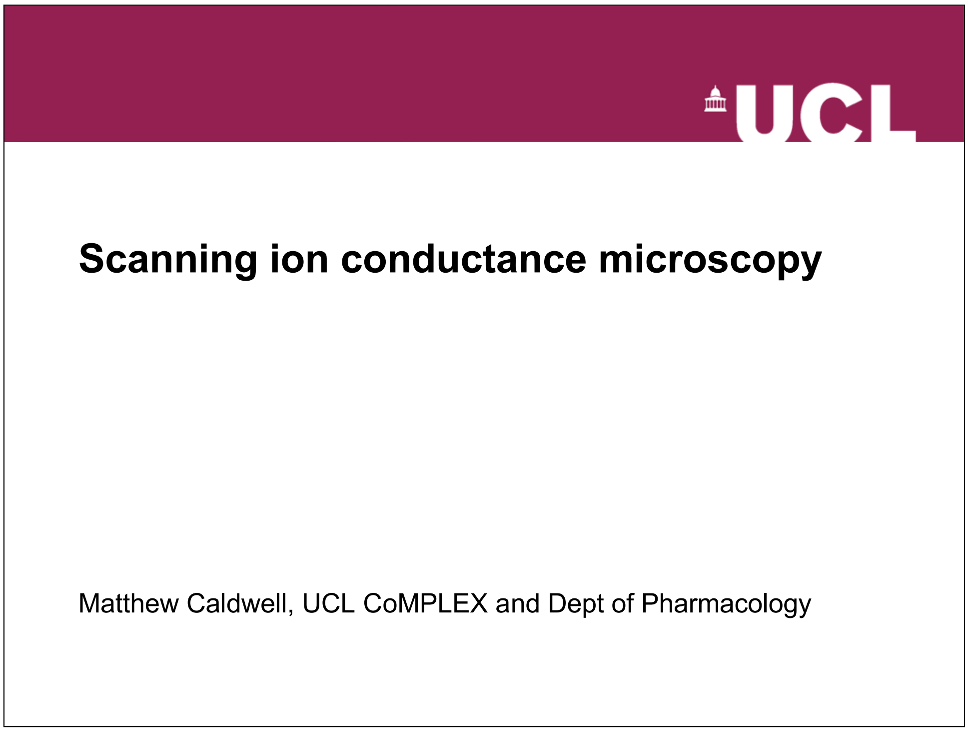
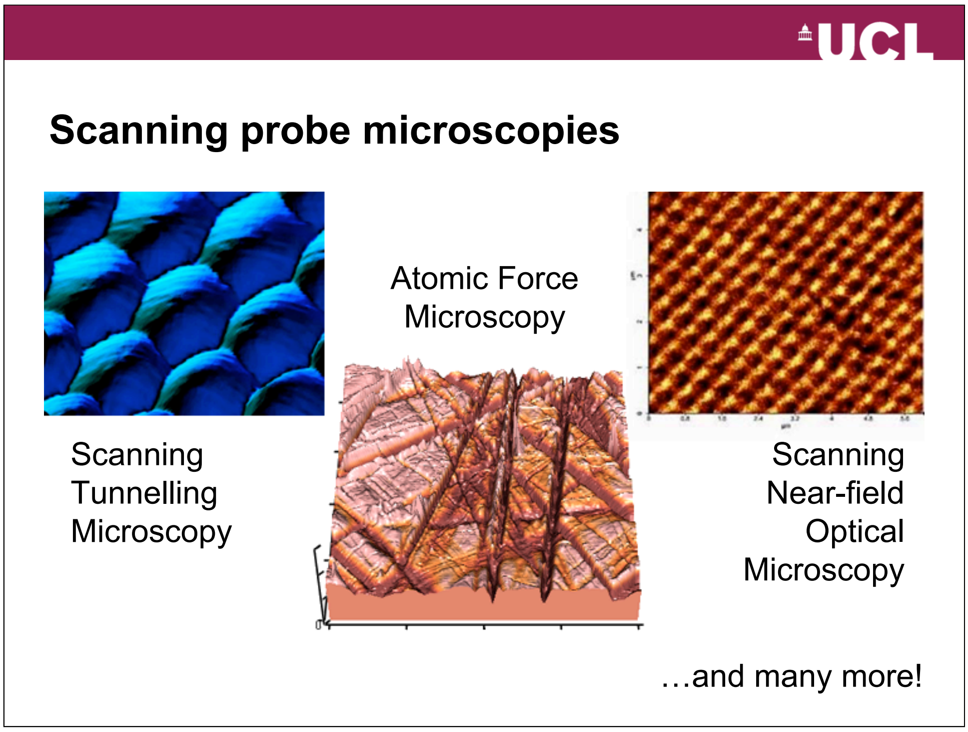


