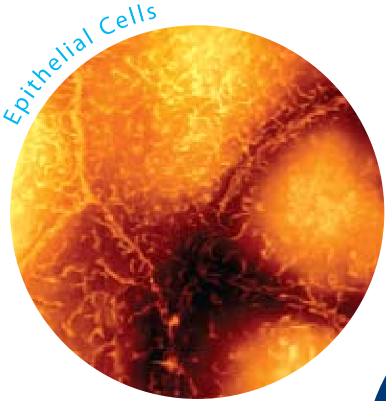
Sperm Cell

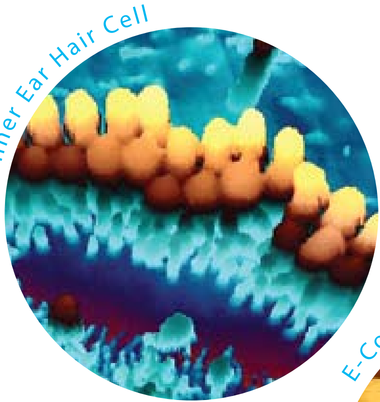
Epithelial Cells



Inner Ear Hair Cells

ionscope

Inner Ear Hair Cell



Neurons

E-Coli Bacteria

Scanning Ion Conductance Microscope

ICnano

About ionscope

The ionscope scanning ion conductance microscope delivers high-resolution topographic imaging and a wide range of additional functionality for life, physical and material sciences. Developed by a team of scientists at Imperial College London and Cambridge University, ionscope's proprietary technology – modulated probe ion conductance scanning – offers uncompromised performance and stability, thus enabling high resolution continuous scanning over long periods.

Innovative Design

Incorporating a host of proprietary design features, the ionscope ICnano has established itself as the next generation research tool for a broad range of cutting edge applications. Glass nanopipettes are pulled to the required dimension using a standard pipette puller making setting up simple and inexpensive. SICM differentiates itself from other nanoscale modalities through its ability to produce these images by sensing ion current and not through attraction or repulsion, and thus involves no mechanical interaction with the surface during scanning.

The ICnano offers a unique, state of the art, closed loop flexure scan system that ensures accurate measurements with low noise levels. Three independent piezos in x, y and z allow accurate measurements in all three dimensions regardless of size, offset, speed, and rotation. The system offers high resolution imaging with great ease and scans up to 100µm in x and y with an exceptional 100µm range in z. Integration with high resolution optics makes finding features and the pipette tip fast and easy.

Versatile Acquisition Software

The ionscope software integrates a host of features with intuitive, straightforward access to all scanning and feedback parameters. Fully automated tip approach combined with real-time scan progress monitoring and automated scan parameter selection makes set up fast and easy. The ionscope image analysis and presentation software provides a comprehensive set of image manipulation tools and multichannel rendering designed to aid analysis, exploration and presentation of data.

Straightforward Operation

The ICnano, with its capacity for high resolution imaging, can be operated as a standalone instrument, presenting a compact and cost effective imaging and probe automation capability. Sample preparation is effortless – simply place the sample in a petri dish and immerse in electrolyte solution. The ICnano provides excellent sample access, easy placement of electrodes and straightforward mounting of the nanopipette. The core algorithm driving the system seeks to avoid any physical surface interaction, whether of the sample or of floating debris, helping to protect the scan, the sample and the system from damage and contamination. Once set up, the system can continue to scan unattended for long periods enabling dynamic processes such as live cell interactions to be followed over days.

Unrivalled Integration and Flexibility

Integration of the ICnano with a number of advanced optical techniques such as scanning confocal microscopy, FRET, and TIRF provide a powerful approach for correlating spatial information with dynamic cellular processes. When combined with scanning confocal microscopy, the pipette tip and laser focus may be locked together. The optical focus tracks the pipette tip, following the sample contours and presents a fluorescent emission map in a single pass eliminating degradation by photo-bleaching.

Replacing micro-manipulators with the ICnano offers radical improvement to patch clamp productivity and effectiveness. Using the patching pipette itself as the scanning probe, a cluster of cells may be imaged and a target cell selected. A single cell may be scanned at higher resolution to identify specific locations of interest. The fine tip aperture can be precisely positioned vertically at the target using computer control. Through this process, the tip is protected from inadvertent contact with the cell surface or with floating debris by the ICnano's feedback control system.

