vitro* selection. Such reagents are a prerequisite for progress in protein-based diagnostics.

There is also a pressing need to go beyond the classical western blot and sandwich ELISA approaches for antibody-based protein detection to obtain improved assay performance. For example, nanoparticles or the combination of antibodies with DNA reporters may lead to higher sensitivity of detection, greater degrees of multiplexing, and also permit evaluation of complexes of interacting proteins or proteins having undergone specific post-translational modifications, as these can reflect functional states of cells and tissues and, for example, response to therapy.

In order to develop powerful new diagnostics, it is essential to obtain a comprehensive, molecular understanding of the disease-causing alterations and of the healthy state. Current technologies can each reveal important aspects of an organism, organ, tissue, cell or molecule, but there is no single (foreseeable) technology capable of achieving the required all-embracing molecular picture. Therefore, development of diagnostic tools will increasingly require quantitative integration of complementary technologies (multimodality). Important for the success of such a synergistic and integrating approach is the adaptation of well designed and well documented protocols for sample treatment, for data acquisition, and for storage and quantitative analysis of the data.

The clear synergy and complementarity between molecular testing and bioimaging in applications in scientific research and healthcare indicates that a coordination of efforts within and between these areas is welcome and likely to yield rapid progress, and may lead to spectacular breakthroughs.

2.2 Role of Europe – strengths and weaknesses

Europe has played a leading role in the development of technologies for imaging and molecular testing, now widely used as diagnostic tools. Traditionally, the European industry has held a world leadership in the field of *in vivo* diagnostic agents. European industry is also at world-class level for imaging equipment, with all 3 top companies in the world now based in Europe (2 being of European origin). This commercial dominance of the market has been possible also thanks to the excellent work carried out in many European universities and research centres. However, this may be subject to drastic changes if Europe does not take appropriate initiatives to further support innovation in the field.

Indeed, at present, the strong position of the European Union (EU) in bioimaging is challenged especially as a result of sharply increased funding of National Institutes of Health (NIH) in the USA in recent years, and the recognition of imaging as an important tool in several critical fields such as advanced clinical diagnosis, image-guided interventions, as well as in the development of new drugs (imaging biomarkers in preclinical research, and in clinical evaluation). The USA has undertaken an impressive program of activities, created national programmes and a dedicated Institute. Some of these recent specific trends in the USA include:

- The establishment of the NIH (National Institutes of Health) roadmap in bringing scientific advances to the bedside, and the specific role of imaging in the key area of translational research;
- The increased attention of various NIH institutes to imaging technologies, ranging from new contrast agents to advanced diagnostics through molecular and cellular imaging, and image-

- guided interventions. Examples are NCI (National Cancer Institute), NIMH (National Institute of Mental Health) and NHLBI (National Heart, Lung and Blood Institute);
- The creation in 2001 of NIBIB (National Institute for Biomedical Imaging and Bioengineering): the first time that "biomedical imaging" appears specifically in the name of one of the NIH institutes.

In 2002, NIBIB was funded with more than 120 million \$ to support projects on innovative biomedical imaging. Similar levels of funding from the NCI are focused on the same objective. NIBIB's 2005 budget is 300 million \$, with a large part for biomedical imaging. According to an assessment study by an independent consulting company (Source: Clinical Reports, Medical Diagnostic Imaging 2002; A.T. Kearney research and analysis), the Molecular Imaging market will see a significant rate of growth (13 % on average) during the next 10 years, depending on the availability of innovative imaging agents.

The establishment of European Research Programmes supporting and strengthening concerted efforts on bioimaging, and on immunological and molecular testing is therefore obviously essential to maintain the competitiveness of European industries and academic institutions with respect to current activities elsewhere in the world.

A number of projects in the field are already being supported in the 6th and 7th Framework Programmes (in particular with Integrated Projects and Networks of Excellence) and others were in previous Framework Programmes.