Scanning ion conductance microscopy

Matthew Caldwell, UCL CoMPLEX and Dept of Pharmacology

A high resolution imaging technique particular useful in experimental biology



Scanning probe microscopies

Scanning
Tunnelling
Microscopy

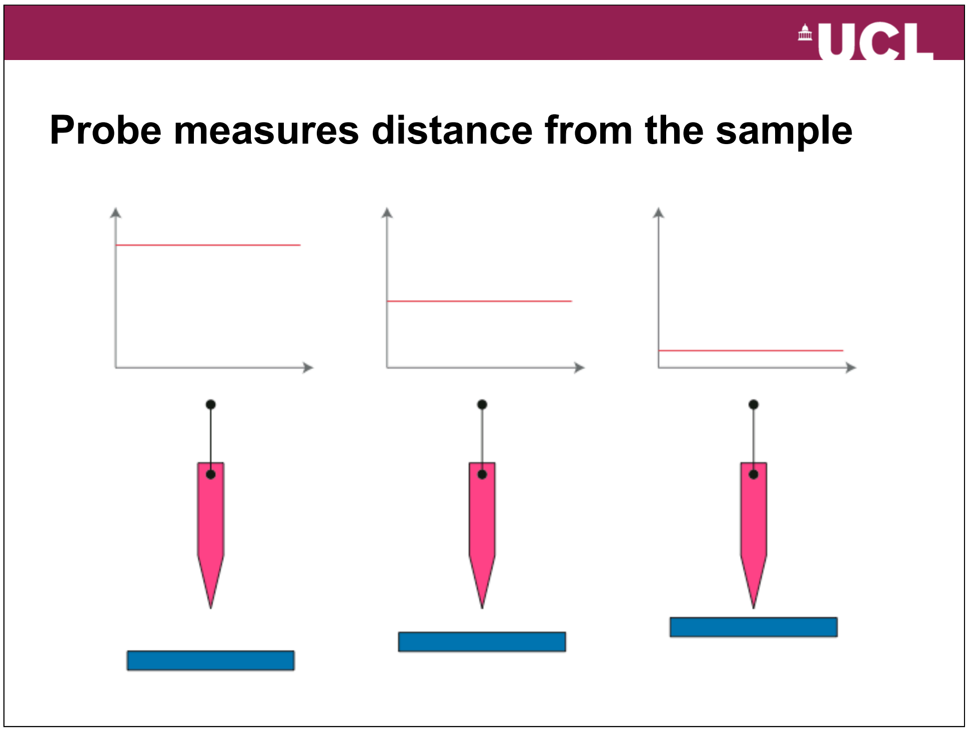
Atomic Force
Microscopy

Scanning
Near-field
Optical
Microscopy

…and many more!

SICM belongs to a family of techniques collectively known as scanning probe microscopy. Some other examples are mentioned here and there are plenty of others.

The underlying principles of all these techniques are basically the same, so I'll briefly outline those before going on to explain the aspects specific to SICM.

