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Scanning Ion Conductance Microscopy for High Resolution Topography of Soft Samples Including Live Cells.

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Scanning ion conductance microscopy (SICM) is a rapidly emerging scanning probe microscopy (SPM) technique particularly suited for high resolution imaging of live cells maintained in their physiological/culturing medium.
In the past few years there has been a growing interest in understanding dynamic cell membrane processes occurring physiologically and upon external stimuli at the nano-scale. Optical and fluorescent microscopy techniques to image live cells are limited in resolution to few hundreds of nanometres. In addition, fluorescence microscopy requires the labelling of specific molecules, which can alter the physiological functioning of the cell. Historically, high resolution imaging techniques like scanning electron microscopy (SEM) have been used, which are also highly invasive and limited to fixed specimens. To obtain nanoscale resolution of cell membrane, atomic force microscopy (AFM) has been utilised for living cells. However, the deformation of the soft cell membrane by the AFM cantilever represents a substantial limitation [1].

SICM, first developed by Prof. Hansma and collaborators in the late 80s, can obtain high resolution topographical images of nonconducting surfaces immersed in electrolyte solution [2-5]. The probe used for SICM is a sharp glass nanopipette filled with electrolyte. The technique uses the measurement of the ion current flowing through the nanopipette tip to track the topography of the sample surface. This unique feedback mechanism allows keeping the nanopipette tip at a fixed distance from the surface of the sample. Therefore, no physical contact with the surface during scanning is needed. This unique feature differentiates SICM from other nanoscale imaging techniques, including AFM, and allows imaging the true topography of a soft sample at a resolution comparable to the resolution that AFM could achieve [6].
The modality of scanning we use, hopping mode SICM, developed by a team of collaborators led by Prof. Korchev in London, involves that the nanopipette approaches the sample from a starting position which is above any of the surface features and retracts away from the sample to a safe distance at every image point. This modality of scanning has made the technique more versatile, as tall/convoluted features can be resolved, features traditionally challenging for raster-scanning SPM imaging techniques [7].
The combined ability to keep live cells in physiological medium and to scan them without damage to their membrane by the probe tip makes SICM particularly suitable for obtaining 3D images of living cells and monitor in time gross cellular movement or finer dynamic behaviour of the cell membrane (Fig 1).
The inner diameter of the nanopipette tip is a critical factor in defining the resolution achievable with the technique. Early studies have reported images of proteins in living cell membrane [8], while recent data show that borosilicate glass nanopipette pulled to an inner diameter of approximately 30 nm allow imaging of features with lateral resolution better than 20nm [7].

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We will show examples of images of biological and non biological materials that illustrate the versatility of the technology in a wide area of life science applications and discuss the limitations in resolutions imposed by the inner diameter of the pipette tip.
Finally, the technique is amenable to integration with complementary techniques (e.g. patch-clamp, scanning confocal microscopy), and the nanopipette can potentially be used to apply mechanical forces for cell stimulation or to deposit material, to mention few of the applications described in the literature.
The development of a new generation of scanning ion conductance microscopes which can smartly integrate with other measurement systems will widen the fields of application for this technology and integrate imaging with functional cellular studies.

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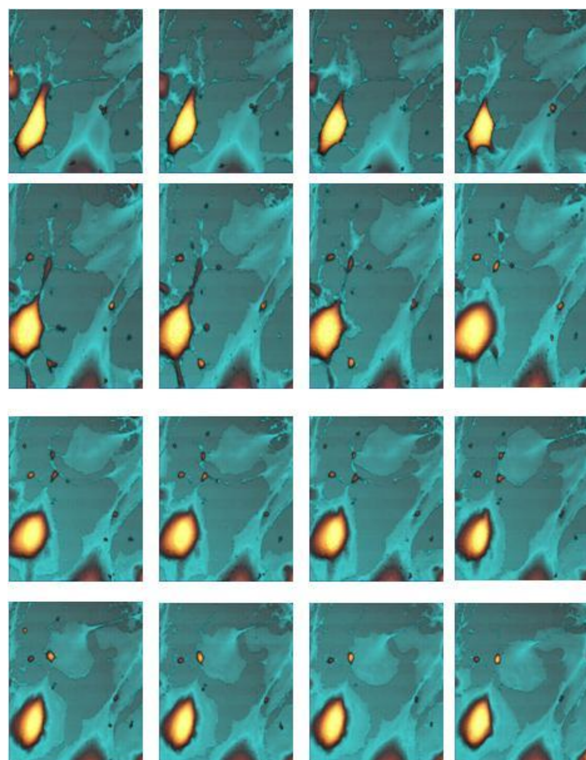

Fig 1: Sequential scanning of mesenchymal stem cells imaged with hopping mode SICM at room temperature. Each scan is about 40 min apart from successive one. Scan size: 95µm x 95 µm; Z range: 5-7.5 µm. Sample courtesy of Dr Iryna Palona, University of Liverpool, UK.

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