The European Society of Molecular Imaging (ESMI) was recently created (2005), in particular by EC project participants, in order to improve the coordination of European efforts in this booming field. Similarly, the recently created European Institute for Biomedical Imaging Research (EIBIR), initially supported within the 6th Framework Programme, aims at helping coordinate research efforts in bioimaging by pooling resources and proposing a Europe-wide research policy. The ESFRI (European Strategy Forum on Research Infrastructures) roadmap for research infrastructures, as updated in December 2008, has included Euro-BioImaging as one of the only 10 projects to be established in life sciences. This initiative is expected to boost research activities in bioimaging in Europe and help retain and further develop talent in this field. In addition, the biobanking infrastructure BBMRI (www.bbMRI.eu) currently in a preparative phase, serves to further motivate a concerted effort to develop improved means for *in vitro* imaging and diagnostics. In order to fully take advantage of high quality sample collections it will be important to develop and provide access to high performance analytical methods. The availability of such samples also means that promising assays and biomarkers can be more rapidly validated and brought to clinical utility.

Also, in the field of molecular testing, European networks play a dominant role in the design, standardisation and clinical evaluation of novel diagnostic strategies and assays. Several of these activities have been supported by the EU Framework Programmes. Thanks to prominent academic organisations (Pasteur Institute, Erasmus Medical Centre, Karolinska Institute, etc.) Europe has reached excellence in research on the diagnosis of infectious diseases and cancer. Increasing collaboration between the academic and private sectors in this field helps Europe play a significant role in the development of new *in vitro* diagnostics (e.g. rapid testing, new markers, protein and DNA chips).

The collaborative attitude of European scientists in biomedical research and the strong European networks in diagnostic and clinical patient care, have contributed significantly to the strong position of bioimaging and molecular testing in Europe. However, Europe does not have a broad and longstanding tradition in collaboration between academia and industry in biomedical research. The current EU support to include SMEs (Small and Medium Size Enterprises) into European networks will significantly enhance the collaboration between academia and industry.

Training and improving public awareness

Expert training of both young and experienced scientists and practitioners is essential for new developments and their effective dissemination. Public awareness and support must be enhanced through a comprehensive outreach programme.

2.3 The Workshops (aims and procedure)

DG RTD's Health Directorate of the European Commission organised, on 4-5 July 2005, 8 June 2006 and 17-18 November 2008, workshops on "Development of New Diagnostics" to prepare the implementation of FP7 in the area of "Detection, diagnosis, and monitoring". Independent experts (see below and annex) were invited to advise the Commission services on research orientations in biomedical imaging and in molecular/genetic testing, for the benefit of European research, citizens (health and quality of life), and industry.

The experts were invited to identify fields with:

1. *European potential:*
 - promising fields, with respect to scientific progress, innovation and medical applications;
 - existence of potential scientific excellence in Europe;
 - critical mass in Europe.
2. *European added value:*
 - complementary national skills can be combined, in particular in interdisciplinary situations;
 - joint research is of interest given the cross-border nature of the questions to be dealt with;
 - co-operation offers positive effects in terms of stimulating public-private partnership.

The workshops consisted in plenary sessions and sessions split into separate groups, where the various aspects were explored. The main conclusions and recommendations are given below.

3. "Molecular Testing"

Recommendations for various fields

3.1 New biomarkers

Background

Reliable biomarkers for each disease category are essential for appropriate diagnostic and clinical management of patients. They are needed at different disease stages, particularly for monitoring individual patients throughout the disease process. Consequently they include:

- disease markers for predisposition, screening, diagnosis, classification (relation with prognosis and choice of treatment) and monitoring (evaluation of treatment response);
- biomarkers at the DNA, RNA, protein, cellular and functional level, including host responses;
- special attention for markers that occur in soluble as well as in cell-bound form and for markers that can easily form complexes with other molecules (*in vivo* and/or *in vitro*).

Justification

For many diseases there is an urgent need for reliable prognostic and diagnostic markers, e.g. for infectious diseases, cardiovascular diseases, common cancers or age-related diseases, like osteoporosis and Alzheimer's disease. Well-defined European Biobanks (already existing or in preparation) can facilitate such investigations.

Type of action needed

Large-scale Integrating Projects (IP) or Small or medium-scale Focused Research Projects (FRP) for specific fields (disease oriented projects).

