Supplementary Data, **Supplementary Methods** and **Supplementary Table 2**). To enable comparison with Breakdancer on real data, we performed a similar analysis using paired-end reads from *Mycobacterium tuberculosis* and, consistent with the simulation data, found that PrInSeS-G outperformed BreakDancer in terms of false positive and false negative rates (**Supplementary Data**, **Supplementary Table 3** and **Supplementary Methods**).

Next we used PrInSeS-G to analyze uncharacterized genomes. First, we analyzed single-end reads from *M. tuberculosis* strain 18b (**Supplementary Methods**) and detected 275 consensus non-SNP variants for which the read depth improved after realigning the reads to the new consensus sequence (**Supplementary Data**, **Supplementary Figs. 2** and **3**, and **Supplementary Table 4**). We then mapped structural variation in *Drosophila melanogaster* whole genomes using a combination of single- and paired-end read data from the *Drosophila* Genetic Reference Panel[4] with an average combined coverage of 31. Initially we identified 121,198 non-SNP variants; 93% of these resulted in improved read alignment (**Fig. 1b** and **Supplementary Fig. 4**). After selecting these and removing overlapping variants (**Supplementary Methods**), we reached a final 'consensus' list of 107,517 non-SNP variants up to ~10 kb, thereby generating, to our knowledge for the first time, a comprehensive catalog of naturally occurring *D. melanogaster* variants (**Supplementary Tables 5** and **6**). Sanger sequencing validation for selected variants confirmed 84% of these (26 out of 31) (**Fig. 1c**, **Supplementary Data** and **Supplementary Methods**). Our data were consistent with those obtained using microarray comparative genome hybridization[5,6] in that we found significantly fewer variants per megabase on the X chromomsome compared to the autosomal chromosomes (741 versus 928, respectively; G test (G) = 21.0, $P < 4.6 \times 10^{-6}$, degrees of freedom (d.f.) = 1; **Fig. 1d**) and in exons compared to nonexonic regions (223 versus 1,148, respectively; $G = 683$, $P < 2.2 \times 10^{-16}$, d.f. = 1; **Supplementary Fig. 5**). In addition, genes with structural variants had significantly more expression variation than those without variants (Wilcoxon rank-sum test, $P < 2.2 \times 10^{-16}$; **Supplementary Fig. 6** and **Supplementary Table 7**), consistent with the notion that genes tend to be closely associated with variants that impact their expression[5].

We expect that imminent read length increases and future software development will ameliorate PrInSeS-G's overall variant detection sensitivity and specificity, and should eventually enable it to characterize heterozygous variants in mammalian whole genomes.

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

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Nano-imaging of membrane topography affects interpretations in cell biology

To the Editor: In their correspondence in *Nature Methods*, Adler *et al.* claim to present the first high-resolution topography of plasma membranes in living cells[1]. Using hopping-probe ion conductance microscopy (HPICM), they show that the plasma membrane contains many subresolution membrane folds and point out serious implications for single-particle tracking studies. We fully underwrite this conclusion and would like to point out that subresolution wrinkling also has serious implications for other, more mainstream, imaging techniques.

We reported in 2002 a simple approach to visualize plasma membrane topography with nanometer precision[2,3] (**Supplementary Fig. 1**). Whereas HPICM equipment is sparsely available, our approach requires only a confocal microscope with precise focus-stepping capacity. In full accordance with the data of Adler and co-workers[1], we showed that plasma membranes contain many subresolution wrinkles. We also showed that subresolution membrane folds should be considered when interpreting lateral fluorescence enrichments at the plasma membrane. For example, apparent microdomains intensely decorated with lipid probes have been interpreted to reflect lateral probe enrichment. However, our results indicated that they rather represent spots with high membrane content, that is, wrinkles in a homogenously labeled plasma membrane[2]. Perhaps the simplest and most effective approach to control for local membrane content is by normalizing the fluorescence intensity of probes of interest to that of a homogenously distributed membrane marker such as the carbocyanine dye DiI.

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

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