EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshop Scheme

Standing Committee for Life, Earth and Environmental Sciences (LESC)

Standing Committee for Physical and Engineering Sciences (PESC)

ESF Exploratory Workshop on

New High-Resolution Multimodal Techniques For The Imaging Of Living Systems

Bordeaux (France), 28-30 September 2011

Convened by:
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SCIENTIFIC REPORT

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1. Executive summary

The ESF exploratory workshop entitled "*New High-Resolution Multimodal Techniques For The Imaging Of Living Systems*" was held in Bordeaux on 28th-30th September 2011. Participation numbered 35 people from 10 countries (France, Germany, Italy, Sweden, China, Taiwan, USA, Belgium, U.K., The Netherlands).
General atmosphere of the workshop was friendly and relaxed but all participants were involved and took part to all discussions. Indeed, considering also the objective of future actions of this emerging community on multimodal imaging in biosciences, all workshop sessions were characterized by a strong involvement of participants and fruitful discussions at the end of each presentation. Coffee-breaks, lunches, and gala dinner were other occasions to continue the discussions and increase the knowledge.

Objectives

The objective was to define new high-resolution multimodal imaging approaches optimized to investigate biosystems at the micro- to nanoscopic scales. Collecting real-time high-resolution concurrent images is critical to understand biochemistry and biophysics, functions, as well as morphology organization at subcellular levels. Several high-performance imaging techniques are available but multimodality is absent or limited with no quantitative capabilities. Moreover, imaging has to fulfill sample safety issues: ionizing radiation dose, thermal heating, stress, etc. To open research opportunities in Europe, this workshop discussed new strategies for the combined analysis of biosystems in order to identify multimodal imaging applications and the required instrumental R&D.

Course of events

The workshop was organized on three days with 17 presentations lasting 45 minutes each as well as two round-tables of about 1h 30 min. The presentations made by experts of the imaging field addressed the last innovations in imaging technology, multimodality opportunities and existing limitations. The round-tables were designed to run the discussion about future multimodal imaging developments. Selected scientists among participants with the aim at addressing expectations of interested academic and research institution animated the first event. The second roundtable was leaded by industrial partners (Bruker, WITec, Horiba, Accurion) with the goal to identify possible crosslinks between the economic objectives of companies with the scientific challenges of the development of multimodal imaging technologies. Another objective of the workshop was also identifying the community (scientists and industrials) interested to work on a long-term research activity on multimodal imaging applications in biosciences.

Scientific outcomes

Presentations dealing with vibrational spectroscopy based microscopy techniques, namely Fourier-Transform InfraRed (FTIR), X-Ray fluorescence (XRF), and Raman, clearly showed that combination with other techniques, notably those providing morphological information from samples, could be of great benefit to foresee new advancements in bioresearches. Applications showing that FTIR imaging could be combined to either AFM or ellipsometry imaging in unique setups also demonstrated the industrial perspectives of multimodal imaging. These examples, together with existing multimodal setups (Raman/AFM or ellipsometry/fluorescence) offer new perspectives in the exploitation of global chemical/molecular information provided by vibrational spectroscopy with morphology (thickness, topography…) rendering. This scenario may open the route towards instruments able to quantify chemical information from a sample at the micro- to nano-meter scales.
Several presentations also addressed the coming prospects of 3D imaging for analyzing small biological objects (from single molecules to small animals) at the nanometer scale, thus opening a new window on inside biology. 3D tomographic or holographic representations of individual cells have been clearly demonstrated by several X-Ray based techniques. However, these new imaging possibilities remain to be addressed in terms of safety issues for the samples, e.g., minimize the dose, but significant efforts have been also devoted towards the possibility of a real multimodal approach. The combination of 3D techniques with physicochemical/molecular modalities is under development in several academic laboratories