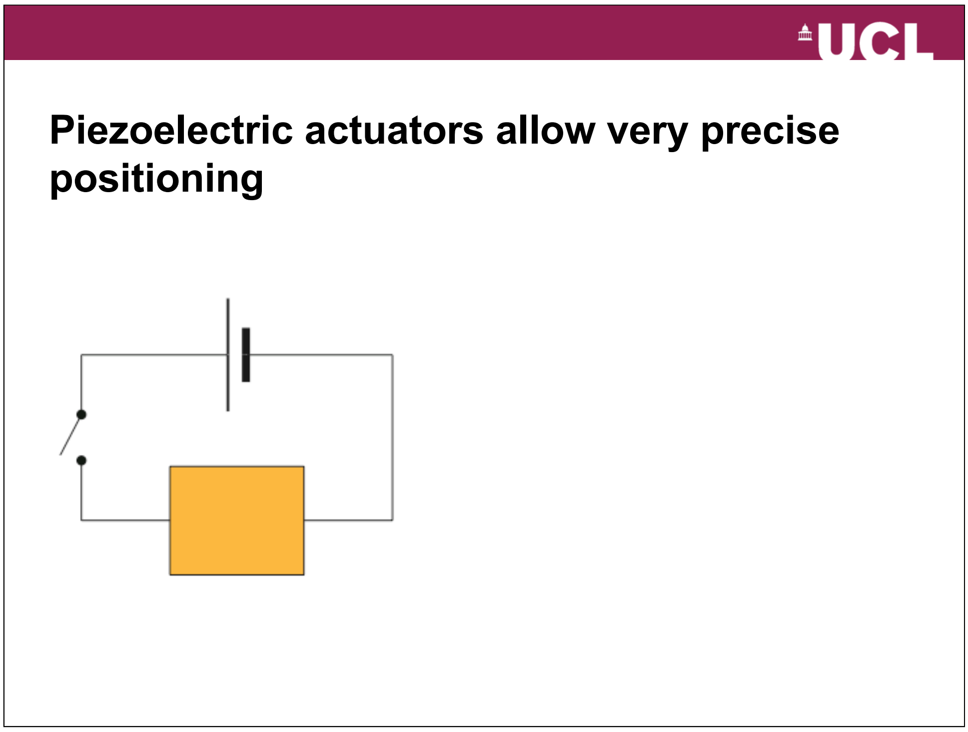


Probe measures distance from the sample

The first thing you need for any scanning probe technique is a *probe*. A bunch of different things can fulfill this role, which is why there are so many different SPMs.

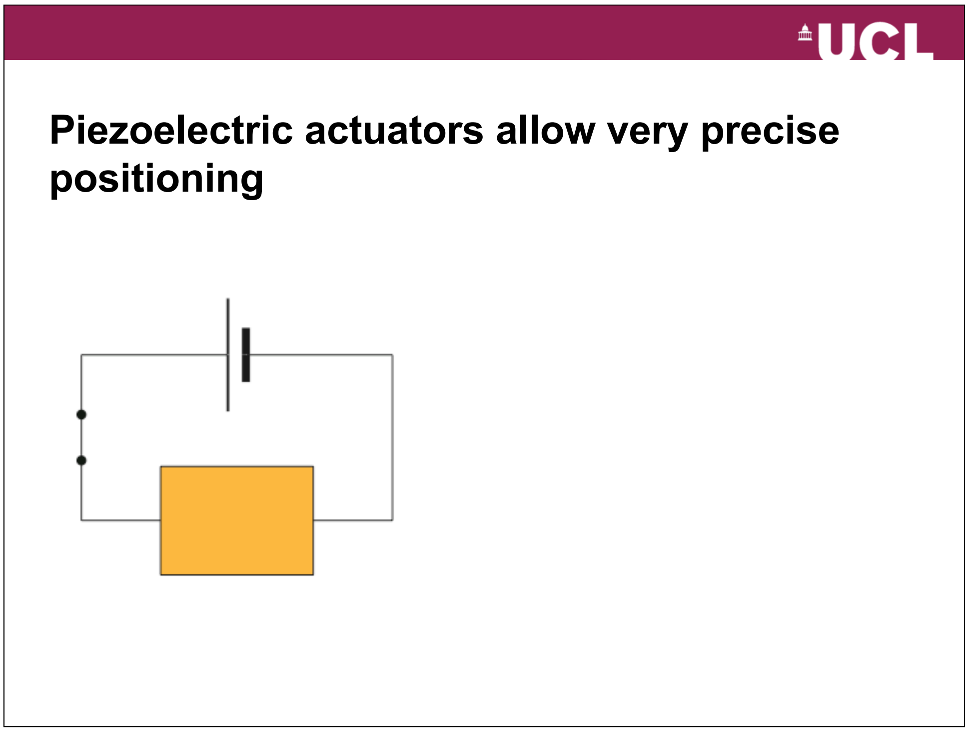
The crucial property of the probe is that it must be able to deliver some kind of signal that varies in a consistent way with the distance between the prope tip and the specimen we want to image.

Typically this signal only works over a very small range of distances, and it may be pretty difficult to translate into an exact distance, but it turns out that this isn’t a big problem, as we’ll see in a minute.