3.2 New tools for the identification of biomarkers

Background

Identification of new biomarkers needs comparison between health and disease, particularly between the affected cells and their unaffected (healthy) counterparts. Consequently, several large-scale technical approaches are needed to support the identification of new biomarkers:

- large-scale, high-throughput comparative methods;
- enrichment procedures for supporting identification of biomarkers;
- methods for the detection of secondary modifications of proteins (e.g. post-translational modifications) and/or interactions with other macromolecules;
- methods for the evaluation of the affinity/avidity of ligand-biomarker interaction in diagnostic applications;
- joint application of *in-vivo* molecular or functional imaging and molecular testing for supporting the identification and differential (tissue-specific) distribution of relevant biomarkers.

Justification

Identification and specification of new yet-undiscovered discriminative biomarkers.

Type of action needed

IP or FRP.

3.3 New methods for measurement of biomarkers in clinical samples, followed by validation of the method

Background

As soon as new biomarkers have been identified for specific disease categories or for specific diagnostic applications, fast and easy assays need to be developed. Initially the original method of discovery might be used, but application in routine practice asks for specific criteria. This implies that new methods need to be developed with special attention for:

- use of new detection methods and improvement of existing ones, including nanotechnology and improved online lab-on-a-chip devices;
- the detection method should fulfil all relevant diagnostic criteria: speed, high sensitivity, specificity, accurate quantitation, etc.;
- suited for “point-of-care” testing, including robustness and automation;
- minimally-invasive methods, e.g. important for cancer diagnostics and prenatal diagnostics;
- evaluation in clinically well-defined samples from patients and suitable controls;
- standardisation and validation in multicentre studies.

Justification

Easy and broad availability of new diagnostics for supporting patient care: diagnosis, classification, disease and treatment monitoring. Diagnostic standardisation and validation studies in Europe can be supported by well-defined Biobanks.

Type of action needed

FRP(s) or IP, ideally collaborating with a Network of Excellence (NoE). FRP(s) or IP should focus on the technical aspects, while the NoE should focus on standardisation, validation and implementation in routine practice.

Specific actions requested from the EC

- strengthening the further development and harmonisation of Biobanks in Europe;
- EC guidelines to harmonise and facilitate the usage of anonymised/pseudonymised patient and control samples and the corresponding clinical data (attention for privacy issues and data protection).

3.4 Development of new devices for diagnosis and/or monitoring

Background

To support the development of biomarkers in clinical samples with methods suited for point of care, automation, theranostics, or diagnosis in emerging or developing countries, efforts are needed from the engineering point of view.

These efforts should focus on developing devices able to include or achieve:

- Automation and/or Point of Care diagnosis
- Multiplex Multi-Modality detection
- Higher sensitivity and/or specificity
- High throughput biomarker screening
- Analysis of samples and analytes from diverse origins and of various composition
- Sample handling, preservation and purification technology
- Elimination of sample transportation
- In vivo testing
- Easy to handle, cheap screening methods
- Low cost diagnosis methods for developing countries
- Smart new combination of existing technologies on a same device.

Justification

Field of high added-value for Europe, in a very competitive setting (with USA primarily), with some of the world's top academic and industrial players being currently in Europe.

Type of actions needed

IP or FRP, ideally including companies, with specific focus on the engineering aspects.

3.5 Developments of new reagents for diagnostics

Background

In several diagnostic fields, assays at the protein, cellular and functional level are based on the application of highly-specific binders/ligands such as antibodies, aptamers, reagents produced by phage or ribosome display, or low molecular weight compounds. High-affinity monoclonal antibodies or high-affinity phage display/single chain antibodies have the advantage of “eternal” production without change in specificity and affinity. Usage of such antibodies is particularly important in the diagnostic fields of immunology, haemato-oncology, endocrinology and pathology.

