Scanning Ion Conductance Microscopy

Imaging Technique Integrating Shear Force Distance Control

Scanning ion conductance microscopy (SICM) is an imaging technique, measuring conductance through a nanometer-sized pipette tip opening that is brought close to a sample surface submerged in electrolyte solution. In combination with an integrated shear force distance control, the local ion conductance can be measured independently of and simultaneously with topography. The design of a shear-force-controlled SICM is presented and a new imaging mode that significantly improves image quality is discussed.

Introduction

For measuring different physical quantities on the nanoscale, a large variety of different scanning probe techniques such as scanning tunneling microscopy and atomic force microscopy have been developed during the past two decades. One of the more advanced and less wide-spread techniques is scanning ion conductance microscopy (SICM) [1].

In SICM, an electrolyte-filled, tapered glass nanopipette with an opening diameter of 100 nm or less serves as scanning probe (fig. 1). An ionic current is driven through this opening. When the pipette tip is approached toward a sample surface, the measured ion current through the pipette opening shows a strong distance dependence at pipette-sample distances similar to the opening diameter of the pipette. This is because the current has to "squeeze" through the narrowing gap between pipette tip and sample thereby decreasing the total ion conductance. The conductance is therefore a measure of pipette-sample distance and can be used as input to a feedback loop keeping the pipette-sample distance constant while laterally scanning the sample surface. With this technique, images of sample topography on fragile samples such as living cells can be generated at a resolution on the order of the tip opening diameter and without mechanical contact with the sample [2].

To measure ion conductance independently of and simultaneously with sample topography we complemented SICM with shear force distance control (fig. 2). For
shear force detection, the pipette is set into transverse vibrations. When approaching the pipette toward the sample the vibration amplitude decreases within a tip-sample distance in the nanometer range due to arising shear forces between pipette tip and sample.

The decrease of the vibration amplitude is detected and used for feedback control, allowing imaging of sample topography. Simultaneously, the ion current through the pipette is recorded and used to generate a complementary image of ion conductance.

**Experimental Setup**

In the centre of the SICM with integrated shear force distance control is the electrolyte-filled pipette whose tip is brought in close proximity to a sample surface covered by electrolyte solution. For the measurement of ion conductance, a voltage is applied between two Ag/AgCl-electrodes, of which the first is placed inside the pipette and the second outside the pipette in the electrolyte bath where the sample is located (fig. 2). For shear force distance control the pipette is brought into transverse vibrations by driving it with a dither piezo. The vibration amplitude of the pipette is measured with an optical read out scheme in form of a periscope design [3]: A collimated laser beam from a laser diode passes down the first periscope tube and is reflected horizontally by a 45° mirror. Then the beam passes through a lens that provides the interface between the air inside the tube and the electrolyte outside the tube. The lens focuses the beam onto the thin end of the pipette. The scattered light from the pipette is incident on a two segment photodiode, which is placed at the bottom of a second periscope tube and which detects the vibration amplitude of the pipette. The vibration amplitude signal is fed to a controller which drives the x-, y-, and z-scanner. The sample topography is generated from the vibration amplitude signal via a feedback loop and the ion
conductance is recorded simultaneously. With this periscope design, the tapered end of the pipette can be completely submerged in the electrolyte and stable imaging for hours is easily possible. This is an advantage over previous shear force designs, in which the sample was covered only by a thin film of electrolyte and where the resonant frequency of the probe was easily affected by fluctuations of the liquid-air meniscus (e.g. by surface waves or by evaporation).

**Imaging Modes**

In "regular" shear force imaging, the tip-sample distance is kept constant by a feedback loop using a fixed setpoint for the shear force vibration amplitude. The challenge with this mode is that the pipette tip permanently interacts with the sample and often causes damage to itself or to the sample while scanning. We therefore developed an alternative imaging mode [3]. In the "triggered z-modulation" imaging mode, the tip-sample distance of the vibrating pipette tip is modulated sinusoidally. The amplitude is periodically sampled only at the points of closest tip-sample approach with a sample-and-hold circuit. This provides a signal of shear force amplitude close to the sample surface while reducing the possibility for tip or sample damage. The sample topography is often imaged with improved quality in triggered z-modulation mode. A similar mode was introduced for scanning near field microscopy imaging in ambient conditions [4].

**Results**

Fixed Madin-Darby canine kidney (MDCK II) cells were imaged with the triggered z-modulation imaging mode. The topography image (fig. 3(a)) shows a closed-packed layer of cells. The cell bodies and cell-cell contacts can clearly be recognized, and substructures on the cell surfaces are visible. The simultaneously recorded ion conductance image (fig. 3(b)) shows a weak dependence on topography, even though there are no conductive paths through the sample. This dependence is mainly due to the finite response time of the feedback loop, causing the average tip-sample distance of the pipette to vary (depending on the local slope of the sample). Another contribution might come from soft protruding structures temporarily occluding the tip opening. Such structures, for example, might be formed by overlapping cell membranes in the regions of the cell-cell contacts.

**Conclusion**

SICM with integrated shear force distance control allows generating images of topography and local ion conductance simultaneously. This technique opens new possibilities for the investigation of a large variety of biologically and technically
relevant samples. In particular, it promises to become a useful tool for the analysis of transmembrane transport processes in living systems.

References:

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