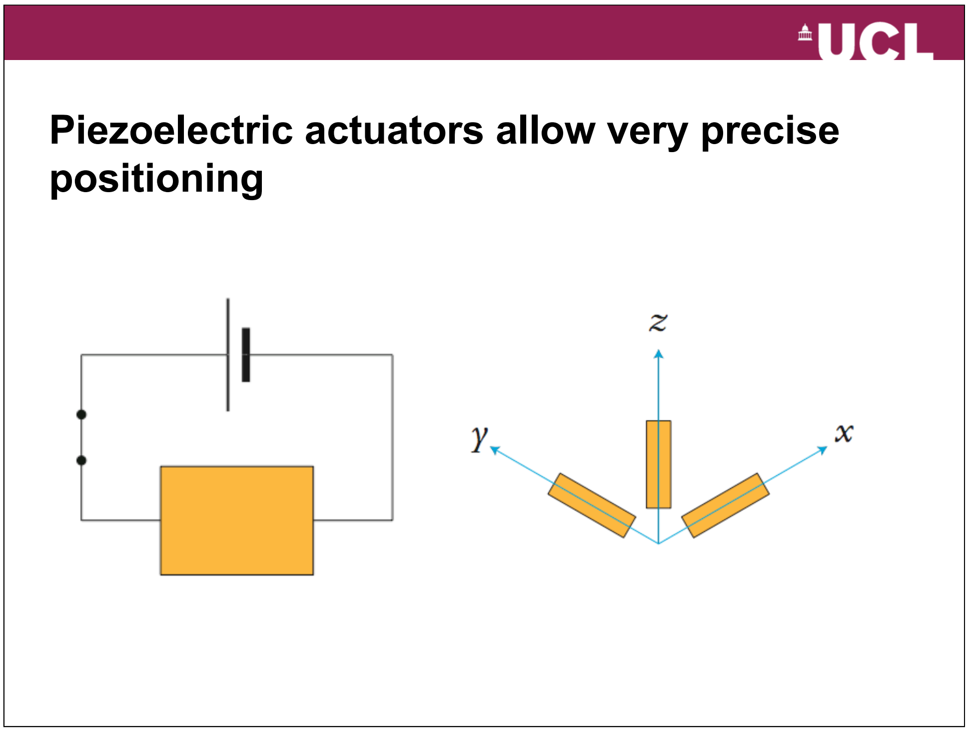
Piezoelectric actuators allow very precise positioning

The next thing you need is to be able to position the probe with incredible accuracy, and this is done using piezoelectric devices. A piezoelectric substance transduces between electrical and mechanical energy. For our purposes, this means that if you apply a voltage to it, it changes size.



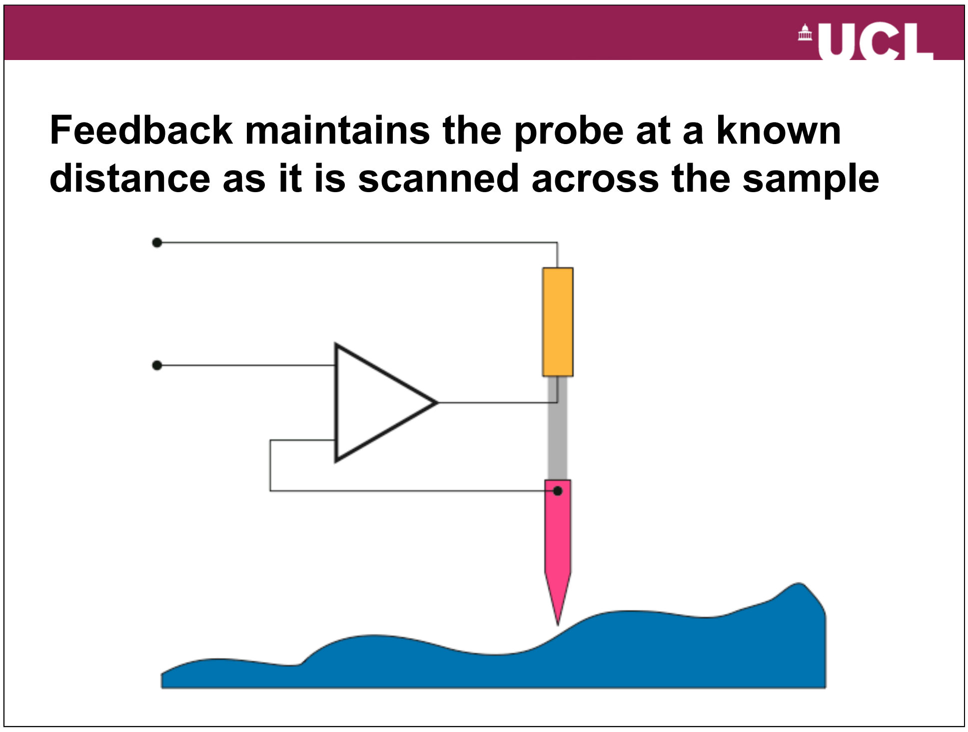
Piezoelectric actuators allow very precise positioning

The size change with voltage is very tiny, but very consistent. This means that with comparatively easy to control large voltages, we can achieve very precise changes in size.