or large research infrastructures (notably, synchrotron radiation facilities), and initiatives involving industrial partners are emerging. Multimodal setups combining X-Ray and optical microscopy techniques are under test (e.g., ellipsometry and diffraction methods such as XRD and SAXS), thus showing the trend towards new instrumentation setups, which should finally lead to the release of new commercial systems for the international market. There is thus a tangible economic perspective for the R&D of multimodal imaging instrumentations.
Another major outcome of this workshop was to address safety issues while using intense and brilliant sources for imaging biosamples. Whatever the source is a laser or an X-Ray beam, different presentations highlighted how the development of nanoimaging instrumentations has to take care of the dose-response of a biological object (ionization, heating, subtle effects…). It was demonstrated that short-to-long term dedicated studies must be performed on these safety issues for highlighting the effects on biosamples, which are major drawbacks of numerous studies that have been published in nanoimaging so far. Such studies are also important because they often times lay the foundation for the development of future applications.
Several examples of applications of multimodal imaging in biosciences have shown or outlined the major role it will play in the future. It was suggested that “multimodal imaging” includes both the use of concurrent imaging techniques on the same sample as well as the use of specific probes allowing collecting as much as possible information at the same time. As a general overview, one has to consider that the role of multimodal imaging is fulfilled when the solution of a biological issue is associated to chemical/molecular information obtained in the most exhaustive context. This means that the development of instrumentations and tools allowing the combination of chemical/molecular with topographic/tomographic information on the same sample is the main target of the future multimodal imaging applications in biosciences. A general consensus rose from the audience on this critical issue and this will be the foundation of the next common initiatives.

Conclusions of the workshop

In this ESF exploratory workshop, it was clearly understood that many biological issues would greatly benefit from the developments of multimodal imaging approaches. In biosciences this is an emerging field requiring further organization and cooperation among different institutions for the most efficient development. As exposed in the Section 4 of this report, it appeared clearly that the multimodal imaging community has to trigger and coordinate a large European action to share all skills and means required to biological investigations. Several discussions between participants also led to formal meetings to come for implementing new collaborations, notably between teams belonging to different scientific fields, at the interface between physics – chemistry – biology.

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2. Scientific content of the event

The workshop addressed current opportunities in the development of imaging modalities for multimodal instrumentations and applications in biosciences. On the one hand, several presentations showed the state-of-the-art of imaging techniques for analysis of living systems and their recent development for allowing multimodal applications. On the other hand, several contributions showed that in many cases, currently available imaging techniques need only minor changes or dedicated tools to match their utilization in biosciences. It rose from roundtable discussions that the development of such tools (e.g., sample holders, data treatment methods, dedicated softwares, fluidic devices…) can be considered among the many partnership objective between academic and industrial actors of multimodal imaging for biosciences.

Vibrational spectroscopy based imaging

Goormaghtigh: *FTIR imaging of cells and tissues: towards protein secondary structure imaging*. We have illustrated here the status of the art of FTIR spectro-imaging in the analysis of protein secondary structures. It was demonstrated how sophisticated spectral data treatments can produce molecular images reconstructed from complex samples, i.e., cells and tissues. Possible routine applications were envisaged.

Kazarian: *Potential of ATR FTIR spectroscopic imaging approaches for studies of live cells*. An overview of recent advances in FTIR spectroscopic imaging using infrared array detectors was provided about the visualization and distribution of chemical components in cells without labeling. Applications of time-resolved chemical imaging to processes in live cells and flows in microfluidics were also presented.

Cricenti: *Near field optical microscopy imaging in the 1-10 micron spectral range: overview and perspectives*. An overview of the near-field techniques applied to biological specimen was given using conventional, synchrotron radiation and free electron laser sources. Current limitations of these approaches have been discussed for further improvements in the scenario of the multimodality.

Cinque: *Diffraction-limited InfraRed MicroSpectroscopy and Imaging at Diamond: state of the art and research examples in Life Sciences*. Experimental capabilities and research examples performed at MIRIAM Beamline via FTIR microprobe at Diamond 3rd GLS facility were given. Research carried out at Diamond in microbiological and biomedical applications were presented, with specific emphasis on the highest spatial resolution optically achievable in IR microscopy and/or and highest S/N spectral quality attained via IR microspectroscopy as well as IR imaging. He presented new perspectives for enhancing the SR micro-FTIR sensitivity and resolution, together with the concurrent characterization of the biomedical sample with other ancillary microscopy techniques e.g. in the visible range, (epifluorescence, bright/darkfield, DIC, etc.).