Exploitation of new knowledge from genomics, transcriptomics and proteomics needs the development of new reagents for diagnostics, such as:

- new reagents against newly-identified proteins, particularly intracellular proteins;
- reagents for detection of secondary modifications of proteins and/or interactions with other macromolecules;
- new reagents for function testing, like RNAi (RNA interference), antisense or other approaches;

- nanoparticles or other structures for *in vitro* and *in vivo* diagnostics.

Justification

Field of excellence for Europe; high economic impact; strategic value for biomedical research.

Type of action needed

IP (or combination of several collaborating FRPs) for the development of new reagents, followed by a NoE for implementation in diagnostics (standardisation, validation and education).

4. “Biomedical Imaging”

General recommendations

Bioimaging allows the visualisation and characterisation of both structure and function. It can, for instance, show the structural and functional alterations that are associated with disease.

Combining imaging technologies that can correlate cellular- and molecular-level information (molecular imaging) with anatomical information will give researchers an extremely powerful tool to diagnose, track and treat a variety of diseases. Bioimaging will also help in the development of treatment strategies (e.g. stem cell, drug or gene therapies) and in guidance of minimally invasive treatments.

Bioimaging requires multidisciplinary teams to push forward science, and to further improve technologies. Essential expertise should cover:

- medical knowledge of the diseases being studied for *(pre-)clinical development and validation*;
- biochemistry and molecular biology of the cellular processes being visualised for developing *novel probes*, including bioinformatics for molecular modelling and design of optimised probes;
- physics of image formation and acquisition for developing *bioimaging technologies*;
- bioinformatics of data modelling, correlation and quantitative analysis for *integration of image modalities, diagnosis and treatment selection*;
- knowledge in image processing for automated image interpretation and development of quantitative imaging biomarkers

One of the main risks of highly multidisciplinary projects is the occurrence of bottlenecks in one of their critical paths, causing an entire programme to slow down while waiting for a particular result. Hence, we propose breaking up the programme according to the broad and partially overlapping areas itemised above. The various consortia should be well aware of each other’s focus, but not be dependent on each other’s progress. Essential for such an approach to work, parallel efforts are required in:

- *standardisation* to facilitate the exchange of protocols and data;
- (advanced) *training and educational facilities* for the exchange of ideas and knowledge, the inspiration of young talents and public support.

Below, we summarise the current state of each of these areas and suggest research fields that should be stimulated at European level.

4.1 Pre-clinical and clinical validation

Background and justification

Medical knowledge is a prerequisite for the introduction of novel bioimaging technologies in clinical research and applications. Several consecutive steps in current research in bioimaging can be distinguished leading to the full utilisation of data from (pre-) clinical experiments across modalities and experimental techniques into an integrated and accessible system:

- the development of animal models
- translational development from animal models into clinical models (with a main emphasis on safety of patients) to population level (disease risk prediction, screening)
- validation of clinical models for the purpose of prognosis, diagnosis, staging of disease, and selection and monitoring of therapy.

Suggested actions

It is suggested to concentrate the development of clinical models within this sub-programme to a limited number of serious major diseases for which early intervention can be foreseen to increase the quality of a patient's life. Suggestions include neurological and cardiovascular diseases and cancer. Concerted efforts supported should lead, through the steps outlined above, to more effective treatment of these diseases.

4.2 Novel probes for visualising molecular and cellular processes

Background and justification

Many bioimaging technologies allow the localisation of specific molecules ('probes') within their biological context. This context can be the living organism, living tissue or even the ultrastructure of the cell. The precise location, state, kinetics and relative concentration of such molecules can give very important information concerning the diagnosis or prognosis of a specific disease. In other applications, probes may allow to evaluate the biodistribution, bioavailability or targeting of potential drugs. Advanced delivery systems are anticipated to render such probes even more specific.

The design of novel probes requires the collaborative effort of molecular biologists and biochemists to establish improved probe designs and for identifying and characterising potential targets, and of synthetic chemists for the design and production of suitable probes. Input from biomedical and pharmacological researchers for target definition, and from bioimaging physicists for optimal probe design is essential for this sub-programme. Furthermore, substantial input from nano-technology is anticipated for the design of high-contrast probes, novel forms of probe delivery and *in vivo* control of probe properties.

