

Integrative Imaging: a New Challenge for Cell Biology

The main feature of cell biology is to apprehend functional and/or structural mechanism in their whole, from the molecular to the cellular aspect. Different microscopy techniques have been developed during the last decades. Each of them is used to answer only one particular aspect of the studied biological mechanism. The combination of different data of the same sample coming from various techniques could help to reveal masked information. The feature of integrative imaging hence is to combine the data at various levels of resolution in order to create a unique model that will reflect the general mechanism. However, there is no easy-to-use tools that allows the fusion of all data present in the images. Integrative imaging implies the development of new tools for registering and overlay heterogeneous data (2D, 3D, structural, chemical) of the same sample, that means an adaptation of protocols of sample preparation. First steps were done with correlative microscopy between optical and electron microscopy. Recently, we have successfully combined transmission electron microscopy, EFTEM and SIMS, opening the way to complete integrative imaging.

Keywords

Image processing and analysis, microscopy, electron microscopy, confocal, tomography

Innovative Imaging Techniques in Cellular Biology

The last decades have shown a tremendous development in cellular biology, especially in innovative imaging techniques. In parallel, the development of computers, in processing capabilities and data storage, have made possible the use of imaging as a basic tool for research in cellular biology, as molecular biology or bioinformatics. Furthermore, the improvements of imaging techniques were quantitative, in terms of number of images that can be acquired and processed, and also qualitative, opening new ways of looking at cells. The major qualitative steps include 3D imaging such as confocal microscopy and electron tomography [9, 6]. Another major step, much less exploited, includes analytical microscopy, such as EFTEM (Energy Filtered Transmission Electron Microscopy) [4], EDX (Energy Dispersive X-ray) [8] and SIMS (Secondary Ion Mass Spectrometry) [5], which permit to obtain a chemical view of the cells.

Ranging from a molecular level with electron microscopy, to cellular level with optical microscopy and at a large end to tissue with URI, organisms can be deeply studied at any resolution level. In parallel to these structural data, analytical data can also be obtained.

Observe Cells in 3D

At the molecular and sub-cellular level, molecular volumes obtained by Single Particles Analysis (a.k.a Molecular To-

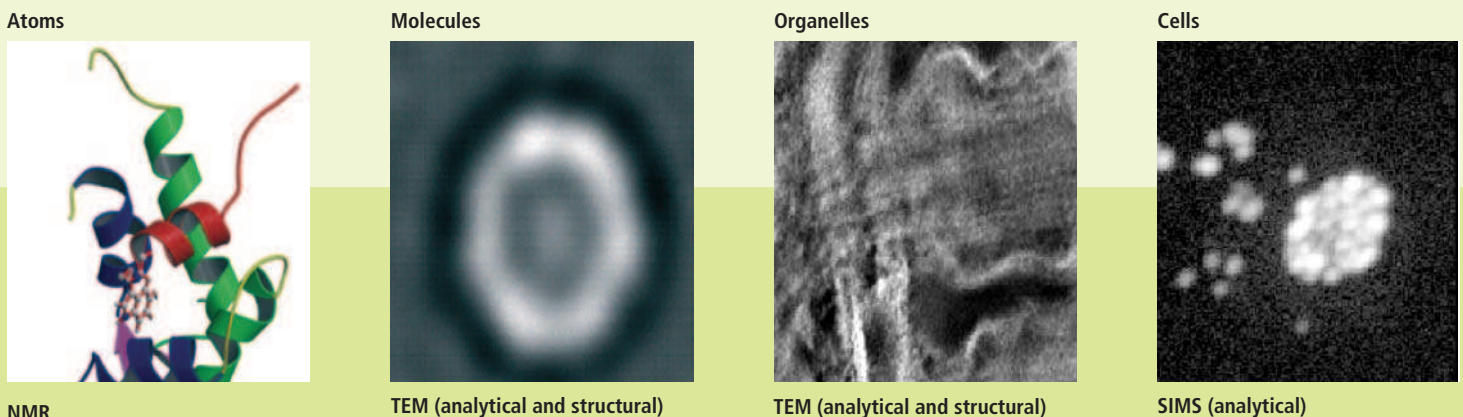
mography) can be fitted into sub-cellular volumes obtained by electron tomography (a.k.a Cellular Tomography) [6]. In cellular tomography, the sample is tilted inside an electron microscope in order to obtain projections of the same sample at various orientations. Then reconstruction algorithms can be applied to obtain a 3D volume of the sample. With this technique, cellular organelles, cytoskeleton, and in general cytoplasmic and nuclear structures can be observed. It is then possible via the fitting of molecular and macromolecular volumes to obtain a functional 3D view of the cell [2].