3D morphological imaging

Pianetta: *Application of hard X-Ray TXM to biological imaging*. State of the art of X-Ray microscopy and latest achievements with synchrotron radiation in the USA were presented. Information on biosamples analyzed at ultimate spatial resolution for 3D rendering were discussed regarding significance in bioscience researches.

Hong: *Differential Phase Contrast Images Analysis in the Hard X-ray Transmission Microscope*. It was presented and discussed novel methods to investigate quantitative differential phase contrast images using hard X-ray microscope system. These methods are important to minimize the X-Ray dose for 3D reconstruction of small and large biosamples.

Lüning: *X-ray spectro-holography for lens-less imaging of biosystems*. Imaging using coherent brilliant X-Ray beam have been presented. Special emphasis has been given to methods under development to manage the radiation damage vs. the spatial resolution in particular on biosamples. Efficiency of X-Ray spectro-holography was presented for future real applications.

Thiesen: *Accurion, a leading company in ellipsometry-based analytical techniques. Can Imaging ellipsometry and Brewster angle microscopy (BAM) identify living cells?* Imaging ellipsometry and Brewster angle microscopy have been presented for time-resolved study of cells at the interface for high-sensitivity and no-radiation damage applications. Combinations of this technique for multimodal developments have been discussed.

Multimodal chemical/morphological imaging

Schmidt: *WITec, a leading company for the development of Raman-based multimodal imaging instrumentation*. Confocal Raman Imaging Applied in Bio-Medical Sciences. View from the industrial side of imaging instrumentation development. Possibilities offered by a multimodal system based on Raman microscopy were emphasized for analysis of biological specimens. Most of presented examples showed the need for a close relationship with academic scientific researches for development of cutting-edge instrumentation.

Dazzi: *Infrared nanoscopy done by photothermal technique*. An innovative IR spectromicroscopy technique coupled to AFM was presented. It allows local IR imaging at the nanometric scale. It was discussed further implementation of the method, the combination with other imaging modalities and the possibility to extend the technique to living systems.

Hwu: *Multimodality imaging commanding on 3D imaging.* Recent progress in 3D imaging by X-Ray PC were presented with all opportunities for coupling with other imaging methods. From cell to animal imaging scales, several relevant biological issues have been presented and discussed. Foreseen international-scale initiatives have been also envisaged.

Safety issues with high-performance imaging techniques

Ramundo-Orlando: *Biological effects of Terahertz radiation*. The sensitivity of biological specimens to low-power THz radiation as well as their nonlinear effects over exposure time was summarized. Safety issues on multimodal imaging technology applied to the analysis of biosamples were also deeply emphasized.

Wu and Wang: *Energy optimization for three-dimensional X-ray imaging of a whole, large cell*. It was emphasized the relevance to develop new phase contrast methods to work at high energy to optimize the contrast of images of large cells and to manage the X-Ray dose vs. resolution in 3D reconstructions. Recent achievements and ongoing projects in China have been presented.

Multimodal imaging applications to biological issues

Acher: *HORIBA, a company developing multimodal instrumentations*. It was presented the opportunity to couple Raman microscopy with other techniques. A contribution for the development of tools and softwares dedicated to biosample analysis were also considered.

Policar: *Photothermal IR-Spectromicroscopy for Subcellular Imaging: an Example of Cellular Mapping of a Metal-Carbonyl Exogenous compound*. This communication has presented both the results of a quantitative analysis of the cellular content by sub-micron resolution FTIR imaging and the chemical mapping of IR-sensitive labels in cells. In the future, this research will be optimized by using high-flux and time-resolved IR sources.

Petibois: *Development of multimodal imaging solutions for disease pattern recognition*. Several multimodal imaging applications to cancerology and virology have been presented highlighting the role of cutting edge imaging technologies in the disease investigation. Developments of benchtop and SR-based techniques for major health issues have been discussed.

3. Assessment of the results, contribution to the future direction of the field, outcome

What we learned?