Suggested actions

It is suggested to support several promising (generic) approaches for the design and application of probes, giving specific attention to the development of probes offering improved sensitivity, specificity or the ability to recognise novel targets, and to contrast agents/enhancers, tracers, ligands and linkers, for use in MRI (Magnetic Resonance Imaging), PET (Positron Emission Tomography), SPECT (Single Photon Emission Computed Tomography), ultra-sound and optical imaging technologies. In general, each bioimaging technology demands probes with special functionalities (magnetic, radioactive, fluorescent, etc.). We foresee that in a number of applications, especially where multiple forms of bioimaging are combined, multifunctional probes will become essential; and research in this area should also be stimulated.

These more chemically-oriented efforts should be complemented with more biological approaches including the identification and validation of novel imaging targets, more versatile probe delivery systems, the development of sensor probes that respond to their biological context, and the

clarification of their kinetics within the animal and human body. Essential in this endeavour is close interaction or combination with the efforts suggested in part 3 of this report: the development of novel molecular tests.

4.3 Biomedical imaging technologies

Background and justification

Applications of bioimaging technologies range from studies of single molecules at atomic resolution, to the localisation of specific molecules within patients at (sub-) millimetre resolution. *In vivo*, high spatial resolution is often limited by the movement of the organs or features to be imaged, which can be remedied by developing novel technologies that allow faster data acquisition times. Other limiting factors *in vivo* include low contrast, reduced binding of imaging probes and poor accessibility of the tissue or organ by the imaging equipment. Hence, *in vivo* imaging and high-resolution *in vitro* studies complement each other and both are required in many applications to get the complete picture.

Some essential biomedical imaging technologies used in patient care and research include:

- Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS)
- Positron Emission Tomography (PET)
- Single Photon Emission Computed Tomography (SPECT)
- X-ray Imaging and Computed Tomography (CT)
- Ultrasound Imaging (US)
- Optical Imaging

Furthermore, new bioimaging methods like photoacoustic tomography, magnetic particle imaging and Terahertz imaging are currently in the research phase. They might prove to be valuable complements to available technologies.

For more detailed in-vitro studies (e.g. of patient cells and tissues), a whole range of complementary forms of microscopy are available. Spanning the spatial resolution range from sub-cellular to near atomic, these microscopies include:

- (multi-photon) fluorescence (confocal) microscopies;
- scanning probe and near-field scanning optical microscopies;
- electron tomography of cells;
- Single particle cryo-electron microscopy.

Additional spectroscopic and (usually X-ray) diffraction analyses allow the visualisation of molecular structures outside the context of the cell.

Developing new and improving existing bioimaging technologies requires a concerted effort of scientists from many disciplines. Development of machine hardware is mainly a task for physicists and engineers, who need continuous feedback from medical experts for issues concerning patient care, and pharmacologists and chemists for issues concerning the specific probes that are to be designed or visualised.

Suggested actions

It is suggested to support concerted efforts aimed at improving the resolution (temporal, spatial, e.g. micro-imaging, etc.), contrast, throughput and/or versatility of bioimaging technologies, paying specific attention to quantification methods. The development of complementary, multimodal technologies will become increasingly important. This includes the development and validation of hybrid systems and co-registration protocols that allow concurrent and consecutive image acquisition, and the use of multiple bioimaging modalities (multimodality), possibly with multifunctional probes (see part 4.2 Novel probes). Examples include combined light- and electron-microscopy, or MRI and optical imaging.

It is important that new developments adhere (where possible) to existing standards, or encourage the development or refinement of such standards. Standards should cover the way in which patients or samples are prepared for imaging, the imaging protocols, image formats and the structures of image databases (see part on Standardisation).

The use of some of the above technologies (e.g. X-rays, CT, PET) is limited by the radiation dose that patients can receive in diagnostic procedures. So, radiation safety also needs to be kept in mind. Novel detectors or detection methods (like quantum X- or γ -ray imaging with specific, spectrally resolved imaging) may provide additional information, and allow new imaging applications (e.g. fast, low dose dynamic imaging of moving tissues or organs) and/or reducing doses. Expertise for this is spread throughout Europe.

