# Scale and Time in Biological and Medical Imaging: the observation and integration paradigms.

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***Abstract* – Biological and medical imaging technologies have made impressive advances over the last decades. Some of their dimensions are health care driven (from screening, diagnosis assessment to therapy), others technology driven (bio-arrays, micro- to macro-imaging) or biology driven (genes to proteins and cells). Computational modelling and information technology in biology and medicine is also becoming to have a major role in the interdisciplinary attempt to elucidate functions of living systems and structure-to-function inter-relations. However, the recursion between description levels, at all space and time scales, for highly dynamical, interacting and complex systems, still requires new breakthroughs in observational tools closely coupled to sound information processing for confrontation to theoretical models.**

## I. INTRODUCTION

Physics, chemistry, applied mathematics and engineering science are all needed to address relevant biological and physiological issues. The targets range from molecules, genes, proteins, cells, substructures, organs and systems up to whole organisms. The understanding of their biological or pathophysiological behaviours is first challenged by the intrinsic complexity to deal with and to the fact that a number of mechanisms are still ill-understood. The elucidation of the intra- and inter-level processes depends on our capabilities (i) to design advanced devices with fast acquisition rates, (ii) to extract, quantify and interpret the information brought by the data, (iii) to build quantitative models and to maintain them as testable working hypotheses.

Structures, shapes and functions are intimately linked in living systems. Their quantification requires unravelling the multidimensional relations they have at multilevel and multiscale, the multiphysics expressions they take, and the intricated spatial and temporal patterns they share. The understanding of dynamic, complex molecular and cellular systems call for the improvement of observational devices among which imaging tools have a major role. We have emphasized [1] for different objects and objectives how it was important, at the same time, to design new paradigms for information processing and to establish sound mathematical models in order to integrate and control the increasing amount of data and knowledge. The main goal was to propose a reading grid while revisiting some aspects of image processing, and to stimulate research in the area. Some basic and generic issues were recently reviewed on motion analysis [2] like motion estimation, object tracking, segmentation and motion coupling. A second paper [3] dealt with motion in medical macro-imaging with the idea to propose new windows on its relations with the image capture and image reconstruction. A third and last paper in this series is devoted to biological imaging, e.g nano and micro imaging [4]. Although a number of concerns are shared when going to these scales, the conditions of observation, the objects under study and the problems to address make this research very challenging by the cautions that must be taken to control the experiments, a mandatory condition to get conclusive results and to infer sound conclusions. The present lecture emphasizes the need for a transdisciplinary research through a few key highlights. There is no doubt that more involvement of engineering disciplines is central to bring together different pieces of knowledge, methods and techniques.

## II. FROM NANO TO MICRO IMAGING

Much has been said on medical imaging [5] and on the emergence of molecular imaging [6]. Thus, we will shortly focus on the techniques available for biology [7]. Let us emphasize first that in biology, and in contrast with medical imaging where the clinical process to acquire images remain relatively simple, the preparation and the conditioning of the samples is certainly a critical step. For instance, the optical properties of a sample rely on the all components that will be used to fix it: the glass coverslip, the refractive index of the immersion medium, the temperature have an important impact in the image formation and the presence of distortions and aberrations.

In addition, these properties are directly enhanced by extrinsic markers, which are themselves time-varying (degradation of the fluorescent probes over time, or photobleaching). Tagging techniques based on probes, or markers, attached to a targeted protein, made a real breakthrough in biology.

Many solutions are today available to get insights into biological objects (refer to a recent special issue [8]). Atomic Force Microscopy (AFM), with the tapping mode in fluid, allows accessing individual biological molecules and thus makes possible the study of their dynamics and their interactions. Electron microscopy (EM) is another mean to explore the structures of biological specimens over a wide range of scales, from cellular structures to single macromolecules. Cryo-electron tomography (CET), with a resolution of about 4-5 nm, provides access to assemblies of multiple proteins with positions, shapes and even conformational changes.

Optical 3D image forming devices are among the most quickly evolving techniques. They have the advantage among others to achieve dynamic studies on hydrated, living cells [9] [10], to track change in protein conformation, to co-localize and detect protein-protein interaction. New advances in sensing are expected with faster scanning (higher than 100 frames/s) and better depth resolution in multiphoton imaging, multiple objective imaging [10] and selective plane illumination or stimulated emission depletion (STED). These technological advances are accompanied by the design of protein probes. They allow the understanding of the cellular and subcellular mechanisms involved in the synthesis and the delivery of specific macromolecules. Many fluorescent labels have been developed and will continue to be. Beyond quantum dots (made of a nanometer-sized semiconductor crystal core and an external protective shell) which have a broad absorption and a narrow emission spectra, resulting in brighter fluorescence, a very active research is devoted to labeling of recombinant proteins and photocontrollable proteins (photoactivable, photoswitchable).

