Cellular Imaging: Imaging the Cell

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Abstract - **A cell is the basic functional unit of any living organism. A cell with a nucleus carries all the genetic information needed to produce all the proteins and other chemicals that are needed for the proper functioning or homeostasis of tissues and organs. The cells without the nucleus like erythrocyte besides carrying the essential chemicals also carry signatures of what is going on in different parts of the body. Therefore, a detailed knowledge of what goes on in a cell is important not only from the fundamental point of understanding life at the basic level but is also important for discovering targets for drugs at the molecular level and uncovering the cellular markers of the diseases that fail to show up in standard biochemical assays. The fundamental technique of imaging the cell is optical microscopy with its many variants like imaging and fluorescence life time imaging. In this very short lecture, aimed mainly at starting post-graduate students and researchers entering the field I briefly review the essential physics of microscopy, image formation and deconvolution of digitalised images with emphasis on spectral imaging and fluorescence imaging. I briefly describe a very flexible experimental set-up that has been operational in my group and the research problems that we are dealing with. I also attempt to delineate future topics of interest.**

I DIFFERENT VIEWS OF A CELL

From the point of view of a photographer, the cell is a colourful object whose picture can be taken with the help of a special lens called microscope objective and special lighting arrangements are needed to show the details faithfully. In the digital age that we live, the final spatial resolution of the image depends upon the maximum distortion-free magnification of the objective and the pixel size of the digital detector. In live cell imaging, the crucial factor is the life of the cell under the intensity of the light needed for imaging to the desired resolution. Thus the sensitivity of the detector and the noise floor of the image acquisition system become the limiting factors. The principal challenges in imaging the cell for the purpose of knowing the state of health of its inner parts arise from the fact that the cell is too thin, too small and almost uniformly transparent. Thus, the parts that we want to see must be somehow coloured differently than the rest of the cell. This leads us to the subject of fluorescence imaging.

There are two aspects of colour imaging. Contrast of an object in a background can be increased by looking at it through a colour filter; this is called spectral imaging. Different parts of the cell may appear with different colour when viewed under a UV light; this is called endogenous fluorescence imaging and the "colour" of different objects may be excited by different wavelengths of the UV light thus providing the tuning method to enhance the contrast. The most common method in pathology is simply to dye the cells; different dyes for different parts. In live cell imaging, however, we have to ensure that the dye does not harm the cell or alter its functioning. Added to this is the problem that some dyes fade away during the observation and therefore quantitative imaging has to take this into consideration. The safest and correct way to "image" the cell is to image it for is for a specific chemical compound. Each compound has vibrational band structure sufficiently specific that its Raman Spectra can be used as its signature. This almost routine technique of research laboratories is already penetrating clinical pathology environments and we hope to see Raman Spectral Photographs in the future medical records. The problem of weakness of Raman Signal is being attacked from several different angles. A single x-y image or photograph does not readily convey the information about the relative depth z of the objects of the photograph. In order to achieve this, z-stacks of digital images taken at different focal planes can be "processed" or deconvoluted. Today, it is possible to obtain contrast figure beyond the diffraction limit and we can delineate features that are below the µm size or in the nanometer range.

II. SET-UP AT DFUA

In the Physics Department we have set-up a flexible system around a microscope that allows us to do a variety of measurements mentioned above. The microscope can be chosen in the upright configuration or the inverted configuration. There is a wide choice of illumination sources, lamps and monochromators as well as lasers in the visible to near infrared region. Besides, the cooled sensitive CCD with sub-micro second shutter, the light output from the focal spot can be analysed in a spectrometer. With the help of a notch filter or a secondary subtractive monochromator the system works as

micro Raman spectrometer. The objective can be moved in the vertical direction with a step of 100nm and thus we can get the depth information on the sample. With the z-stack of high resolution images and optimized deconvolution algorithms it is possible to determine the relative z-positions of anomalies in the images of a single red blood cell (approximately 8µm across); the anomalies itself are in the nm range. We have also made a cell chamber connected with parastaltic pump that allows us to do live-cell imaging and change the surrounding fluid without disturbing the cell. A photograph of the main part of the system is shown below.

Fig1 Set-up

III. SOME INTERESTING PROBLEMS AND POSSIBILITIES

The system that has been described above has been used for material science studies as well as to understand some problems that are important in biology and health sciences. Below we give a sample of some of the problems we have studied and others that we are studying.

A. Toxicological Studies

One of the first applications of the system to the biological problems was in assessing the damage caused by the environmental Al3+ on the functioning of Na-K/ATP trans-membrane protein responsible for the cell viability. For this we did live-cell imaging of cellular Na+ through its fluorescence indicator at regular intervals after introducing the Al salt in the extra-cellular medium. Our studies clearly show the effect of Al3+ on the pump.

B. Studies on pathology of erythrocyte

In many diseases, a routine analysis of blood or even a micrograph of single red blood cell cannot properly characterise the pathology, its progression and variants. With spectral imaging technique it was possible to increase the contrast to the level that the statistical analysis of the geometrical features of the pathology across several patients became feasible; the contrast enhancement permitted an efficient use of image analysis routines of Matlab toolbox. Recently, we have used the deconvolution via an optimised PSF of a z-stack of images to infer the relative z-displacement between the pathological features of the cell.

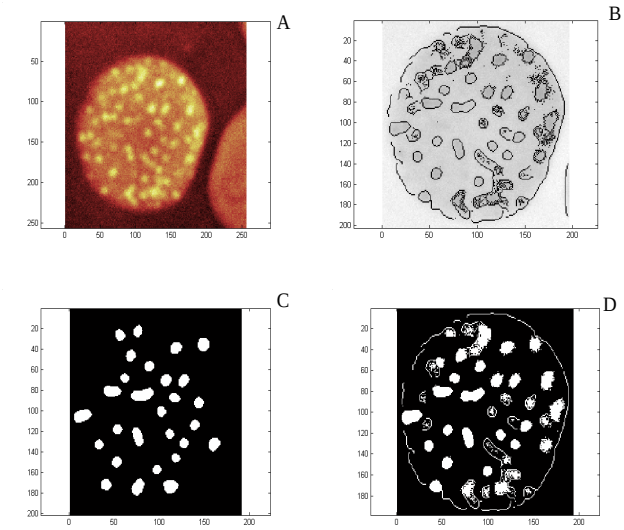

Fig2. Image analysis of pathology of erythrocyte

C. Effect of EM Field

The set-up described above is quite open and flexible thus allowing to undertake studies commonly not possible in a commercial turn-key system. One of the interesting problem that is being investigated is the effect of electromagnetic fields on the electron-transfer reactions that take place in a cell. A cell chamber is being constructed that will permit applying a variety of fields to the cell while it is being imaged in the fluorescence mode, for example. These studies are important because while EM fields are being used for therapeutic reasons at the tissue or organ level, a clear understanding of the underlying cellular mechanism of their effect is lacking.

D. Substrates for Surface Enhanced Raman Scattering

Though the Raman Scattering is one of the main non-invasive probe with adequate specificity it is not being

widely used because the signal is very low. In our case we are applying Lock-In techniques to “see” the signal in the noise and use cooled sensitive APD arrays in the photon counting mode. Another approach is to enhance the signal by surface plasmon modes on a metallic surface with adequate nano size roughness features.

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