4.4 Integration of image modalities

Background and justification

Currently, bioimage analysis requires expert knowledge. It is difficult, and often impossible for a non-expert, to combine images or data obtained by multiple technologies into a comprehensive picture, let alone into a quantitative (mathematical) representation. However, the workshop participants strongly agree that substantial increase in insight can be achieved through such analyses. The development of quantitative mathematical models and simulations has already had a major impact in many other fields, and we are convinced that its impact in bioimaging will prove to be similar. Current advances in systems biology will grind to a halt if the physical structure of life is not taken into account, and bioimaging is probably the only discipline that can provide the structural and functional data required. Structural knowledge of the interplay of molecules within the living cell is critical to put the large amount of data from genomics projects into an integrated and comprehensive system of knowledge that would be usable by scientists and medical practitioners.

A major effort is advised in methods for quantitative data analysis, requiring three partially overlapping developments:

- development of data handling routines, including standard data structures and protocols for image storage, retrieval and visualisation;
- expert systems that guide researchers towards the relevant aspects of new (multimodal) data;
- mathematical models and simulations of biological structures and their dynamics in all resolution ranges relevant for functionalities and functional pathways in health, disease and therapy.

Suggested actions

It is suggested to stimulate European efforts in this field through supporting collaborative programmes in these three areas, paying specific attention to the sharing of existing data standards, algorithms and mathematical models between existing disciplines (see part 4.5 Standardisation).

4.5 Development of new quantitative imaging biomarkers

Background and Justification

Quantitative imaging biomarkers are expected to have a large impact in medicine, e.g. for better understanding the mechanisms of disease, for drug discovery and development, screening, improved diagnosis, and monitoring of treatment. In drug discovery, they have the potential to be used as surrogate endpoints in clinical trials. In screening and diagnosis, they may lead to more sensitive and specific diagnosis. In treatment monitoring, they may be used to assess the response to treatment at an early stage. Ultimately, imaging biomarkers enable individualised patient management and treatment.

Modern biomedical imaging allows the in vivo visualisation of human anatomy, function and pathology at multiple scales of observation: from the molecular and cellular to the organ and body level. The sheer quantity and complexity of the multiscale and multimodal image data that result from these new imaging experiments pose a major challenge. Novel image analysis techniques, which

achieve a high degree of automation, accuracy and reproducibility, are required, to fully exploit the richness of information that is available in these imaging data.

Suggested actions

Biomarkers in this context are either newly developed molecules, novel technological approaches or established imaging technologies with novel applications having the potential to be translated into clinical use: The newly developed imaging biomarker(s) should improve diagnosis, predicting or monitoring treatment response, or monitoring safety (toxic effects) at an early stage.

The qualification and validation of imaging biomarkers should also be addressed.

A main aspect would be to develop and validate quantitative image analysis techniques which accurately and robustly extract anatomic, physiologic/functional, metabolic, biochemical, biophysical or molecular parameters that can be used to determine the presence and state of disease.

4.6 Image-Guided Therapy

Background and Justification

Image-guided therapy is the use of biomedical images to guide therapeutic interventions, including surgery, radiotherapy/radiation oncology, ablation therapy, and drug or cell delivery. With the exception of ultrasound, x-ray/CT fluoroscopy and some (ultra)fast MRI techniques, biomedical images currently used for this purpose are essentially static and provide “snapshots” in time, rather than continuous images over time. This limitation presents difficulties for image-guided therapy in regions of the body where motion occurs. Image-guided therapy in the 4 dimensions (space and time) requires techniques to quantify motion and delineate its impact on organ movement and deformation, and provide motion-correcting algorithms to guide more accurate interventional procedures.

The main objective of 4-dimensional image-guided therapy is safer and less invasive interventions that also yield greater success. A successful therapeutic intervention is ultimately the removal or healing of diseased tissue, without damaging normal tissue to the degree that the patient’s well-being is compromised. Another objective of 4-dimensional image-guided therapy is the development of real-time validation of treatment end points (biomarkers) to quantitatively assess patient outcomes and level of discomfort.