To Combine Images for Complementary Information

At cellular level, duo-labelled probes have been developed in order to combine images from fluorescence and electron microscopy [3]. These two techniques bring complementary information at cellular and sub-cellular level. Optical microscopy can give information of the cellular distribution of a protein targeted with a fluorophore-labelled antibody, while electron microscopy can give information of the ultra-structure around the protein.

Idea of the Chemical Composition

In parallel with structural techniques, analytical techniques can bring important functional information such as the distribution of vital chemical elements including P, O, Ca, or S. In electron mi-



croscopy, sorting of electrons according to their energy loss (EFTEM) allows one to have a precise idea of the chemical composition of a sample. Combined with electron tomography (ET) it is then possible to obtain a 3D chemical distribution of the sample [7].

At a higher level (μm), SIMS permits to obtain up to 5 images of the distribution of almost any element (such as O, Ca, I, or Si) in parallel with a structural image (distribution of C and N atoms). The precision of the technique can also permit to detect isotope elements such as ^{15}N and ^{18}O .

Despite the rapid evolution of these individual techniques that yields an increase in raw data, this does not necessarily yield an increase in pertinent information. Some questions are still unresolved due to the lack of bridges between imaging techniques.

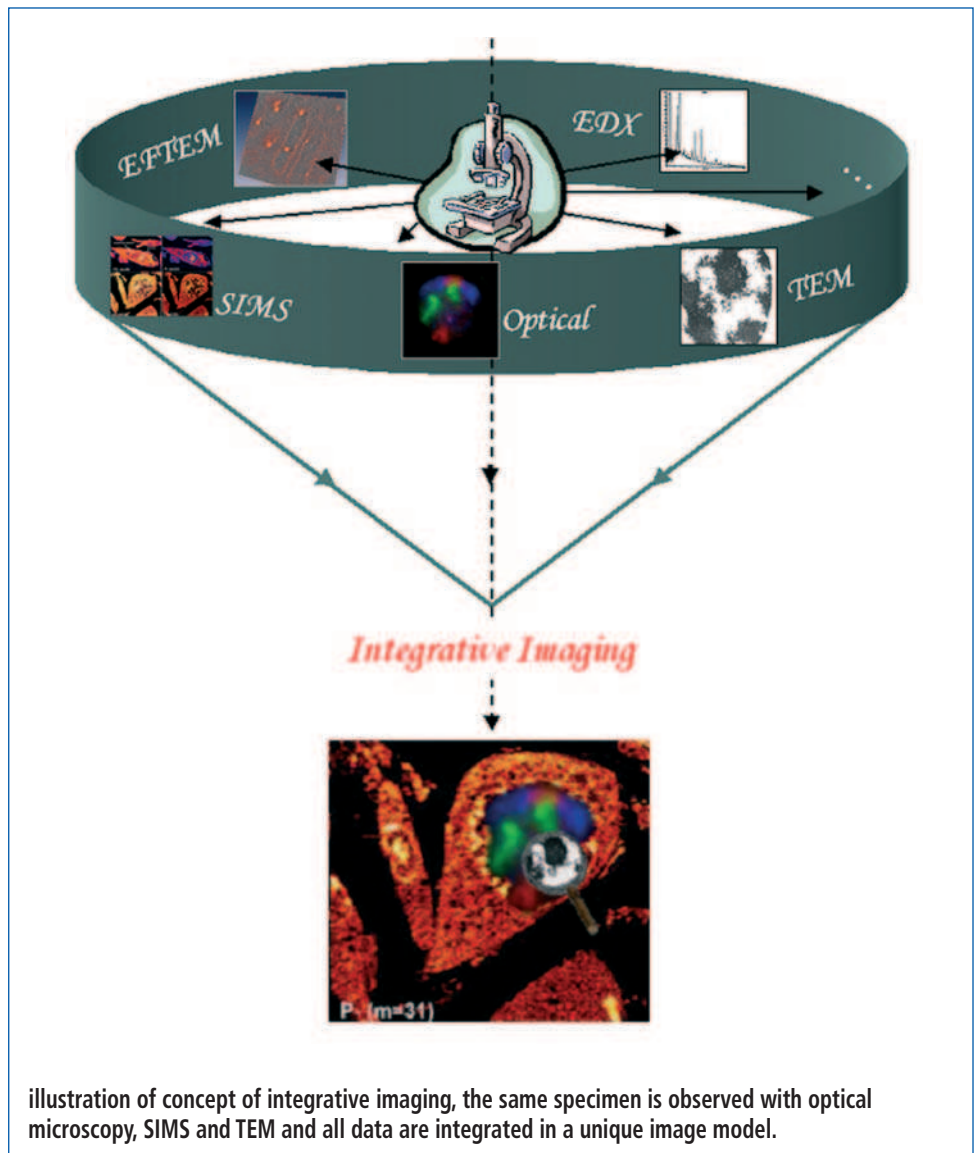
Birth of Complete Integrative Imaging

A first bridge between imaging techniques was built with correlative microscopy between electron and optical microscopy. Another bridge concerning ultra-structure technique is also possible. We have successfully combined ET, EFTEM and SIMS on a preparation of bacteria containing iron granular inclusions giving new ideas about the functional interest of these granules.

Hence combining classical and ultra-structural correlative microscopy gives birth to complete integrative imaging. The goal of integrative imaging is to obtain, from a serie of images coming from various techniques of microscopy, a representative 2D or 3D model of a unique sample, that can then be visualised at various resolutions and modalities.

A Challenge for Cellular Imaging

The integration of all the data present in the images will be a challenge for cellu-

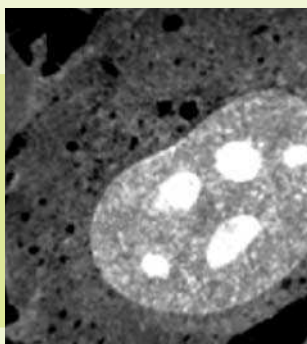


lar imaging. This will imply the development of new protocols for sample preparation and new image analysis tools.