Exploiting the hybrid functionality of the nanopipette provides additional powerful research flexibility through delivery of defined chemical, electrical and mechanical stimuli. Combining scanning ion conductance microscopy with a hydraulic pressure system allows quantitative pressure to be applied at precise locations to probe surface mechanical properties using the same pipette as is used for scanning. The nanopipette can also be filled with specific reagents or biomolecules and used for controlled, local, voltage-driven application for deposition or response mapping. Drawing out multi-barrelled pipettes offers further extension of these applications.

Configuration Options

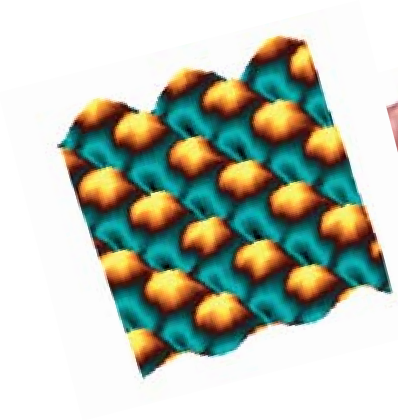
Sample scan – ICnano S

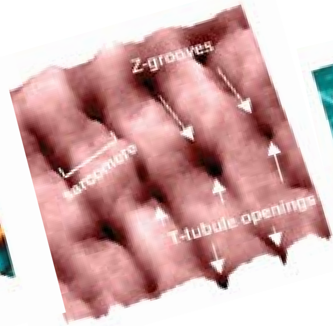
In the ICnano S scan head the probe is held in a fixed position and the sample is moved. As the sample is scanned in X and Y, the control system adjusts the Z position to maintain a fixed distance from the probe tip. If used with an inverted optical microscope, the ICnano S scan head is simply placed onto the existing stage. In addition to providing easy mounting and removal, this configuration enables the recording of fluorescence at the same time as surface topography – the sample surface is always within the confocal volume.

