Bowdon, Thomonde, Gurabo, Charcol Azul; and (v) Pacific: San Ignacio, Imperial, Cueva de Angostura, unnamed units on Tres Marias Islands and Baja California. T. M. Cronin *et al.* [*Palaeogeogr. Palaeoclimatol. Palaeoecol.* **47**, 21 (1984)] summarize age data for many Atlantic formations. See W. Bold [in *Sixth International Ostracode Symposium*, H. Löffler and D. Danielopol Eds. (Junk, The Hague, 1977), pp. 175–186] on occurrences of *Puriana*. Most fossil material was collected by T.M.C., but other samples were provided by W. Blow, W. A. van den Bold, A. L. Carreno, J. E. Hazel, K.-H. Paik, J. T. Smith, T. R. Waller. *Puriana* samples from modern continental shelves off the eastern United States, Hispañola, Veracruz, Campeche Banks, Belize, Gulf of California, Texas, Baja, Mexico, and California were examined. Specimens were provided by R. H. Benson, M. Kontrovitz, P. R. Krutak, F. M. Swain, J. W. Teeter, and P. C. Valentine. For detailed locality maps see Cronin (*10*).

8. These species were studied: *carolinensis* Hazel, 1983; *convoluta* Teeter, 1975; *elongorugata* Howe, 1936; *floridana* Puri, 1960; *gatunensis* (Coryell and Fields, 1937); *horrida* Benson and Kaesler, 1963; *matthewsi* Teeter, 1975; *mesacostalis* (Edwards, 1944); *pacifica* Benson, 1959; and *rugipunctata* (Ulrich and Bassler, 1904). *Puriana congestocostata* van den Bold, 1963 and *P. fissispinata* Benson and Coleman, 1963 belong in *Coquimba* [W. Bold, *Bull. Am. Paleontol.* **79** (No. 312) (1981). *Puriana* aff. *elongorugata* is an undescribed species. For illustrations of most taxa, see J. E. Hazel, *U.S. Geol. Surv. J. Res.* **5**, 373 (1977); T. M. Cronin and J. E. Hazel, *U.S. Geol. Surv. Prof. Pap. 1125-B* (1980); and J. E. Hazel, *Smithson. Contrib. Paleobiol. 53* (1983), p. 81.
9. M. A. Buzas, *J. Paleontol.* **40**, 585 (1966); R. E. Blackith and R. A. Reyment, *Multivariate Morphometrics* (Academic Press, London, 1971); N. A. Campbell and R. A. Reyment, *Math. Geol.* **10**, 347 (1978). Data analyzed on an Amdahl 470/V7 with CANDISC [SAS Users Guide: Statistics (Statistical Analysis System, Cary, N.C., 1982)]. For analysis of *Puriana*, classes were seven species groups; for analysis of *floridana* and *mesacostalis*, class was age.
10. T. M. Cronin, *U.S. Geol. Surv. Prof. Pap.*, in press.
11. S. M. Stanley, in *Paleontologia Comme Scienza Geostorica*, E. M. Gallitelli, Ed. (STEM-Mucchi, Modena, 1982), pp. 179–191.
12. D. E. Schindel, *Paleobiology* **8**, 340 (1982).
13. N. Eldredge and S. J. Gould, in *Models in Paleobiology*, T. J. M. Schopf, Ed. (Freeman Cooper, San Francisco, 1972), pp. 82–115.
14. W. L. Prell, *Init. Rep. Deep Sea Drill Proj.* **68**, 455 (1982); R. H. Fillon and D. J. Williams, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **42**, 7 (1983).
15. T. J. M. Schopf, *Evolution* **36**, 1141 (1982); M. J. Novacek and M. A. Norell, *Syst. Zool.* **31**, 366 (1982).
16. S. Wright, *Proc. 6th Int. Genet. Conf.* **1**, 356 (1932); *Evolution* **36**, 427 (1982).
17. G. L. Bush *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3942 (1977); M. J. D. White, *Modes of Speciation* (Freeman, San Francisco, 1978); A. R. Templeton, *Evolution* **34**, 719 (1980).
18. E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, Cambridge, 1963); S. J. Gould and N. Eldredge, *Paleobiology* **3**, 115 (1977).
19. J. W. Valentine and D. Jablonski, in *Evolution, Time and Space: The Emergence of the Biosphere*, R. W. Sims *et al.*, Eds. (Academic Press, London, 1983), pp. 201–226.
20. B. Charlesworth *et al.*, *Evolution* **36**, 474 (1982).
21. S. M. Stanley, *Macroevolution: Pattern and Process* (Freeman, San Francisco, 1979).
22. G. Oster and P. Alberch, *Evolution* **36**, 444 (1982); S. J. Gould, in *Perspectives on Evolution*, R. Milkman, Ed. (Sinauer, Sunderland, Mass., 1982), pp. 83–104; P. G. Williamson, *Nature (London)* **293**, 437 (1981).
23. See D. C. Potts [*Bull. Mar. Sci.* **33**, 619 (1983)] for study of Indo-Pacific corals and Milankovitch cycles.
24. H. L. Carson, in *Mechanisms of Speciation*, C. Barigozzi, Ed. (Liss, New York, 1982), p. 411.
25. J. H. Campbell, in *Perspectives in Evolution*, R. Milkman, Ed. (Sinauer, Sunderland, Mass., 1982), pp. 190–201.