Concerning image analysis, new and easy to use software needs to be developed in order to successfully register and overlay all the images at different levels (nm to μm) that can be 2D, 3D,

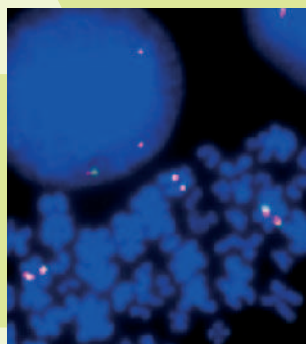
structural or analytical. The challenge will then be to try to match features in images at different resolutions. Once all the images are registered, a synthetic model should be built integrating all the information. An effort should also be made in the field of visualisation.

Cells



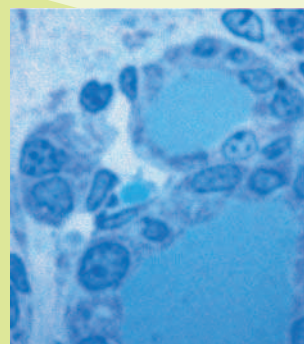
SIMS (structural)

Cells



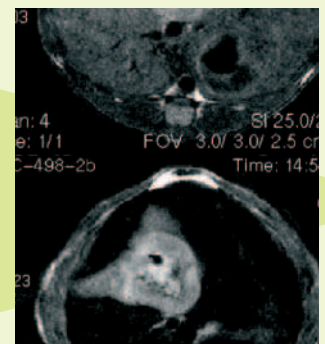
Optical Microscopy

Tissue



Optical Microscopy

Organism



MRI (analytical and structural)

Samples Fit in various Instruments

In the same way, experimental procedures need to be set up to be able to prepare a sample in order to fit the requirements of the various instruments. For instance, the sample must be prepared / fixed for optical microscopy not on classical glass slide but on a grid compatible with electron microscopy or SIMS. After observation using optical microscopy, the sample will need to be further fixed for electron and SIMS microscopy. However, one other challenge will be to find adapted protocols for cell fixation, including cryo techniques, which will modify as least as possible the internal structures of the sample [1].

Shed Light on all Information in Cells

Integrating data from different images sources such as fluorescence, SIMS and electron tomography can give global and complementary information from sub-cellular to tissue level. For example, some drugs used in chemotherapy can produce chromosomal abnormalities (for instance etoposide in acute leukaemia). First, SIMS images can give information about the localisation of isotope-labelled drugs in cell compartments (for instance in the nucleus). Second, fluorescence images (for instance 3D-FISH images) can give information about the localisation of genes involved in abnormalities. Third, electron microscopy can give information about chromatin context – euchromatin vs. heterochromatin – around genes of interest and/or drugs. Furthermore, macromolecular complexes involved in nuclear processes (such as chromosomal repair) could eventually be fitted inside the electron image or volume.

In conclusion, new bridges should be built between actual imaging techniques. As the amount of integrated information is greater than the sum of independent information, integrative imaging that aims to combine different imaging data will be one main challenge for cell biology.

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