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Surface Imaging by Self-propelled Nanoscale Probes

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An alternative approach to scanning probe microscopy is to replace the tip of a macroscopic cantilever with an autonomous, self-propelled probe-"robot" of microscopic dimensions, which moves across the surface in a random path [1]. We can then derive information about the surface simply by tracking the "robot", provided that its position can be detected accurately and that its *path* or the detected *signal* is sensitive to surface properties. Conceptually this procedure is similar to mathematical Monte Carlo techniques utilizing random sampling to calculate for instance definite integrals.

Here we experimentally demonstrate the feasibility of this novel approach to surface imaging using a particular probe-"robot" and a suitable detection method. The proposed probe-"robots" are fluorescent microtubules moving across a surface coated with the motor protein kinesin. This so-called gliding motility assay has been originally developed to study motor proteins *in vitro* [2, 3]. The position of several hundred microtubules is simultaneously recorded using a fluorescence microscope and a CCD camera. Previous experiments have shown that surface topography influences the *path* of an individual microtubule, since the stiff microtubules avoid climbing steep walls [4, 5]. Elevated surfaces are therefore much less accessible to the microtubules which first bind in a recessed region (Fig. 1). In this situation, the *path* of the microtubules (acting as probes) is confined by the surface topography, and the positions of the microtubules at one time-point correspond to a sample of the accessible regions. Repeated sampling gives an increasingly reliable estimate of the surface topography.

In the experimental realization presented here, an 85 μm x 68 μm (636 x 510 pixels) image of the surface of a thin (20 μm) polyurethane (PU) film, patterned by replica-molding with posts of 10 μm diameter and 1 μm height, was constructed. First, the surface was coated with the motor protein kinesin. After the microtubules were added and landed on the PU surface, 500 fluorescence images of the adsorbed microtubules are acquired in 5 s intervals using a CCD camera and an epi-fluorescence microscope (100x objective). The acquired images were thresholded to identify all microtubules. In a second step, stationary microtubules were excluded by subtracting from each frame the previous frame. The resulting stack of binary images was then summed up to show which parts of the surface were visited by the microtubules (Fig. 2).

Stochastic sampling of a surface by a large number of independent, self-propelled probes is an alternative concept to scanning probe techniques. Its main advantages are that no macroscopic cantilever is needed to move the probes, enabling us to image internal surfaces, and that an overview of the surface properties is quickly generated, which becomes more detailed as sampling proceeds. The microscopic probes also do not have to be retrieved from the surface after imaging. Together with the absence of a physical connection between probe and detection system, this may be beneficial for the handling of contaminated samples. Extending the resolution to the nanoscale is in principle possible, since a probe itself can have nanometer dimensions. By employing microtubules moved by the motor protein kinesin across a patterned surface, we have demonstrated an experimental realization of the proposed concept.

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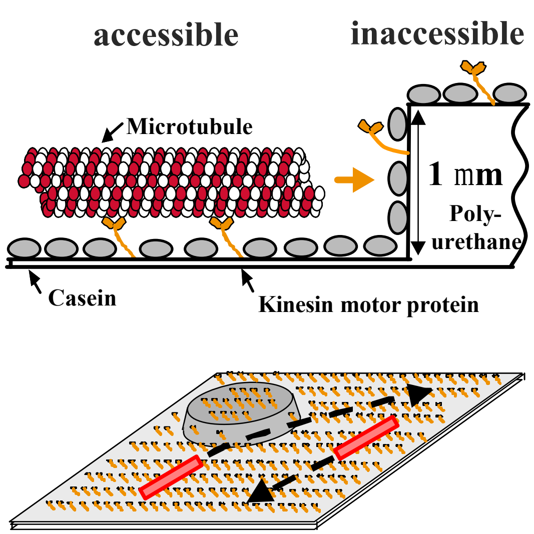

Figure 1: Fluorescently labeled microtubules are moved by the motor protein kinesin homogeneously adsorbed to a polyurethane (PU) surface coated with casein. Microtubules are guided around elevated regions (1 µm high) of the PU surface since they resist a sharper turn upwards due to their large stiffness. This makes the plateaus less accessible.

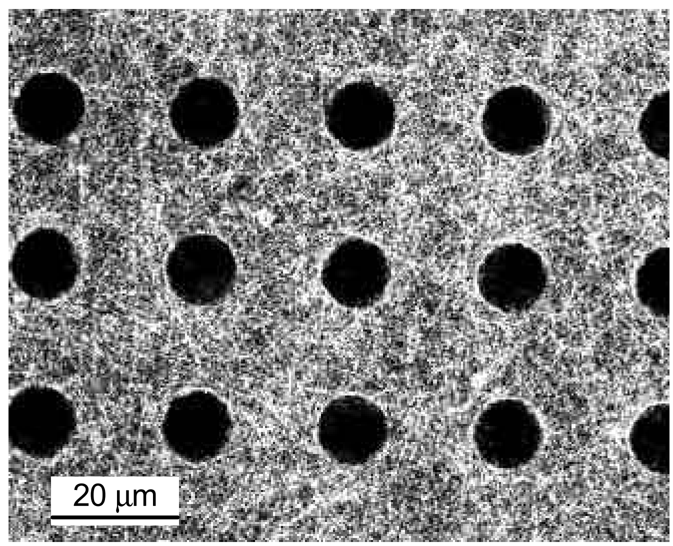

Figure 2: Sum image of a surface patterned with circular posts of 10 µm diameter and 1 µm height. The posts cannot be climbed by microtubules moving on the surface between them. The position of several hundred microtubules moving with a velocity of 0.25 µm/s in random directions was detected every 5 s for 2500 s. After thresholding of the individual images and selecting for moving microtubules, all 500 frames were summed up.

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