Suggested actions

Four-dimensional image-guided therapy is rapidly evolving, and its rate of development often outpaces the clinical validation of any particular approach. On top of direct research in the area, a collaboration of research teams would help validation research keep pace with technological evolution by accelerating the clinical evaluation of a particular approach (e.g. to motion quantification and correction).

Examples of important research: Intra-operative real-time imaging of tumour margins with high resolution and specificity, aiming at allowing complete tumour removal ; Image-guided drug delivery, aiming at novel imaging methods to control and visualise in vivo drug delivery and release, and at monitoring therapeutic effects ; Image-guidance for radiotherapy planning, guidance and monitoring, preferably in real-time (egg. by developing imaging agents/probes reporting on relevant biomarkers, such as partial oxygen pressure or angiogenesis). In the latter, all radiotherapeutic approaches should be considered, including ion-beam (or hadron) -therapy and targeted radionuclide therapy.

4.7 Standardisation of technologies, protocols and data formats

Background and justification

Without standardisation, it is impossible to achieve an efficient exchange of methods, tools and information. Hence a European effort is required to formulate and document preferred standard practices in:

- patient preparation prior to imaging;
- sample preparation prior to imaging;
- imaging protocols;
- image storage formats;
- data models and mathematical modelling techniques;
- image analysis and interpretation;
- guidelines for therapy selection and monitoring.

Suggested actions

Given the state of the field, now is the time to address these questions at a European scale. It is suggested to set up sub-groups according to the items listed above, for formulating these standards or improving existing ones.

4.8 Teaching and training facilities

Background and justification

Due to the multidisciplinary nature of biomedical imaging, there is a world-wide shortage of talents trained in biology, medicine and technologies. In Europe, the talent drain to the USA has further accentuated this shortage. Having a sustained talent pool in Europe is critical for maintaining and increasing the competitive advantage of Europe in this area, which is innovative and crucial to the future of healthcare and hence welfare of EU citizens.

Suggested actions

- Enable cross-training opportunities in bioimaging that provide opportunities for medical as well as science and engineering trainees to learn fundamentals of each other's disciplines, by establishing and encouraging proposals/projects that provide training positions in key laboratories and industrial Research and Development (R&D) setups.
- Initiate discussions with member states on multidisciplinary training programmes, such as MD-PhD programmes in Health Science Technologies (HST).
- Encourage and enable member states and private partners to promote awareness of the relevance of bioimaging through outreach programmes in secondary schools, (science) museums and exhibitions, publications, and on television and the Internet.

5. Synergies between Bioimaging and Molecular Testing

5.1 Synergies in Applications in Health Care and Scientific Research

Background

There is obvious synergy in the development and application of bioimaging and molecular testing. For example, an initial diagnosis by a molecular test may be confirmed and refined by bioimaging, by precisely localising the area that is affected by disease and characterising any structural or functional

abnormalities. This in turn may lead to more precise molecular testing on (biopsies of) affected areas. Subsequently, the location and nature of lesions caused by a disease, combined with molecular test data characterising the failure at the molecular level, may suggest or guide therapeutic interventions.

Similar types of synergy exist in the applications of molecular testing and bioimaging in scientific research that aim at acquiring knowledge of the underlying processes and their aberrations in disease.

Suggested actions

In view of these synergies between the application of bioimaging and molecular testing in scientific research and health care, we strongly suggest that programmes aimed at the discovery of novel biomarkers (3.1 and 4.5) establish links with programmes aimed at (pre-) clinical validation of novel bioimaging probes and technologies (4.1).

5.2 Synergies in Technology Development

Background

In order to develop novel technologies, both in molecular testing and in bioimaging, the identification of potential molecular markers is essential. In molecular testing, the appearance of these markers in the 'bulk' of samples may be indicative of molecular processes *in vivo*, and the specific distribution and local concentration of these markers can be visualised by bioimaging using probes. Often it is not straightforward to use the same marker for both technologies, in which case secondary markers may still allow correlating the data.

