x-y, *x-z*, and *y-z* cross sections of the cell (Fig. 2, C to E, and movie S1) (*27*).

To characterize our cell imaging resolution more quantitatively, we identified point-like objects in the cell that appeared as small clusters of localizations away from any discernible microtubule filaments. These clusters likely represent individual antibodies nonspecifically attached to the cell. The FWHM values of these clusters, which were randomly chosen over the entire measured *z*-range of the cell, were 22 nm in *x*, 28 nm in *y*, and 55 nm in *z* (fig. S2) (*27*), similar to those determined for individual molecules immobilized on a glass surface (compare fig. S2 with Fig. 1C). Two microtubule filaments separated by 100 nm in *z* appeared well separated in the 3D STORM image (Fig. 2F). The apparent width of the microtubule filaments in the *z* dimension was 66 nm, slightly larger than our intrinsic imaging resolution in *z* and in quantitative agreement with the convolution of the imaging resolution and the independently measured width of the antibody-coated microtubule (Fig. 2F). Because the effective resolution is determined by a combination of the intrinsic imaging resolution (as characterized above) and the size of the labels (e.g., antibodies), improved resolution may be achieved by using direct immunofluorescence to remove one layer of antibody labeling, as we show in the next example, or by using Fab fragments or genetically encoded peptide tags (*29*, *30*) in place of antibodies.

Finally, to demonstrate that 3D STORM can resolve the 3D morphology of nanoscopic structures in cells, we imaged clathrin-coated pits (CCPs) in BS-C-1 cells. CCPs are spherical cage-like structures, about 150 to 200 nm in size, assembled from clathrin and cofactors on the cytoplasmic side of the cell membrane to facilitate endocytosis (*31*). To image CCPs, we adopted a direct immunofluorescence scheme using primary antibodies against clathrin doubly labeled with Cy3 and Alexa 647 (*27*). When imaged by conventional fluorescence microscopy, all CCPs appeared as nearly diffraction-limited spots with no discernible structure (Fig. 3A). In 2D STORM images in which the *z*-dimension information was discarded, the round shape of CCPs was clearly seen (Fig. 3, B and D). The size distribution of CCPs measured from the 2D projection image, 180 ± 40 nm, agrees quantitatively with the size distribution determined using electron microscopy (EM) (*32*). Including the *z*-dimension information allowed us to clearly visualize the 3D structure of the pits (Fig. 3, C and E to H). Figures 3C and 3E show the *x-y* cross sections of the image, taken from a region near the opening of the pits at the cell surface. The circular ring-like structure of the pit periphery was unambiguously resolved. Consecutive *x-y* and *x-z* cross sections of the pits (Fig. 3, F to H) clearly revealed the half-spherical cage-like morphology of these nanoscopic structures that was not observable in the 2D images. These experiments demonstrate the ability of 3D STORM to resolve nanoscopic features of cellular structures with molecular specificity under ambient conditions.