26. I thank R. H. Benson, M. A. Buzas, R. Z. Poore, D. E. Schindel, and S. Wing for commenting on the manuscript; T. A. Ager, J. E. Hazel, Y. Okada, K.-H. Paik, N. Ikeya, T. Hanai, and E. Compton for helpful discussion; and colleagues listed in (*7*) for loan of specimens.

1 June 1984; accepted 17 October 1984

Direct Imaging of Live Human Platelets by Flash X-ray Microscopy

Abstract. *A 100-nanosecond pulse of long-wavelength x-rays was used to produce high-resolution stop-motion images of living human platelets. Although some aspects of the structure conform to those seen in dehydrated specimens, novel features are apparent. The technique should permit detailed stop-motion examination of the interaction of platelets with their surrounding medium as well as exploration of the phagocytic and secretory activities of a wide variety of other cells.*

Long-wavelength (soft) x-rays may be used with x-ray–sensitive material (x-ray resist) to produce high-resolution images that reflect the photon absorbance patterns of specimens (*1, 2*). The technique, termed contact x-ray microscopy, can provide a bridge between light microscope and electron microscope observations, since the resolution obtainable is 5 to 10 nm and, with a slight loss of resolution, specimens as thick as 10 μm may be studied (*3*). Furthermore, contact images appear to provide unique morphological information not available when light or electrons are used for imaging.

Because of their utility for studying fixed and dried material, soft x-rays have frequently been considered for the imaging of living material (*4, 5*). Exposure times with conventional and synchrotron sources have proved to be too long to prevent natural and radiation-induced motion of the living specimen from blurring the image (*6*). Flash x-ray sources with plasmas as emitters are of sufficient intensity to produce resist images in nanosecond periods (*7, 8*). With this range of exposure times, a specimen may remain alive at the instant of exposure and its image captured before the specimen is destroyed by the exposure. We report here what may be the first soft x-ray image of this type, that of a living human blood platelet, produced with a flash x-ray source that emits a 100-nsec pulse of soft x-rays. The wavelengths used to image the hydrated specimens were between 24 and 43 nm, a region in which the relative absorption of water is low compared to that of protein (*3*). The images reveal details not previously seen in images of fixed or dried platelets.

Human platelets were isolated and resuspended in buffer at a density of 10^9 cells per milliliter (*9*). A 10-μl portion of the suspension was placed on the surface of a Si_3N_4 window 100 nm thick that had been coated with a 1-μm layer of poly-