This short overview is far from covering the all modalities already at our disposal and the exceptionally fast evolution they have. It would have been of interest to mention variants such as Fluorescence Correlation Spectroscopy (FCS), Image Correlation Spectroscopy (ICS), etc. One of the most promising technique is certainly represented by Multifocal Multiphoton Microscopy (MMM) for imaging in 3D the living cell [11], overcoming the diffraction barrier set by Ernst Abbe in 1873. Another example is given by Secondary-Ion Mass Spectrometry (SIMS) and the new Multi-isotope imaging mass spectrometry (MIMS) which offer a way to investigate and quantify tagged molecules in subcellular volumes [12]. In all cases, combination of these techniques will occur that will lead to a new generation of instruments.

## III. INTEGRATED MULTIMODAL IMAGE SENSING

The trend toward multimodal data acquisition is not new. It has been widely emphasized in macro-imaging for years, either by coupling in the same device different techniques (for instance, Computed Tomography and Positron Emission Tomography) or by registering data sets (3-D/3-D and 2-D/3-D) recorded at different times. It will take more importance with the emergence of *Nanomedicine* [13]. This new area aims as "the comprehensive monitoring, repair and improvement of all human biological systems, working from the molecular level using engineered devices and *nanostructures* to achieve medical benefit". The same report identified *nanomaterials* and devices, *nanoimaging* and analytical tools, novel therapeutic and drug delivery systems, as the major technological components to address. The objective is the *in vivo* measurement and characterization of biological processes at the cellular and molecular level, and to be more precise, beyond the standard anatomical and functional mapping, the *in vivo* detection and quantification of molecular disease markers or therapeutic agents via specific probes. It is expected that early disease manifestations will be detected by enzymes or signalling molecules. Succeeding in such challenges should take time of course and should address many faces among which patient-specific patterns and adverse drug reactions.

As an example of what can be expected in optical imaging, the advances brought by FRAP, FRET, FLIM lead up to high dimensional observations in cellular biology with access to the fluorophore environment (Ca concentration, membrane potential, etc.). Their applications range from cellular signalling to physico-chemical parameters and metabolic studies. Their extension to multiphoton imaging, which has the advantage of non-linear near-IR in terms of spatio-temporal resolution and prolonged tracking, deserves today a special attention. Multi-Harmonic Light microscopy with active non-linear chromophore is another example of new tools for dynamic, non-invasive and in-vivo exploration of cells that can complement recent microtechnologies (multiple micro-arrays). The multi-modal dimension is, here too, of concern with the capability to couple optical systems like FRET and FLIM with electrophysiological techniques or others. In fact, beyond this example, it is believed that the design of devices capable to simultaneously capture chemical, electrical, mechanical, etc. characteristics, at a given entity level or even better at several levels, is one of the major challenge for the future.

## IV. THE MULTILEVEL INTEGRATION

*"Integrative"* is certainly one of the most popularized term today in almost all Life Sciences and particularly in Biology and Physiology. It is opposed to the "*reductionist*" approach whose goal consists to identify always finer molecular and cellular events studied in

isolated systems (like it is performed in genomics, proteomics, biochemistry and cell biology). *"Integrative"* is seen as the studies targeted to the understanding of physiological functions in the context of organ or organ systems. Behind these views, there is the perception that molecular biology can not provide all the answers to understand the genetic, proteomic and cellular mechanisms involved in tissue organization, growth, differentiation, etc. However, fundamental questions are posed at the same time by this debate. One of the key points is how to derive findings or to extrapolate the observed behaviours to global, in-vivo, organs or systems at specific life stages. Let us take epilepsy as an illustrative case. The data that we may access ranges from the properties of membrane ion channels observed through patch clamp techniques, to neuronal *in-vivo* characteristics available by means of multiple micro arrays (MMA), populations of neurons using stereo-electro-encephalography (SEEG) or electro-corticography (EcoG), up to extended brain activities with high density EEG and magneto-encephalography (MEG). Beyond single channel data, the higher levels of data are all too rough or too information-sparse to reflect the continuum we are looking for, among which are synaptic delays, excitation and inhibition, afferent and efferent connections and distant loops, etc. These data types provide only insight into electrical mechanisms, a first step of the frame required to understand the intra-level coupling and the inter-level transitions. This understanding will perhaps open other pathways where not only genomic features but also cellular interactions (cellular microenvironment, tissue structure) are involved. Much remains to discover at nano-, micro- and macro-levels in living systems and re-assembling them into global pictures in order to capture their key collective properties remains to be carried out.

