Surface Wave Microscopy

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This describes some of our work, it was started when I was in the UK and has been developed recently since I arrived in PolyU.

Although microscopy and light microscopy, in particular, seems a highly venerable subject developments in the field are coming at a rapid rate, the driver for developing new microscopies comes from the fact that scientists and engineers need to be able to visualize the structures they create and examine. Most lay people will know about the development of 'superresolution' microscopy so that features far smaller than the so-called 'Abbe' limit can be visualized. Structures below 100nm (or 1/10000 mm) can now be routinely visualized and such instruments are now commercially available and indeed PolyU has recently taken delivery of such an instrument. Despite these high profile developments there has been something of a quiet revolution, which involves the way people think of microscopy and imaging. It is no longer simply 'taking a picture' but more about performing an experiment on a local region and producing maps of these local experimental results. Complex interactions combined with substantial processing are now routinely used to form the points in the images. This may not sound dramatic but it changes the way people think of microscopy and it flavours the way new ideas are developed.

One theme of my research group has been involved in is the development of so-called evanescent wave microscopies. Each wave has its so-called wavelength which determines the distance over which it repeats itself, microwaves have wavelengths of a few cm, optical waves in the visible range with wavelengths around 1/2 a micron, and X-rays typically 100 times smaller. Evanescent waves occur when the wave is squeezed in one direction to a size smaller than half the wavelength. In this case the wave decays very rapidly in at least one other direction. For those of you familiar with electronics a useful analogy for an evanescent wave is the capacitor and inductor in a tuned resonant circuit, where energy is stored but does not dissipate. One situation where evanescent waves are formed is in total internal reflection, when a wave from a higher refractive index (glass) hits a sample such as a cell at an oblique angle greater than the critical angle. An evanescent wave is produced at the interface between the glass slide and the cell which interacts with the cell membrane, the evanescent wave thus forms a highly localized probe of the surface of the cell. Localizing our signal to this region has major importance since the membrane is the 'gateway' to the cell where infective agents…or drugs can enter. Moreover, proteins at the cell membrane are vital in signalling to other cells and play a crucial role in determining the functioning of the whole organism.

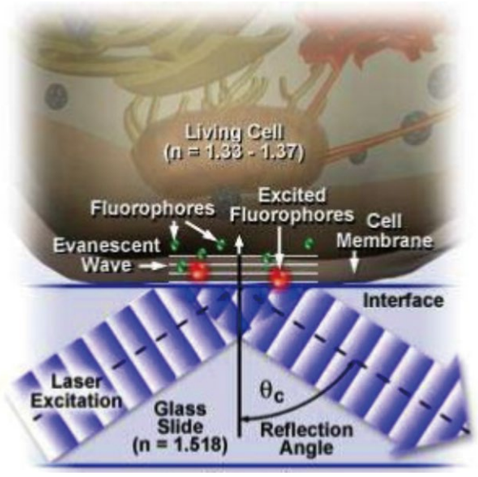


Figure 1 : Schematic showing total internal reflection. The evanescent wave penetrates through the cell membrane but will not interact with the body of the cell.

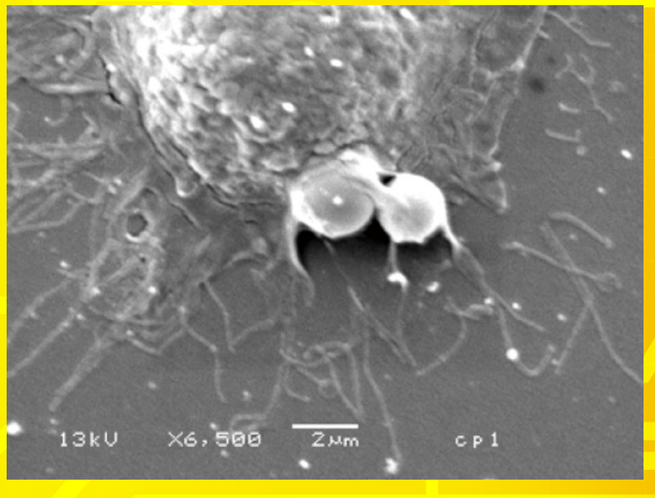


Figure 2: Scanning electron microscope images of particles being consumed by a cell.