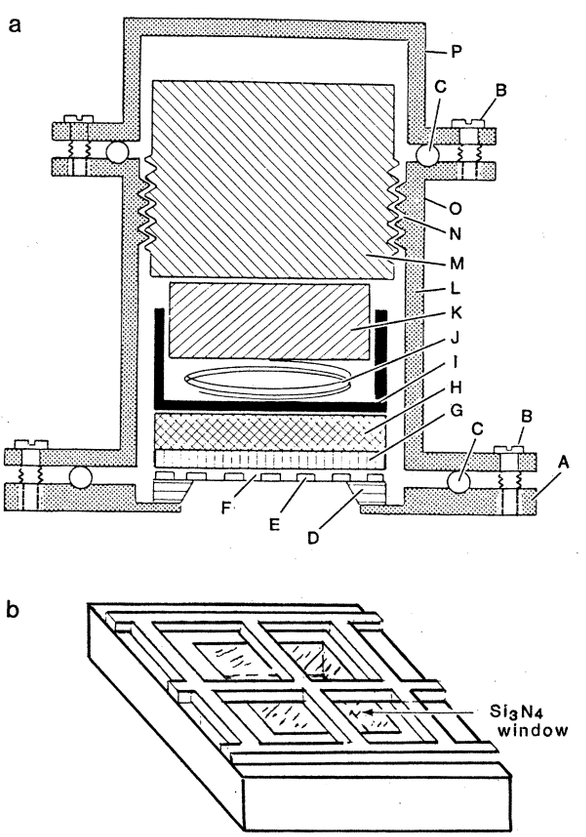

Fig. 1. (a) Diagram of the wet chamber, showing the (A) substrate holder, (D) substrate for vacuum window, (E) resist honeycomb, (F) specimen area, (G) recording resist, (H) substrate for resist, (H to K) spring mechanism to hold resist in position, (B, C, and L) body of wet chamber with O-ring seals, (M and N) threaded spring compressor, and (P) top of chamber. (b) Honeycomb subchamber [enlarged view of (E)].

methyl methacrylate (PMMA). Over a 1-mm^2 area the PMMA layer had been etched down to the Si_3N_4 in a honeycomb pattern (Fig. 1b), forming a meshwork of wells 1 μm deep and 10 μm^2 in area (Fig. 1a). After the drop of liquid had been blotted (Whatman No. 4 filter paper) until only a thin film remained, a 1-cm^2 silicon wafer coated with 400 nm of copolymer resist, PMMA–methacrylic acid, was inverted onto the window so that the resist and the window were opposed. The copolymer served as the detector. Total time between blotting and exposure was less than 5 minutes.

The entire assembly was mounted in a gas-tight holder (Fig. 1a) 20 cm from the x-ray source and exposed to a 100-nsec burst of soft x-rays produced by an imploding gas jet plasma. This x-ray source (*7*), called LEXIS (Maxwell Laboratories), produces 300 J of 2.7- to 10-nm radiation in a 140-nsec pulse (full width at half maximum). X-ray diode measurements show that 150 J of the radiation emitted is in the 2.7- to 42-nm region. This results in x-ray intensities greater than 0.75 J/cm^2 at the specimen surface.

Scanning electron microscope (SEM) images of the developed resist (*10*) showed platelets in various stages of activation (Fig. 2, a and b) (for comparison, Fig. 3 shows an x-ray image of a dried and fixed blood platelet). Both the SEM images and the x-ray replicas are of high quality, and objects as small as 10 nm are visible. The absence of salt crystals around the platelets and the lack of elevated rims of dried protein (*11*) support the conclusion that the cells were hydrated at the moment of exposure. As noted in whole mounts of air-dried human platelets (*2*), pseudopods contained a central core of photon-absorbant material that passed through the platelet membrane and was connected to a similar network that ramified throughout the platelet cytoplasm. Although this cytoplasmic network is a prominent feature of platelets with few or no pseudopods, in these platelets neither dense bodies nor α-granules were distinguishable. The flocculent material associated with the bases of pseudopods and scattered throughout the cells (arrows in Fig. 2, a and b) has not, to our knowledge, been observed previously by electron microscopy or contact x-ray microscopy of air-dried whole mounts.

The very high quality and resolution of the images indicate that the technique provides adequate contrast between the cell substance and an envelope of water as thick as 1 μm or more. Direct high-resolution visualization of morphological events associated with many cellular processes occurring in submillisecond time should now be possible.