References and Notes

1. S. W. Hell, *Nat. Biotechnol.* **21**, 1347 (2003).
2. S. W. Hell, *Science* **316**, 1153 (2007).
3. M. G. L. Gustafsson, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13081 (2005).
4. M. J. Rust, M. Bates, X. Zhuang, *Nat. Methods* **3**, 793 (2006).
5. M. Bates, B. Huang, G. T. Dempsey, X. Zhuang, *Science* **317**, 1749 (2007); published online 15 August 2007 (10.1126/science.1146598).
6. E. Betzig *et al.*, *Science* **313**, 1642 (2006); published online 9 August 2006 (10.1126/science.1127344).
7. S. T. Hess, T. P. K. Girirajan, M. D. Mason, *Biophys. J.* **91**, 4258 (2006).
8. A. Sharonov, R. M. Hochstrasser, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 18911 (2006).
9. A. Egner *et al.*, *Biophys. J.* **93**, 3285 (2007).
10. H. Bock *et al.*, *Appl. Phys. B* **88**, 161 (2007).
11. P. Torok, T. Wilson, *Opt. Commun.* **137**, 127 (1997).
12. W. R. Zipfel, R. M. Williams, W. W. Webb, *Nat. Biotechnol.* **21**, 1369 (2003).
13. M. Nagorni, S. W. Hell, *J. Struct. Biol.* **123**, 236 (1998).
14. M. G. L. Gustafsson, D. A. Agard, J. W. Sedat, *J. Microsc.* **195**, 10 (1999).
15. A. Egner, S. W. Hell, *Trends Cell Biol.* **15**, 207 (2005).
16. W. E. Moerner, M. Orrit, *Science* **283**, 1670 (1999).
17. R. E. Thompson, D. R. Larson, W. W. Webb, *Biophys. J.* **82**, 2775 (2002).
18. A. Yildiz *et al.*, *Science* **300**, 2061 (2003); published online 5 June 2003 (10.1126/science.1084398).
19. L. S. Barak, W. W. Webb, *J. Cell Biol.* **90**, 595 (1981).
20. J. Gelles, B. J. Schnapp, M. P. Sheetz, *Nature* **331**, 450 (1988).
21. A. M. van Oijen, J. Kohler, J. Schmidt, M. Muller, G. J. Brakenhoff, *Chem. Phys. Lett.* **292**, 183 (1998).
22. M. Speidel, A. Jonas, E. L. Florin, *Opt. Lett.* **28**, 69 (2003).
23. P. Prabhat, S. Ram, E. S. Ward, R. J. Ober, *Proc. SPIE* **6090**, 60900L (2006).
24. E. Toprak, H. Balci, B. H. Behm, P. R. Selvin, *Nano Lett.* **7**, 2043 (2007).
25. H. P. Kao, A. S. Verkman, *Biophys. J.* **67**, 1291 (1994).
26. L. Holtzer, T. Meckel, T. Schmidt, *Appl. Phys. Lett.* **90**, 053902 (2007).
27. See supporting material on *Science* Online.
28. M. Bates, T. R. Blosser, X. Zhuang, *Phys. Rev. Lett.* **94**, 108101 (2005).
29. I. Chen, A. Y. Ting, *Curr. Opin. Biotechnol.* **16**, 35 (2005).
30. B. N. G. Giepmans, S. R. Adams, M. H. Ellisman, R. Y. Tsien, *Science* **312**, 217 (2006).
31. V. I. Slepnev, P. De Camilli, *Nat. Rev. Neurosci.* **1**, 161 (2000).
32. J. E. Heuser, R. G. W. Anderson, *J. Cell Biol.* **108**, 389 (1989).

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Supporting Online Material

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Materials and Methods
Figs. S1 and S2
Movie S1
References

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An Association Between the Kinship and Fertility of Human Couples

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Previous studies have reported that related human couples tend to produce more children than unrelated couples but have been unable to determine whether this difference is biological or stems from socioeconomic variables. Our results, drawn from all known couples of the Icelandic population born between 1800 and 1965, show a significant positive association between kinship and fertility, with the greatest reproductive success observed for couples related at the level of third and fourth cousins. Owing to the relative socioeconomic homogeneity of Icelanders, and the observation of highly significant differences in the fertility of couples separated by very fine intervals of kinship, we conclude that this association is likely to have a biological basis.

There has been long-standing uncertainty about the impact of kinship or consanguinity between spouses on the total number of offspring they produce (completed fertility). Consanguineous unions among humans increase the probability of a zygote receiving the same deleterious recessive alleles from both parents, with a possible adverse effect on fertility through an increased rate of miscarriage, infant mortality, and morbidity (*1*–*3*). Conversely, consanguineous unions may confer greater completed fertility through earlier age at marriage, as well as the socioeconomic advantages associated with preserving land and wealth within extended families. (*4*, *5*). In other species, lower fitness has been observed in offspring of distantly related individuals, which appears to be a result of the breakdown of coadapted gene complexes (*6*).

Previous studies examining the relationship between kinship and fertility in humans have focused on relatively close relationships between couples, rarely evaluating relationships more distant than second cousins (who share two great-grandparents) (*4*). Such studies have tended to be

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