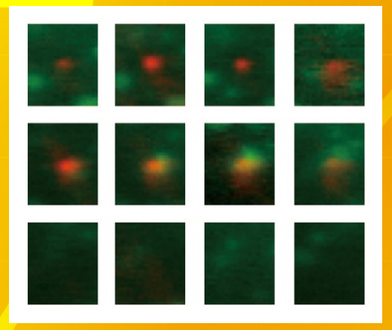


Figure 3: Red shows presence of particle green presence of clathrin. Sequence of images taken at 2-minute intervals showing how the protein and the particle are aligned in time and space disappearing together.

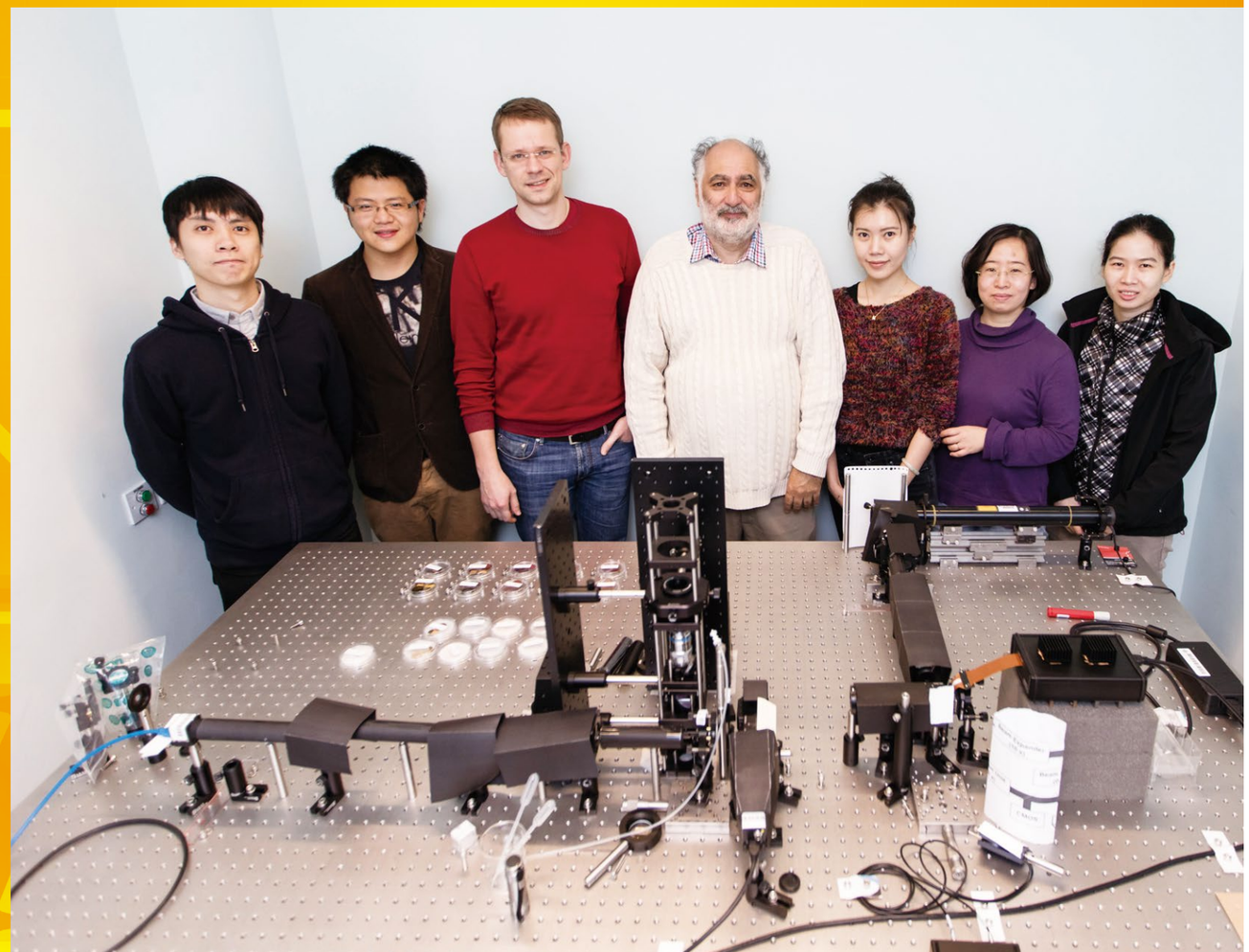
As the evanescent wave penetrates the cell it can be scattered into propagating light, it can also excite fluorescent molecules. We have developed a microscope with two channels one that can detect the scattered light and another channel that detects the fluorescent molecules, the presence of these molecules is related to the chemical processes in the cell. This system was developed in collaboration with pharmaceutical scientists who are interested in drug delivery. We present particles of different sizes and chemical composition to the cell, the scattered light channel detects the physical presence of the particle and shows it being absorbed into the cell, whereas the fluorescence channel shows whether certain chemicals (in this case a protein called clathrin) are being produced by the cell. If the physical and chemical responses are present together in both position and time we can understand the mechanism by which particles are taken into the cell, if they not we know some other mechanism is responsible. This is very important if want to understand how drugs will enter the cell because the method of entry also has a great influence on the way the particle behaves once it is in the cell. Figure 2 shows particles being 'eaten' by a cell taken in a scanning electron microscope, but, of course, in this case the cell that is imaged is dead and coated with gold! Figure 3 shows small section of the surface of a cell where the physical and chemical response of the cells are correlated in time and space, when we used larger particles the chemical signals were not located at the site of the cell indicating different mechanisms were involved. We have recently published this work in American Chemical Society journal Molecular Pharmaceutics and we are pleased that is was chosen as 'Editor's choice' from all the different journals published by the Society.

