Plasma membrane topography and interpretation of single-particle tracks

To the Editor: Many contemporary models of the plasma membrane are based on single-particle tracking (SPT) by light microscopy on live cells. Whereas the analysis of single-particle tracks typically presumes that the cell surface is locally flat, both the ability of cells to rapidly swell and electron microscopy evidence suggest considerable folding[1]. Even the interface between the plasma membrane and a coverslip is not necessarily flat[2]. However, there is a lack of hard evidence about the topography of living cells. Accordingly, we examined cell topography using hopping-probe ion conductance microscopy (HPICM)[3], a high-resolution, noncontact method. HPICM, like all scanning-probe microscopes, has a limited speed (**Supplementary Fig. 1**), but by scanning many small square blocks, rather than sequential lines, HPICM ensures that the relative heights of adjacent pixels are accurate. Of the 70 cell types we examined using this approach on living cells (**Supplementary Table 1**), none had flat plasma membrane subregions (**Fig. 1a**,**b** and **Supplementary Figs. 1–3**). This observation has serious implications for SPT.

It is well established that molecules on cell surfaces appear to diffuse appreciably more slowly than on planar artificial membranes. Similarly, when we analyzed simulated movement over the non-flat surface of a live cell and a fixed cell in two dimensions (2D), the standard form of SPT analysis, the apparent rate of diffusion dropped; the effect varied with the local topography (**Fig. 1b**,**c**). Diffusion over simulated geometric surfaces also had a reduction in apparent movement[4] (**Supplementary Note** and **Supplementary Figs. 4**,**5**) and diffusion over a real anisotropically textured surface proved to be similarly anisotropic[5]. When analyzed in 2D, pillars emulated features of hop diffusion[6], where barriers divide a surface into separate domains in which movement is locally unconstrained (**Fig. 1d**) but from which particles may be excluded or within which they may be confined. The vertical sides of pillars appeared to trap particles (**Fig. 1d**), a phenomenon we call apparent topographical trapping; for thin pillars, this resembled binding (**Supplementary Fig. 6**). In these simulations, exclusion, confinement and locally unconstrained movement are artifacts produced by following movement over a non-flat surface in 2D. On membrane blebs, hop diffusion vanishes and diffusion rates are similar to those on reconstituted planar membranes[7], which is compatible both with the loss of diffusion barriers and with smoothing of the membrane.

The impressive precision with which particles can be localized, down to ±1.5 nm but more typically tens of nanometers, depends generally on localization of a tag rather than the molecule of interest. Cellular topography could cause an underappreciated problem in this respect as well. On a smooth surface the tag and molecule probably maintain a stable alignment, but topography produces an offset of up to ± the tag's radius (**Fig. 1e**), which is substantial given the typical diameter of tags (5–40 nm). Membrane folds may also trap or exclude tags.

Analysis in 2D of movement on non-flat cellular membranes can cause simple diffusion to show apparently complex patterns that necessitate complex explanations. High-resolution tracking in 3D is possible[8]. As the topography of live cells is measurable, a topographical map might be used to provide a general

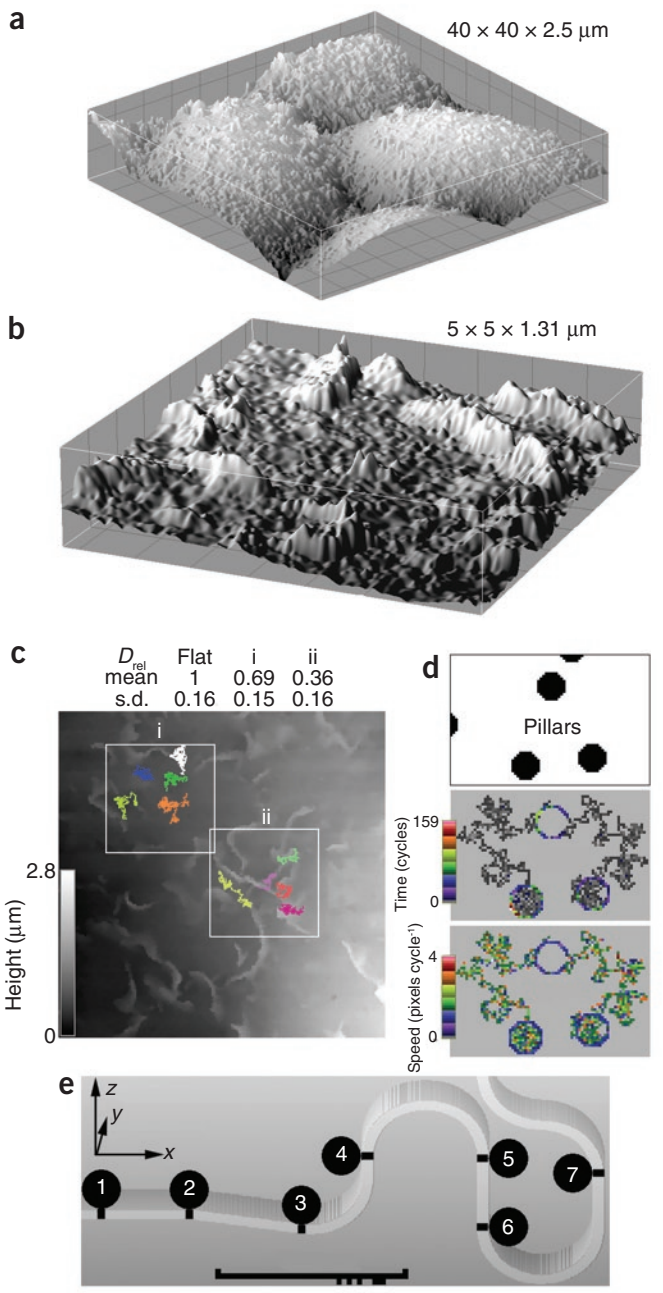

Figure 1 | Cell topography compromises interpretation of SPT data. (**a**,**b**) HPICM images of live FRSK cells rendered in 3D at low (**a**) and high (**b**) resolution. The image in **b** was used to simulate particle diffusion, which was expressed as a fraction of that on a flat surface ± s.d. (0.56 ± 0.16 (n = 25) for tracks originating near the center, 0.43 ± 0.14 (n = 30) for tracks originating near a ridge). (**c**) Height-coded HPICM image in 2D (10 × 10 µm) of a fixed FRSK cell used to simulate diffusion. Simulated, individually colored tracks originated in the two subregions (i and ii), with an equal probability of starting at any pixel in the central third of each region. Diffusion coefficients for subregions i (n = 26) and ii (n = 31) are expressed relative to free diffusion on a horizontal surface (D_{rel}; n = 99). (**d**) Two-dimensional analyses of the residence time (middle) and speed (bottom) in the region shown at the top (height, 40 pixels) for a single particle. Each simulated movement cycle consisted of four random moves. (**e**) Sequential positions of a membrane-bound molecule with a tag (diameter, 20 nm), depicted on a surface with a radius of curvature of 30 nm. Scale bar, 100 nm (see **Supplementary Note** for details of scale bar).

correction. An alternative would be to concurrently track multiple particles[6] with different properties and to use particles known to diffuse freely to construct a topographical map of the surface. Fluorescence correlation spectroscopy and fluorescence recovery after photobleaching experiments may be similarly compromised by topography.

Although we are not suggesting that the plasma membrane is homogenous or that movement of molecules in the membrane occurs only by simple diffusion, we do suggest that either SPT be performed in 3D over an established topography or that the assumption of local flatness be validated.

Note: Supplementary information is available on the Nature Methods website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemethods/.

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