Currently, many targets are extracellular or exposed on the outside surface of cells and most probes are antibodies. However, most cellular proteins are located intracellularly, and antibodies against specific diagnostically-relevant intracellular epitopes are not easy to develop.

Suggested actions

Synergy between technology development in molecular testing and bioimaging may be gained from coordinating efforts, including in:

- finding and characterising novel markers (3.1 and 4.5) and finding novel targets for probes (sub-programme of 4.2);
- developing new tools for the identification and characterisation of novel biomarkers (3.2) and for bioimaging technologies (4.3) ;
- developing and validating new tools for measuring biomarkers in clinical samples (3.3) and developing new tools in (sub-)cellular bioimaging (sub-programme of 4.3), including antibodies against intracellular proteins (3.5);
- developing novel reagents for diagnostics (3.5) and synthesising novel probes (sub-programme of 4.2).
- medical devices (3.4 and most of 4).

At a somewhat later stage (when data integration in bioimaging (4.4) is more mature), further development of a more comprehensive integration in systems biology between bioimaging data and molecular testing data can be foreseen.

An example could be the development of high throughput molecular diagnostic imaging, aiming at integrating high throughput molecular testing with detailed cellular imaging for advanced diagnosis and monitoring of disease, in a quest for new powerful clinically-relevant biomarkers. This would concern innovation at the convergence of medical imaging and "in vitro diagnosis" or biological therapeutic delivery, perhaps by combining high throughput microscopic analysis of cellular structure and of the presence and/or (spatial) distribution of specific biomolecules. It could lead to point of care and/or theranostic applications.

5.3 Other common issues

Intellectual property rights and European patents

Without patents for protection of intellectual property, it would not be possible to obtain investments for product development. Therefore, more attention is needed for:

- education of scientists in recognition of patentable “intellectual property”;
- stimulation of scientists to file patents through some form of reward.

The current European patent system has become out-of-date, with old-fashioned national procedures that block fast and straightforward protection of intellectual property at the European level. If Europe wishes to fulfil the aims of the Lisbon agreement, the system needs fast adaptation:

- filing patents in Europe is too complex (compared to USA) and single filing in a single language for all EU member states is required. Preferably English should be used so that no extra costs are needed for filing in USA, Canada, Australia, and many other countries;
- filing patents in Europe is too expensive (compared to USA) and costs should be cut 10-fold to become competitive;
- the introduction of an “*EU provisional patent*” would reduce the threshold for young scientists, provided the costs are low.

To promote exploitation of academic-derived intellectual property, the European Commission should consider extra support for:

- projects with solid protection of intellectual property via patents;
- academia-derived start-up companies.

Protection of individual rights and confidentiality: patient samples, patient data

Without well-defined patient samples it is not possible to develop novel diagnostics. The vast majority of patients in Europe (in contrast to USA) have no problem with the fact that their “remaining” material from diagnostic procedures as well as their coded or anonymised patient data are used to support the development of new diagnostics for improvement of future patient care. To make the most of this advantage, common European guidelines are needed for the collection and usage of well-defined patient material from diagnostic procedures. These guidelines should be clear but simple, with sufficient attention for the privacy issues. A standard procedure is needed, requiring signing a single document that is straightforward, efficient and provides legal protection for patients, but also doctors and scientists.

6. List of abbreviations

CT	(X-ray) Computed Tomography
DG-RTD	Directorate General Research and Technological Development (or “DG-Research”)
DNA	Deoxyribonucleic Acid
EC	European Commission
EU	European Union
FP7	7 th Framework Programme
FRP	Collaborative Project (Small or medium-scale Focused Research Project)
HST	Health Science Technologies
IP	Collaborative Project (Large scale integrating project)
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NIBIB	National Institute for Biomedical Imaging and Bioengineering
NIH	National Institutes of Health
NoE	Network of Excellence
PET	Positron Emission Tomography
R&D	Research and Development
RNA	Ribonucleic Acid
SME	Small and Medium Size Enterprise
SPECT	Single Photon Emission Computed Tomography
US	Ultrasound

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NB: not all participated in all workshops (the details are not given here)

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