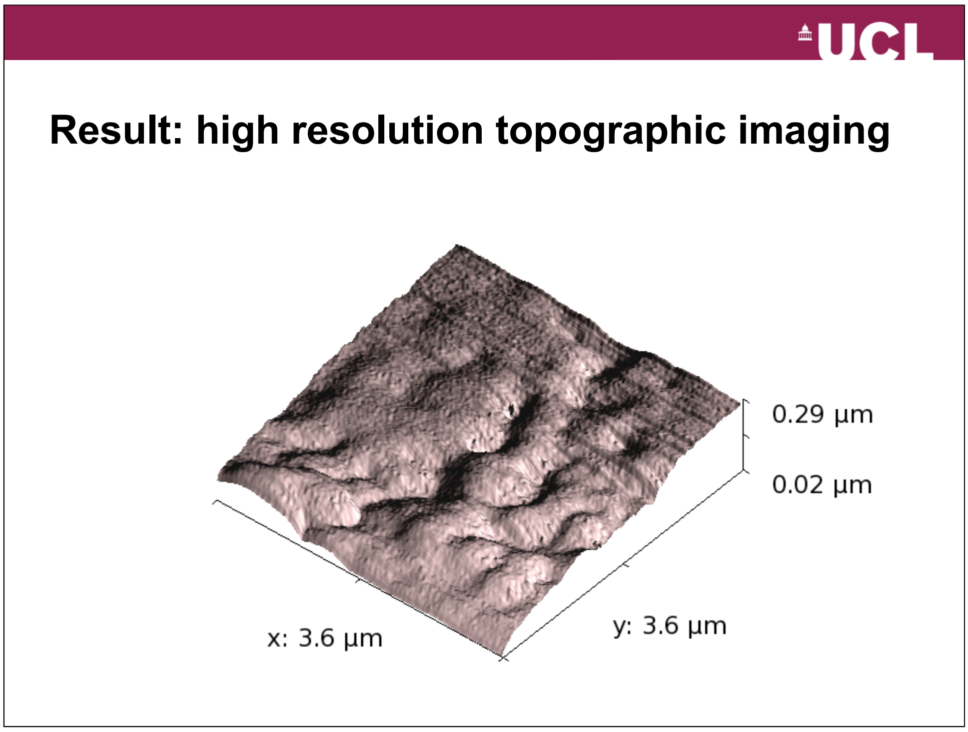
Piezoelectric actuators allow very precise positioning

If you mount something -- say, our proximity probe -- on three separate piezos in three perpendicular direction, voila, we can position it very accurately.



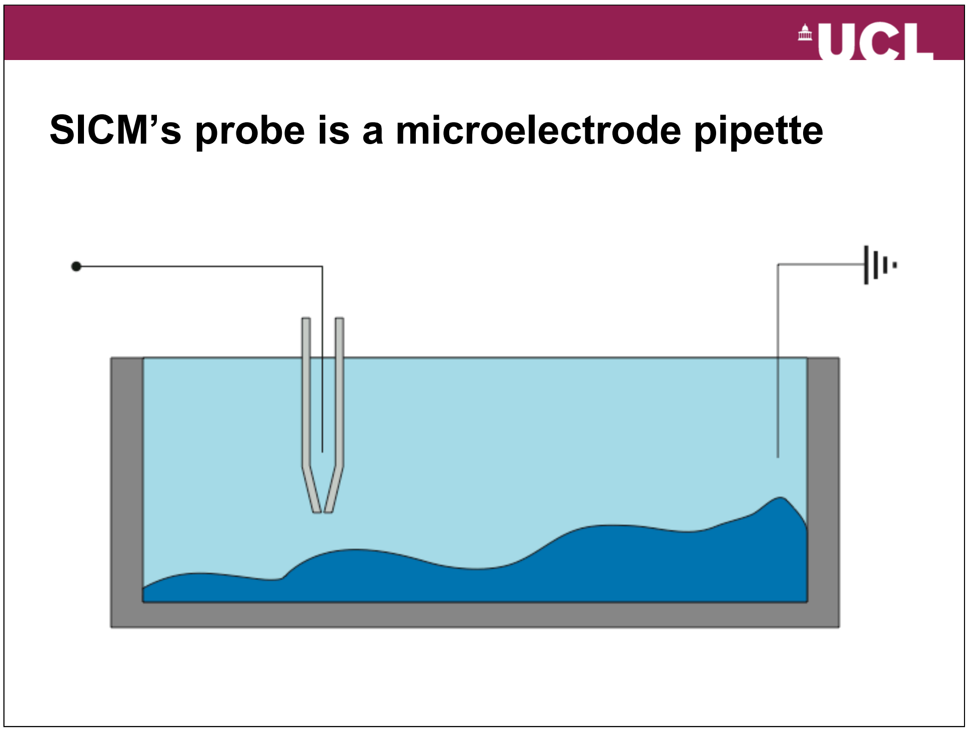
Feedback maintains the probe at a known distance as it is scanned across the sample