## V. SIGNAL AND IMAGING EXPECTATIONS

If technologies are crucial to study spatial and temporal properties in subcellular and supracellular organizations, they are not enough. They all suffer from limitations that must be corrected before any analysis. Instrumental noises (photon shot, background, thermal agitation, etc.) and distortions (out-of-focus, illumination fluctuations, non-stationnary attenuation due to self absorption, ...) are first to be reduced. In optical imaging for instance, photo-bleaching impacts the image intensity and the observation time when absorption and scattering effects limit the excitation and detection depth (the refractive index is space and time dependent). Sophisticated deconvolution, denoising and calibration solutions are required in order to extract reliable quantitative measures: to be efficient, they should make use of physical characteristics of the devices. In the same vein, registration or mosaicing has to be applied when multiple fields of view are acquired.

Segmentation and tracking operations remain however the most challenging tasks to achieve. They must deal with large time-varying shapes, high image intensity variations, objects moving in and out, time-dependent object features (fluorophore or chromophore propagation and disparition), photobleaching, etc. Deformable models are confronted to low contrast boundaries and region-based segmentation is only valid on subparts of the objects. To take one example, tracking multiple molecules (or compounds) in living cells (which can move themselves) relies on the detection of single elements that will be further linked over frames. If the former is already difficult, the latter is even more demanding due to object density and detection errors, object fusion and splitting. Spatial proximity, similarity in appearance and motion, priors on motion modes (that may change over time) and object types can fail to establish the time-stamped correspondence (performed either locally on successive images or globally over the image sequence) and to derive sound individual track features (possibly in 3D) and to set motion classes (path signatures) in subsets of objects.

Any quantitative assessment requires multiple sample analysis and reproducible confrontation. Solutions to these problems call for revisiting and improving the all available schemes at our disposal and the classical assumptions onto which they rely. The present methods, with often empirical parameter tuning, are still unable to face this complexity. In-depth validation of preprocessing, segmentation and tracking algorithms is mandatory for opening windows to live cell functions and subcellular processes.

## VI. THE DIAGNOSTIC AND THERAPEUTIC CHALLENGES

An important issue is to avoid "data for data" search or "modelling for modelling's" sake. The practice in biological and other experimental sciences is to start with an hypothesis about a variable, an object or a system, and to design the right experimental protocol to test if the hypothesis is valid (meaning not invalidated by the particular experiment) or is disproved by the resulting observations. This is a non-trivial, closed loop approach requiring multiple iterations. It may involve new sensing techniques and innovative information processing methods.

The requirements for clinical studies are in principle similar, except for the huge difference that a patient is there, requiring a decision with respect to treatment. Experiments and analyses in the biology laboratory can yield genetic, proteomic and cellular knowledge that may lead to new drug designs, new diagnostic tests, and improved therapy. For the patient in the clinical trial, the testing of the therapy again becomes stochastic or at least probabilistic. Given that patients respond differently from one another, maybe some of the stochasticity of the gene regulatory network remains in play. This is beyond the Physiome projects per se [14].

There is a wide gap between in vitro observations and in-vivo animal or human trials, between a cell and an organ in its whole functioning environment with limited

access to the information that would be required. This incompleteness of data, combined with many other factors like the multiple interactions between functions, diseases, environmental conditions, drug actions, etc. makes highly difficult to establish a link between modeling and clinical requirements, i.e patient-specific diagnosis and therapy. Nevertheless, and apart integration, indexing, retrieval of knowledge, there are several ways in doing that. In some cases the reconstruction, or inverse problem, can be solved in order to fit the observations to the models of concern. Prior knowledge of structures and functions can be used to parameterize and initialize the models: such approach has been widely used for registration and matching purpose between multimodal patient data and atlas maps for instance. Inverse problems are central to electromagnetic source identification in electroencephalography and magnetocencephalography. And the capability to identify multiple closed-loop models from physiological signals allows predicting the effects of a given action (drug for instance) on the whole system as far as relations between some variables with the action are known.

## VII. CONCLUSION

The effort made some time ago to acquire full anatomical and morphological data through the "Visible Human" demonstrated the need for detailed information on the human being. The new breakthrough, the Virtual Physiological Human, as sketched by the STEP project [15], has a long way to go before it can provide sufficiently quantitative data to help in physiological analysis, but it did launch a series of studies around the world to picture the whole body qualitatively in three dimensions. The intensive use of imaging techniques and the corresponding processing like segmentation, rendering, shape modelling is leading to detailed descriptions of organs, and needs to be extended to providing quantitative measures not only of dimensions but also of composition and material properties. It is recognized today that understanding the functions is the next goal and that this should take into account the all scales, from genes to supra-organs.

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