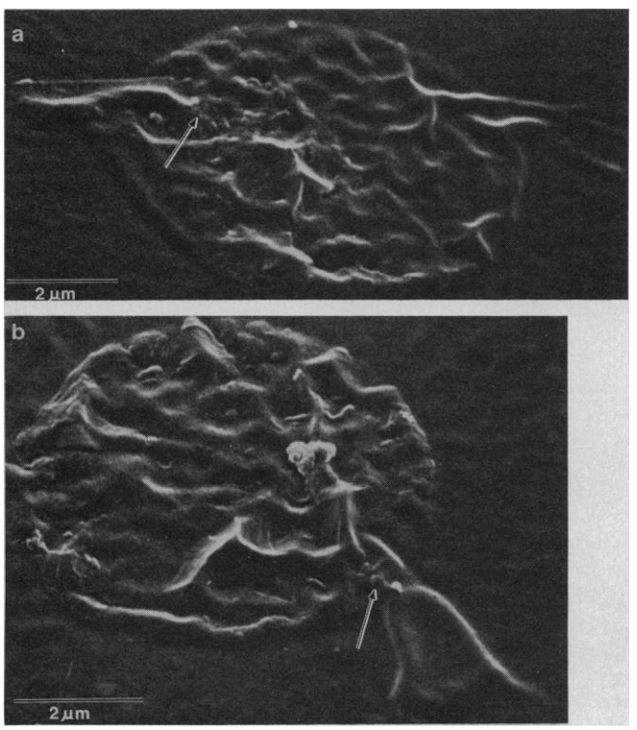

Fig. 2. (a) X-ray image of a wet, living blood platelet. The periphery lacks the continuous elevated rim that characterizes the air-dried platelet shown in Fig. 3. (b) X-ray image of another living blood platelet. Note variations in the contours of the rim and the early stage in pseudopod development (lower right portion of cell). Arrows, in (a) and (b), indicate flocculent material at the base of the pseudopod which is initiated from inside the platelet.

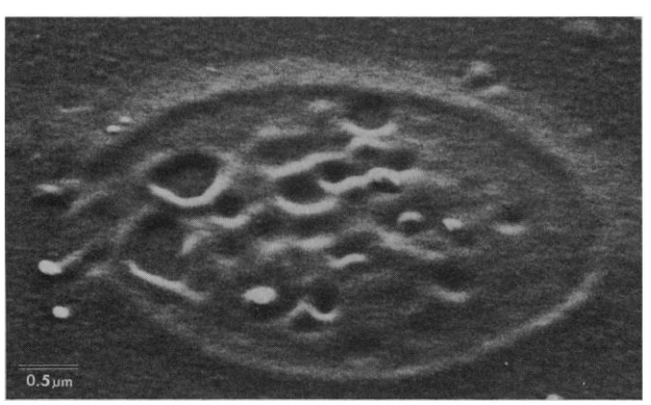

Fig. 3. X-ray image of whole-mounted (air-dried) blood platelet. The SEM picture was taken at 45°. Note the elevated periphery of the cell, a rim of dried protein. The spherical bodies are believed to be dense granules and the depressed areas vacuoles.

RALPH FEDER
VERONICA BANTON
DAVID SAYRE
IBM T. J. Watson Research Center, P.O. Box 218, Yorktown Heights, New York 10598
JOHN COSTA
National Institutes of Health, Building 10, Bethesda, Maryland 20205
MARIO BALDINI
BYUNG KIM
New England Deaconess Hospital, Boston, Massachusetts 02215

References and Notes

1. E. Spiller *et al.*, *Science* **191**, 1172 (1976).
2. R. Feder, J. L. Costa, P. Chaudhari, D. Sayre, *ibid.* **212**, 1398 (1981).
3. D. Sayre, J. Kirz, R. Feder, D. M. Kim, E. Spiller, *Ultramicroscopy* **2**, 337 (1977).
4. R. Feder, J. S. Pearlman, J. C. Riordan, J. Costa, *J. Microsc. (Oxford)*, in press.
5. B. Panessa *et al.*, *Ultramicroscopy* **6**, 139 (1981).
6. J. W. McGowan *et al.*, *J. Cell Biol.* **80**, 732 (1979).
7. J. Pearlman and J. C. Riordan, *J. Vac. Sci. Technol.* **19**, 1190 (1981).
8. J. Bailey, A. Ettinger, A. Fisher, R. Feder, *Appl. Phys. Lett.* **40**, 33 (1982).
9. Whole blood was collected in citrate-EDTA [T. C. Detwiler and R. D. Feinman, *Biochemistry* **12**, 252 (1973)] and platelet-rich plasma was prepared by serial differential centrifugation [D. L. Murphy, R. W. Colburn, J. M. Davis, W. E. Bunney, Jr., *Life Sci.* **8**, 1187 (1969)]. Platelets were pelleted (1000*g* for 20 minutes) and resuspended in a small volume of tris-citrate containing bovine serum albumin [J. L. Costa, D. L. Murphy, M. S. Kafka, *Biochem. Pharmacol.* **26**, 517 (1977)].
10. The exposed resist was developed in a 1:1 solution of ethyl cellusolve acetate and ethyl alcohol for 2 minutes. The resist was then coated with 10 nm of evaporated AuPd.
11. W. Hui and J. L. Costa, *J. Microsc. (Oxford)* **115**, 203 (1979).
12. We thank J. Pearlman, J. Riordan, S. Robb, C. Alliota, and K. Hopkin for their support. Supported in part by DOE contract DE-ACO2-83ER60107 to M.B.

19 June 1984; accepted 17 September 1984