The final piece of the puzzle is to use a feedback system to constantly reposition the probe so that its signal is the same -- so it’s always the same distance away from the sample. We can record how much voltage we have to apply to the piezo to do this, so we know exactly how far the probe is extended. And since it’s always the same distance away from the sample, we know that’s the shape of its surface.



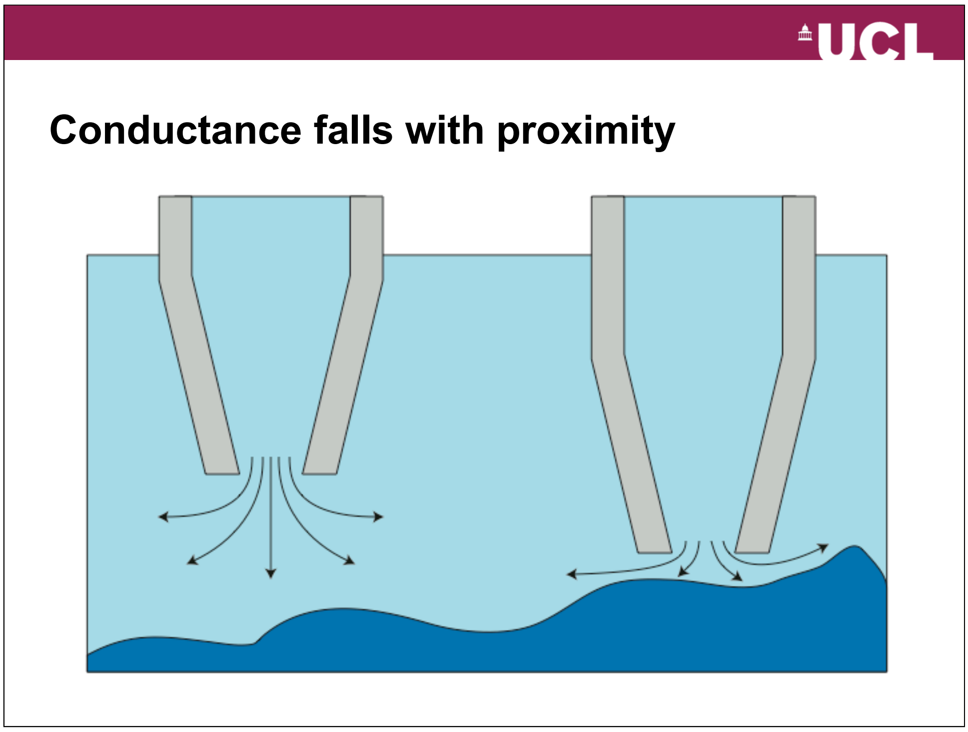
Result: high resolution topographic imaging

Do this for row after row of scans across the surface, and you wind up with a very detailed picture of what the surface looks like.