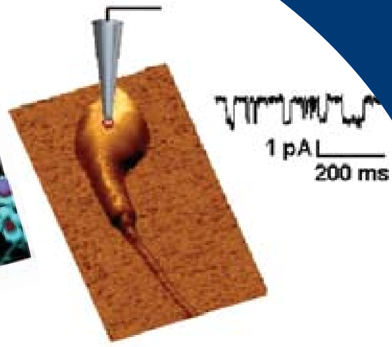
Pipette scan – ICnano P

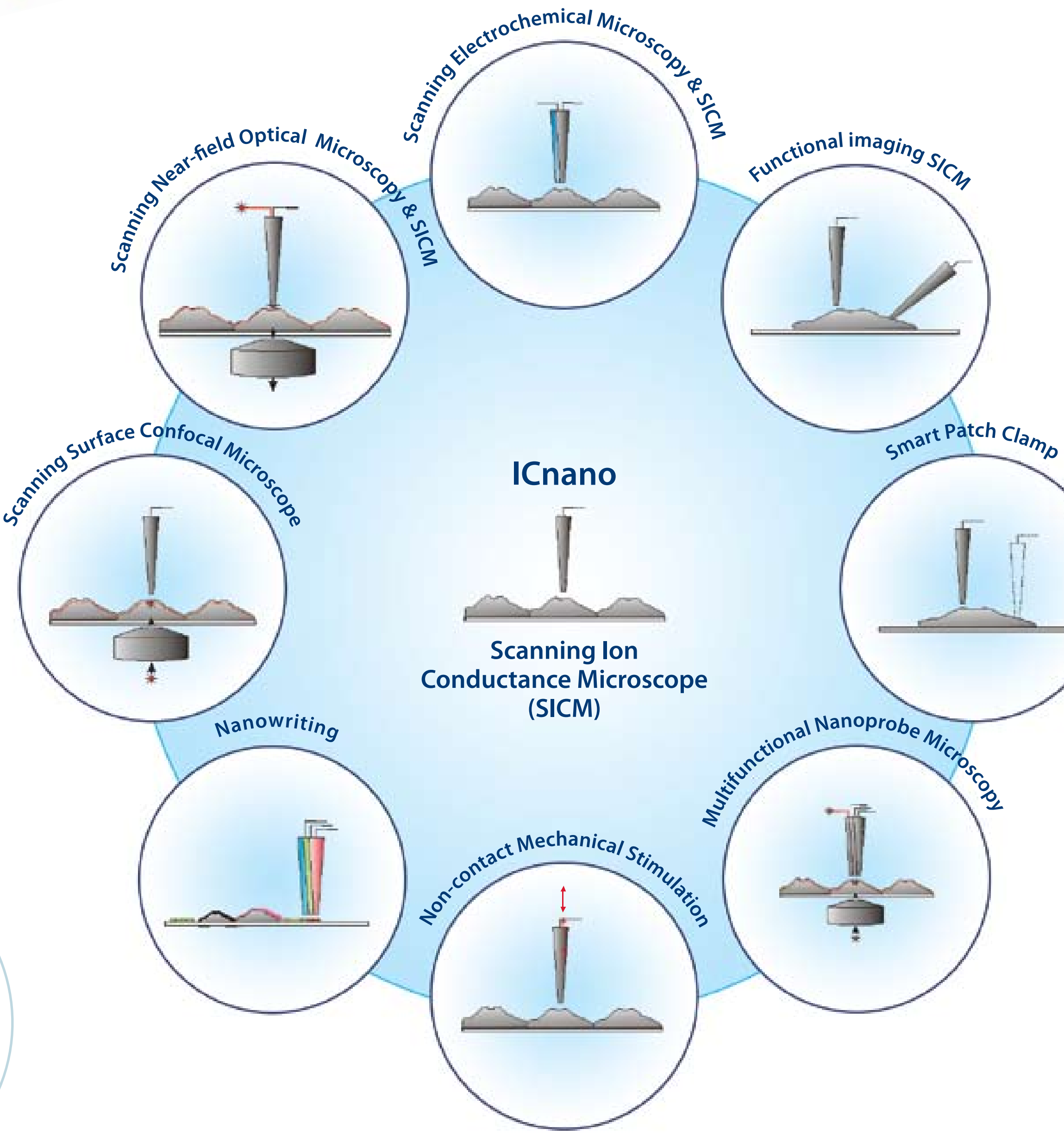
In the ICnano P scan head the sample is held in a fixed position and the pipette is scanned across its surface. The sample is placed onto the optical microscope stage, allowing the use of an existing holder, perfusion chamber or incubator. This configuration is also appropriate if additional probes or microelectrodes are required to engage the sample. The ICnano P scan head is bolted onto the fixed frame of the optical microscope and comes with an adaptor plate appropriate to each specific model.

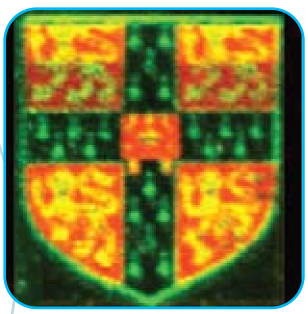
Scanning Ion Conductance Microscopy : Hybrid Techniques

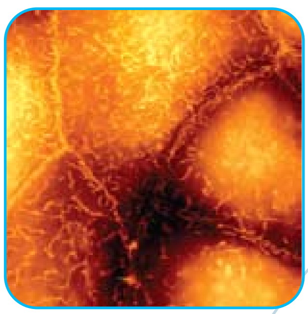










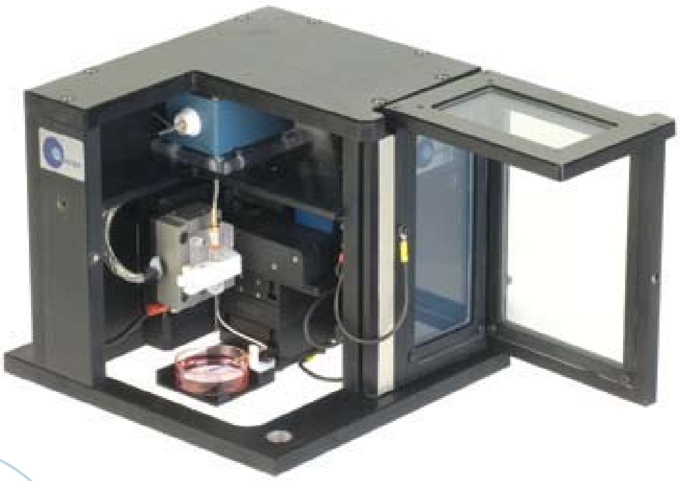


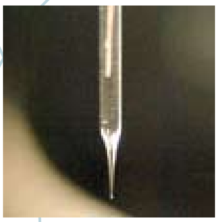
Images top left to bottom right : S-Layer protein from Bacillus sphaericus 40nm; Cardiac Myocyte 8µm; Neurons 65µm; Sperm cell 14x8µm; Two colour printing using DNA 60µm; Greyscale printing using DNA 60µm; Hearing Cell 7µm; Kidney endothelial cells 40µm.