I mentioned at the beginning that we often want to perform local experiments on the surface of a sample to get a very sensitive measure of what is sticking to a surface. A wave called a surface plasmon is exceptionally effective for this purpose. All one needs to do to produce a surface plasmon is to put a thin gold layer on a glass coverslip and it will appear when you hit the sample with light at the correct angle. On the other side of the gold the sample can be placed where there is an evanescent wave like the one described earlier. The properties of this surface plasmon change as the proteins stick to the surface. This is important as the binding of proteins provides a very powerful means to perform disease diagnosis and is also widely used to identify potential new drugs in pharmaceutical research.

The problem with surface plasmons is, as their name suggests, that they travel along the surface and they are not ideally suited for microscopy as they do not stay put and we cannot be sure exactly which region they are looking at. To address this we realized the trick is not to change the way the surface plasmons behave but simply to control where we look at them. We have done this by modifying the so called confocal microscope which is usually used for three dimensional imaging of cells. We now only detect the plasmons that travel a very well defined distance. This has enabled us to develop a modified microscope that can look at protein binding to surfaces but over far smaller distances that has been possible before. This is truly an example where we use a microscope to perform a local experiment that was previously performed in a related way but over a much larger length scale. We are pleased that other groups have followed our approach to surface plasmon microscopy and are producing interesting results for cell imaging and ultrasensitive imaging of nanoparticles.

We have recently been awarded an ITF grant to develop the present system in PolyU towards a commercial prototype and I am very hopeful that we will able to have a system on the market within a short period of time.

The work on evanescent wave microscopy has been the subject of several plenary, keynote and invited talks in the last two years in Xian, Nanjing, Guangzhou and San Francisco amongst others.



Research Breakthrough