As outlined in the scientific outcomes of this ESF exploratory workshop, we could identify the main role of multimodal imaging in biosciences as the combination of physical/chemical/molecular with topographic/tomographic information to face and possibly solve challenging biological issues, and more specifically, for disease pattern recognition. We also realized the huge amount of work remaining before multimodal imaging setups will satisfy the reliability parameters of routine instrumentations, i.e., those that can be used with the confidence of obtained results and safety of a sample at the time of the measurement. However, using spectroscopic and morphological techniques at the nanoscale, it seems obvious that safety considerations must be considered for proper interpretation of obtained results. Established methodologies are also required to allow analyzing the same sample with two or more imaging techniques without altering or modifying the sample during the sequence of acquisitions. It also appeared clearly that new data treatment methods must be developed for combining several sample imaging means at concurrent moments and with different lateral spatial resolutions, specificity, and sensitivity. The development of unique setups combining the different sample information at the same location and at the same time appears as the only solution to achieve such objective. It follows that software allowing both to drive these multimodal instrumentations with similar time and lateral/spatial resolutions as well as common image reconstruction is the major expectation in the field. It was also pointed out that imaging the same sample by different techniques could require the development of specific tools (sample holders, fluidic devices…), which can be experimentally dependent. An effort is expected by scientific community to limit the “home-made” tools usually found in laboratories, and which reduce our capability to compare studies and methods, for proposing more standardized devices.

New research objectives

Identifying the analytical capabilities of MI setups and methods is a key-issue for any foreseen commercial development of new instrumentation. As discussed with industrial partners, bilateral or multi-lateral initiatives should occur after the ESF workshop. It has been pointed out that developing multimodal imaging in biosciences would require the convergence of a lot of skills and knowledge for each application. Every application in academic research is case-dependant and cannot be simply applied to other topics without any arrangement. Furthermore, it is clear that scientific teams and industrial partners cannot share their respective research subjects and technological developments without taking care of the fundamental issue of the intellectual property. There is then a mismatch between the complexity and specificity of scientific projects requiring the use of multimodal imaging methods and the development of new instrumentations by industrial partners with generic utilization objectives. However, there was a common understanding that efforts must occur on both sides (academic and industrial) to see the advent of new multimodal imaging setups. The international competition with Asia and United states shows that Europe must continue to feed the technological innovation to remain at the forefront of microscopy and imaging techniques not only in life sciences. One way to contribute to this strategic effort is to define common actions that can help understanding the best applications of multimodal imaging that will lead to commercial end-products for the instrumentation market. As a general outcome of the ESF workshop, the development of common procedures, e.g., testing the combination of different techniques to foresee what multimodality provides in terms of new information on (bio)samples and tools such as sample holders, data treatments, software codes, etc. … to strengthen MI applications has been considered as a possible starting issue for sharing academic and industrial objectives. One may also consider that developing common strategies to determine which applications in biosciences will benefit from multimodal imaging approaches is a major way to avoid unavoidable duplications of R&D between companies.

Organizing the scientific community

One important results of the workshop was to realize how industrial partners pay attention to academic expectations. Discussions between participants have been fruitful orienting the development of multimodal imaging technology and application to biosciences. All industrial participants claimed their interest to eventually contribute to the set-up of structured research projects or programs on multimodal imaging in biosciences. On the other hand, the scientific community has to identify clearly technological combinations potentially useful for the investigation of biological samples ranging from molecules to small animals. It was also highlighted the major role of large research infrastructures such as synchrotron radiation facilities for testing new setups, modifying existing instrumentation, test limitations of source effects on biosamples (dose-response issues…), validates new analytical approaches. Therefore, unifying the efforts of scientific teams working with conventional sources and those performing researches at large research infrastructures will result in a benefit for any future action. The participants have shown their will to continue organizing and structuring the research activity in the field of multimodal imaging applied to biosciences actually implementing a larger European initiative, under the form of a COST action. The purpose will be strengthen the existing cooperation and expand this interdisciplinary community already bringing together physicists, chemists, and biologists interested in the development of multimodal imaging, as well as the related European companies, to envisage the necessary instrumentation developments.

