Imaging Dynamic Processes in Cells and Tissues

Live cell imaging has been around in one form or another for many years, but recently, interest in the technique has really grown. To cover all aspects of live cell imaging would be a huge task and well beyond the scope of this article. However, this overview will give a brief introduction to the possibilities and more specialised areas will be looked at in future articles. Developments in three main areas are driving the revolution in live cell imaging. Molecular technology, microscope technology and the need to know. Let's start with the last one first. Microscopists have always tended to want things to sit still whilst they look at them, or had to stabilise them to withstand the rigours of intense light or electron bombardment – or just to stop them going off. So they used (and still do) a wide variety of, by definition, noxious chemicals to stabilise living samples. However if we want to really understand the delicate interplay between cellular components, these fixatives are going to have to go.

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Anyone who uses a microscope will be familiar with the use of fixatives such as methanol, acetone, aldehydes and osmium tetroxide. All introduce some degree of artefact and ideally the methods should be optimised such that the artefact is reduced as far as possible. In practice, as long as this limitation is recognised and reproducible this has not been a major problem. However, when dealing with samples that have been fixed in some way, whether looking at cells from culture or sections of tissues, the information that can be gained is of the 'this is what it looks like' or 'that is the change caused by ….' variety. As we get to understand more and more of the molecular basis of cellular architecture and protein function these observations lead onto the next question. I can see what happened but how did it happen? If we then add the further question 'what does this protein do?' then microscopists have had to move from the use of fixed samples to looking at the real thing. This is where live cell imaging comes in.

It is possible to image live and moving cells or even small organisms by transmitted light, often with contrast enhancement by phase contrast or DIC, and this is still giving valuable information. Indeed the vexed question of how motile cells respond to stimuli is still not clearly settled and this has considerable implication for our understanding of the process of metastasis whereby cancer cells leave their original site and migrate to new locations. However, following the fate of individual proteins in cells requires a way to identify them and this is where the molecular revolution comes into the picture. The fluorescent proteins of jellyfish and some corals have been cloned and sequenced – that insultingly short sentence covers decades of work and a Nobel prize – so it is now easy to transfect cells with plasmids that will persuade the cell to make the protein of interest with an attached fluorescent protein (FP) tag. In fact there are now a number of different with different excitation and emission wavelengths so that multiple proteins can be imaged within one cell. The range of possibilities is enormous. Not just individual plant and animal cells be persuaded to produce FP-labelled proteins. Pathogens (viruses, bacteria, fungi and parasites) can be made to express FP's; cell lines can be made where they constitutively express FP's so every cell has the labelled protein; even complete organisms from the simple C elegans to mice or rabbits can all be engineered to express FP-labelled proteins.

There has to be a snag and of course there are plenty. Whilst a large number of proteins are quite able to function normally with a large fluorescent molecule stuck to them, there is an ever-present danger that the FP-labelled protein is not functioning correctly. Furthermore, these methods can cause the protein of interest to be expressed in abnormally high amounts and then there is a very real risk that the normal cellular processes are swamped. Thus you get a stunning live cell video of cells doing abnormal things which is a bit pointless. Another snag is that cells and tissues are sensitive to light and that imaging them over long periods with intense light will generate sufficient free radicals that will kill them. Every part of the imaging procedure must be optimised to reduce the level of illumination of the cells, otherwise yet again the result is a video of cells doing abnormal things – in this case being damaged or even killed by light.

Similarly, the fluorescent markers themselves can be affected by the excitation light and lose their fluorescent intensity, called bleaching. Again, keeping everything optimised will allow the incident light levels to be reduced sufficiently to keep this to manageable levels. It is also possible to turn this to our advantage and there are techniques which rely on bleaching FP's in order to see if the protein-FP complex is static or motile within a cell. Recently there have been a number of modifications developed to the structure of fluorescent proteins and this includes ones that are photo-activatable. That is they are not fluorescent until given a dose of light. This can be used to activate the protein in a defined area such as an organelle so that the movement of a subset of the protein can be followed.

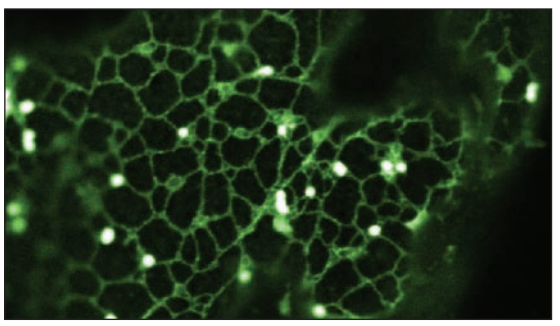

Figure 1. Image of a live Tobacco leaf epidermal cell where the Golgi (dense areas) and endoplasmic reticulum (reticular pattern) have been labelled with Green Fluorescent protein (GFP). The ER-Golgi relationship in plant cell is highly dynamic and can only be appreciated in living cells. Image kindly provided by Prof C Hawes, Oxford Brookes University and Petra Boevink, SCRI.

In all areas of light (and indeed electron) microscopy the user has benefitted from rapid technological advances and whilst some have been driven by the ease of computer control there have been some really innovative developments by far-seeing scientists that the microscope manufacturers have then had the nerve to develop. So cell biologists have to hand a wider range of techniques than ever. For live cell imaging there are a number of choices of technology and each has their own strengths. There is the choice between a widefield camera- based system or a confocal microscope approach. The confocal microscope can be laser scanning or spinning disc and so on. The choice of technology will be driven by the application.

Recently there has been a lot of interest in a technique called TIRF. This stands for Total Internal ReFlectance microscopy. One of the questions asked in cell biology concerns the behaviour of molecules at the cell surface – either molecules or particles entering or leaving the cells, or processes that occur just below the cell membrane. For these studies TIRF is ideal. By some clever manipulation of a laser beam, the excitation of fluorescent molecules can be restricted to a region of up to a couple of hundred microns above the coverslip surface (yes, you do need flat cells). This gives excellent resolution and extremely high sensitivity of fluorescent detection of even single molecules.

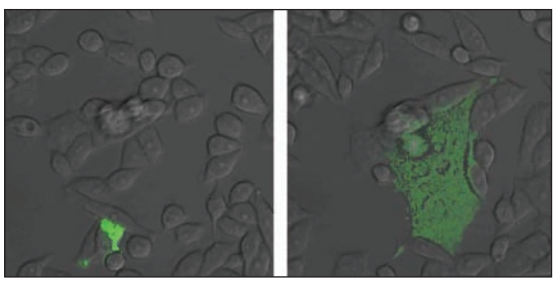

Figure 2. Cells infected with a modified form of Rinderpest virus, which expresses GFP so that infected cells fluoresce green. Rinderpest is a viral disease of cattle and is related to measles. The cell culture was infected and imaged for 40 hours. The left hand image was at 18 hours post infection and the right hand image was 10 hours later. Cells infected with the virus may fuse to form large syncytia and these experiments help us to understand the process of cell migration and fusion. Figure kindly provided by Dr AC Banyard, Institute for Animal health.

Applications cover the whole of cell biology, from the rapid movement of calcium waves in cells through monitoring the interactions of proteins by FRET (Fluorescence Resonance Energy Transfer), all the way to using a two photon confocal microscope to look at fluorescent (or luminescent) cells in living tissue.

Clearly this is a huge topic but it is an area where a lot is happening, and generating whole new ways of looking at proteins, organelles, cells and tissues. You might almost say life, the universe and everything.