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EDITORIAL

Morpho-spectral imaging for investigation of disease progression

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The search for novel bioimaging techniques is essentially driven by the need to increase our knowledge about diseases, with the objective of investigating their critical molecular basis in their environment. This usually occurs at the cellular or sub-cellular level, making spatial resolution and time–resolved observation critical issues. Many techniques have failed because of their inability to reach targets revealing the development or occurrence of a disease and/or because they damage or alter the samples. The development of modes of imaging for biological research requires the merging of specific achievements in imaging technology and related methods for reliable, safe, and reproducible analysis of biosamples.

UV-fluorescence-based microscopes have become routine tools for biological research because of their exceptional analytical performance. First, their main strength is to remove any doubt about the result. The number of molecular probes is growing every day, giving the sensation that almost everything can be probed. They can also be coupled to morphological (3D confocal) or topographic (3D non-confocal) techniques with satisfactory resolution, well below 1 μm, thus enabling determination of the location of probes down to the sub-cellular level. Another important advantage is their capability of time-resolved and kinetic measurements, thus making live imaging possible. However, these approaches are rather limited by the number of probes that can be used at the same time, usually no more than 2–4. Another major limit is the predetermined way of investigating a disease: probes usually target given molecules, which can be relevant for diagnosis, but this remains largely questionable for exploratory studies. Other limits are also reducing interest in these techniques, for example photobleaching of fluorescent probes over time, and the possibility of false-positive results.

For these reasons, probe-free techniques able to provide global information about the sample are now being developed as non-oriented means of analysis in the biological sciences. Spectroscopic techniques are among the best candidates for such coupling to 3D imaging; some have also been developed for non-destructive analysis of biosamples. Fourier-transform infrared (FTIR), X-ray fluorescence (XRF), and Raman techniques enable laterally or spatially resolved chemical mapping of biosamples. The recent commercial release of multimodal systems combining AFM with FTIR or Raman microscopy shows the trend toward utilization of morpho-spectral imaging in the biosciences. Other initiatives include the use of ellipsometry, another non-destructive technique, for rapid 3D analysis of thin samples; this technique can be also coupled to IR microscopy for safe multimodal analysis of biosamples. Ellipsometry, and X-ray microscopy with a CCD camera, can also be regarded as valuable tools for time-resolved studies, at least down to the scale of seconds. Coupling of X-ray fluorescence microscopy to X-ray phase-contrast (XR-PC) imaging in synchrotron radiation facilities has also been tentatively proposed; this would enable elemental characterization of samples with 3D rendering. Therefore, several hybrid imaging arrangements are—or can be—proposed for characterization of biosamples with time-resolved, high-resolution, and in-vivo applications, potentially all combined.

Biosafety issues have also become a major issue in routine in-vivo imaging and now concern all applications, from fundamental biological research to clinical routines. Morpho-spectral imaging approaches can result in great achievements in biological research only if biological

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samples are designed for imaging purposes, i.e., suitable for highly sensitive imaging results and enabling efficient recognition of the moieties of interest without altering the sample. Compared with all other imaging methods, which use molecular probes subject to ionization (UV-florescence probes) or radioactive contrast agents (PET), the only questions about spectroscopy-based techniques are about the source they use, with specific concerns about lasers and X-rays. The X-ray dose applied to a biological specimen is obviously a severe limit for in-vivo applications of X-ray microscopy. Confocal Raman microscopy also requires determination of the limits of laser biosafety for samples, notably in the development of cell-imaging research.

The development of dedicated methods of preparation of biosamples is also a major expectation for morpho-spectral imaging applications. Basically, molecular processes occurring at the cellular level must be visualized in vivo with one or more modes of imaging. The impact of multimodal imaging (e.g. PET-CT and MRI-CT) on medical diagnosis is impressive because it enables early diagnosis and real-time monitoring of pathological development. Such in-vivo achievement without alteration of the biological specimen, combining disease diagnosis with potential therapy, can become of huge interest. To this end, the development of multimodal contrast agents has become of utmost importance. These agents will be sensitive to different physical properties in respect of X-ray attenuation, IR absorption, UV-fluorescence … etc., thus enabling use of several techniques to probe different phenomena in the same environment. In this way, biological applications of morpho-spectral imaging will be enlarged to cover a wide range of disciplines. Drug delivery will most certainly benefit in a variety of ways from such multimodal contrast agents: image-guided drug delivery, triggered drug release, drug-delivery monitoring, or therapy monitoring by imaging. Even if the development of multimodal contrast agents again generates questions about biosafety, specificity, and multiple utilization, it will also lead to new types of bio-imaging because of sensitivity to both morphological and molecular techniques.

The development of morpho-spectral imaging instrumentation, and related imaging procedures, is expected to grow markedly in the forthcoming years as new and powerful tools reveal the potential of multimodal imaging in biological research, and possibly in medicine and clinics. This is clearly a multidisciplinary field, in which contributions from chemists, physicists, biologists, physicians, mathematicians, and imaging technologists must merge to design new approaches for better understanding of major diseases.

The 4th international workshop “Imaging Techniques with Synchrotron Radiation”, held from 24th–30th September 2011 in Bordeaux (France) and supported by the European Science Foundation (ESF), was a unique opportunity to discuss the usefulness of new multimodal imaging instrumentation for determining major analytical limits. The development of morpho-spectral imaging systems was one topic of the workshop, shedding new light (and waves) on living systems to unravel the interface between morphological and chemical/molecular properties of pathologies. These new arrangements can provide new information about disease progression, notably for cancers, neurological disorders, degenerative diseases … etc., i.e., where time-resolved and kinetic investigations are required. The Bordeaux Workshop has also shown that further developments can be foreseen for routine and diagnostic applications of morpho-spectral imaging instrumentation, thus contributing to enhancing our capacity to better understand human diseases.

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