Towards a COST-action – preliminary proposal

Following the exploratory ESF workshop, the coupling of morphological and spectroscopic techniques has been recognized as a ground for future opportunities of R&D and scientific researches. To identify which techniques can be usefully coupled in the biosciences field, a COST-action has been considered with this working title:

Title: Multimodal morphochemical imaging at multi-scale in biosciences

Acronym: 3M-live.

COST domains: 1st = MPNS with multidisciplinary research; 2nd = BMBS.

Key-words: multimodal, multi-scale and complementary, physicochemical, morphological, live, biology, 3D, nano-science, functional/molecular imaging

Short description: Over the last decades, biology has made significant advances in providing a rational understanding of the molecular mechanisms governing life's processes at cellular and sub-cellular levels. However, new imaging instrumentations are emerging for a closer look at chemical/molecular pattern of changes in their environment. Physicists and chemists developed these imaging systems and the required probes to obtain targeted information from complex samples. However, with the advent of "*omics*" age, biosciences have demonstrated the major role of multiparametric information to unfold the intrinsic complexity of living systems during disease occurrence and progression. Recent technological developments allow combining spectroscopic and morphological techniques in unique powerful setups for time-resolved, high-resolution, and live investigations of biosystems, notably for unraveling molecular bases of diseases in their environment. We propose to accurately test the potential of multimodal imaging setups in order to develop new methodologies and tools optimized for bioscience investigations, from molecular interactions to small animal imaging. Combination of spectroscopy-based imaging/microscopy techniques (FTIR, Raman, X-Ray fluorescence) with morphological techniques (based on X-Ray microscopy and holography, ellipsometry, AFM…) will be considered both on model cases and living biosamples.

The proposed Action fulfills the need of a European coordinated research and development of challenging technologies, aiming to bridge the gap between imaging modalities optimization and practical utilization with a future significant impact on biophysics, biochemistry, biomedicine and nanobiotechnology.

4. Final programme

Wednesday 28th September		Thursday 29th September		Friday 30th September	
S1 - Opening session		S5 - Multimodality advantages (Marcelli)		S9 - Optical imaging (Cricenti)	
08:30 - 09:00	Welcome by convenors	08:30 - 09:15	Dazzi	08:30 - 09:30	Luning
09:00 - 10:00	Introductions Bauderon/Dufourc	09:15 - 10:00	Hwu	09:30 - 10:00	Thiesen
10:00 - 10:30	Coffee break	10:00 - 10:30	Coffee break	10:00 - 10:30	Coffee break
S2 - Chemical imaging (Petibois)		S6 - Multimodal imaging (Pianetta)		S10 - Multimodal imaging (Policar)	
10:30 - 11:15	Goormaghtigh	10:30 - 11:15	Schneider/Schade (cancelled)	10:30 - 11:15	Petibois
11:15 - 12:00	Schmidt	11:15 - 12:00	Cricenti	11:15 - 12:00	ESF - Funding opprotunities
12:00 - 13:30	Lunch	12:00 - 13:30	Lunch	12:00 - 13:00	Lunch
S3 - 3D imaging (Hwu)		S7 - IR & THz imaging (Kazarian)		13:00 - 13:30	Conclusions and perspectives
13:30 - 14:15	Pianetta	13:30 - 14:15	Cinque		
14:15 - 15:00	Hong	14:15 - 15:00	Ramundo-Orlando		
15:00 - 15:30	Coffee break	15:00 - 15:30	Coffee break		
S4 - Chemical/morphological imaging (Dazzi)		S8 - nano-imaging (Goormaghtigh)			
15:30 - 16:15	Policar	15:30 - 16:15	Acher		
16:15 - 17:00	Kazarian	16:15 - 17:00	Wu/Wang		
17:30 - 19:00	Roundtable multimodal Imaging	17:30 - 19:00	Roundtable Industry		
		20:00 - 23:00	Gala diner at Château Huchey-Halde		

5. Final list of participants

Name	Surname	Mail	Institution	Country, town
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6. Statistical information on participants

Participants were 45-years old on average, with M/F participation of 32/8 and equilibrated repartition between physicists, chemists and biologists. They came from 10 countries: France, Germany, Italy, Sweden, China, Taiwan, USA, Belgium, U.K., and The Netherlands.