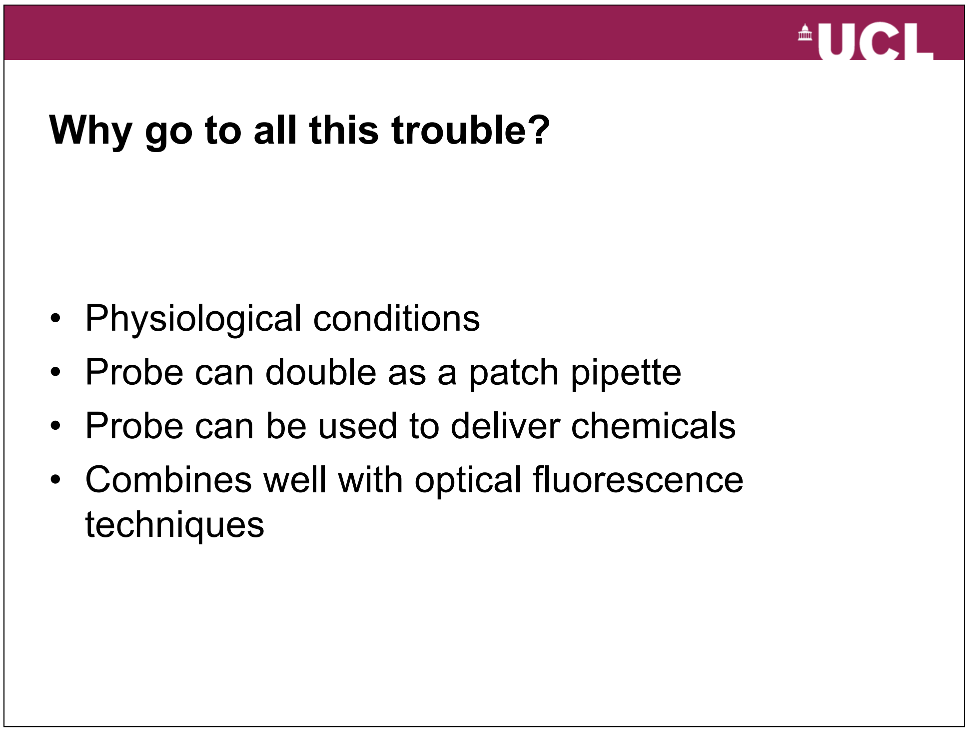
SICM’s probe is a microelectrode pipette

In the case of SICM, the probe is a micropipette -- (show example) -- just a thin glass tube that’s been melted and pulled out until it’s very thin -- immersed in an electrolyte. When a voltage is applied, a current flows through the pipette, in the form of ions. That current is the signal the we use to measure distance from the sample.



Conductance falls with proximity

The ions have to pass through the tip of the pipette. When the pipette is just freely moving around in the bath, the conductance is limited by the geometry of the pipette tip itself -- which is always the same for a given pipette. But when the tip is very close to the surface, the ions’ path is occluded and the conductance goes down. That’s what gives us our signal.

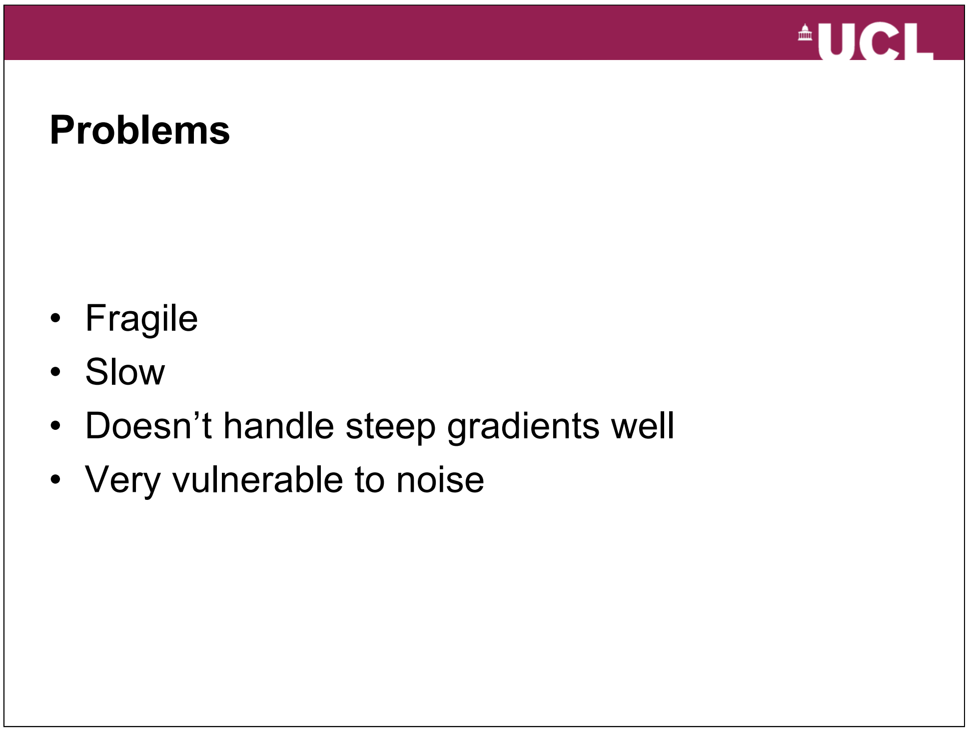


Why go to all this trouble?