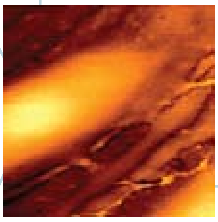
Scanning Ion Conductance Microscopy

How Does It Work?

Scanning Ion Conductance Microscopy is used to acquire topographic images of surfaces immersed in electrolyte liquid, often a physiological buffer. The scanning probe is a glass or quartz nanopipette filled with electrolyte, which measures the ion current passing through its tip. That current is limited by the tip aperture; the potential difference that drives it is applied between reversible silver-silver chloride electrodes, one inside the pipette and one in the sample dish. The current decreases as the probe tip approaches the surface. The sample, under the pipette, is raster scanned while the probe-sample distance is maintained by monitoring the ion current, and its feedback control. This means the pipette tip never makes unintended contact with the sample.







Simple to use

- No sample preparation required, just immerse the sample in a dish containing electrolyte solution
- Nanopipettes are easily prepared using a standard pipette puller. Electrodes are inserted into the pipette and the sample dish
- Automated tip approach for rapid and easy scan start

Superior Performance

- Using our proprietary distance modulated imaging mode, ionscope offers the highest resolution imaging on living cells
- Fast, sensitive feedback allows prolonged imaging and follows dynamic processes over long periods, for example 24 hours
- Easily integrates with optical techniques such as confocal fluorescence, allowing direct functional correlation of data

Dynamic Scanning

- 100µm scan range in x,y and z is advantageous for imaging biological materials
- Accurate image offset and zoom capabilities are achieved without changing hardware
- Uses ultra low noise independent closed loop x, y and z piezo flexure stages for accurate planar imaging with low cross talk

Powerful and Flexible Research Tool

- The nanopipette can be used as a smart patch clamp for electrophysiology measurements on defined cell regions selected from the scanned image
- Precise deposition of molecules to target sites on the sample can be achieved through voltage control of the nanopipette
- Mechanical stimulation and force measurements can be achieved through positive and negative hydraulic pressure in the pipette

Specifications

System

Magnification	1,000x – 100,000x
Scan resolution	20nm XY; 5nm Z
Scan time	50s – 20min (typical, 5µm x 5µm and 90µm x 90µm respectively)
Image resolution	64x64 – 1024x1024 pixels
Image formats	IMG (16-bit greyscale)

XY scan

Range	100µm x 100µm
Resolution	20nm overall (1nm for stage)
Linearity	1%
Max pixel step rate	1ms per pixel

Z scan

Range	100µm
Resolution	5nm overall (1nm for stage)

Pipette

Tip diameter	≥100nm borosilicate; ≥10nm quartz
Typical surface proximity	25nm for 50nm ϕ; 100nm for 200nm ϕ
Body diameter	1mm

Positioning

Range	15mm XY; 25mm Z
Resolution	100nm

Electrodes

Pipette	silver/silver chloride wire
Bulk electrolyte	silver/silver chloride pellet

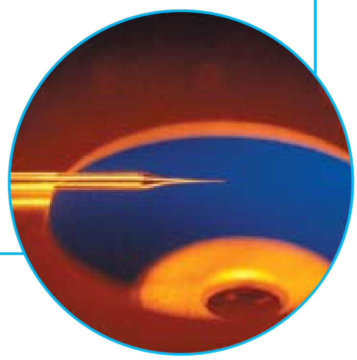
Scan head assembly

Translation stage	Physik Instrumente PI Hera P-6xx, 100µm; PI LISA P-753, 25µm
Motorised positioning	Physik Instrumente M-111/112 DC motor translation stages
Overall dimensions	Approx. 13cm (W) x 17cm (D) x 15cm (H)

Amplifier

The ICnano SICM is supplied with an Axon Instruments CV-5 1GU headstage amplifier to buffer the low current signal from the scanning pipette. However, if an external amplifier is to be used, e.g. for patch clamping, it may be connected instead.

Feedback resistor	1GΩ
Noise	25µV/sqrt(Hz) typical at 10KHz
Input bias current	1pA max at 25°C
Power supply	±12V



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