- Physiological conditions
- Probe can double as a patch pipette
- Probe can be used to deliver chemicals
- Combines well with optical fluorescence techniques

This all may seem a bit Heath Robinson, and it is. But it has several benefits for biological imaging, the most important being that it can operate in pretty much physiological conditions. Most cells naturally sit in a bath of electrolyte the whole time; it keeps them happy and alive. In contrast, most other high resolution imaging techniques require the cells to be killed and fixed and kept super-dry or super-cold, which is a bit of a problem if you’re interested in what the cell actually *does*.

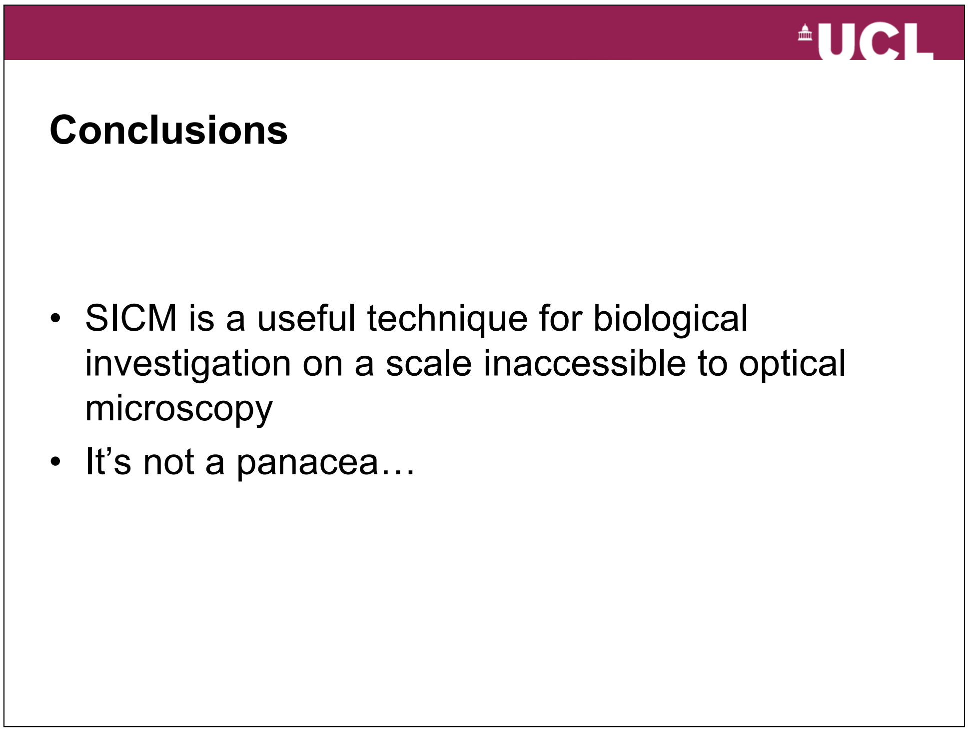
There are some other benefits as well -- you can simultaneously record other information with the same equipment -- but the physiological conditions one is the biggie.



Problems

- Fragile
- Slow
- Doesn’t handle steep gradients well
- Very vulnerable to noise

Of course, it’s not all good news -- the process is rather slow and can also be very flaky. The probe is blind to the sides, so steep gradients can cause it to crash or go haywire. Mechanical or electromagnetic noise -- people walking about, the mains frequency, mobile phones going off -- can interfere with the imaging.



Conclusions

- SICM is a useful technique for biological investigation on a scale inaccessible to optical microscopy
- It’s not a panacea…

But ultimately, despite its problems, SICM constitutes a very useful tool for biological investigation.

Conclusions

- SICM is a useful technique for biological investigation on a scale inaccessible to optical microscopy
- It’s not a panacea…

- …but at that size, what is?

Questions?