marked a subpopulation of stem cells that Weissman, Christa Muller-Sieburg, and Cheryl Whitlock, also of Stanford, had partially purified in 1986, yielding what Weissman now thinks is a pure population of cells.

Other groups of investigators have used different methods to isolate mouse stem cells including separation techniques based on differences in cell density and labeling type I major histocompatibility antigens. These proteins are important in immune system recognition and are found on stem cells. As yet, these approaches have not yielded a pure population of stem cells, says Heimfeld.

In March, Irwin Berstein, Robert Andrews, and Ronald Berenson of the Fred Hutchinson Cancer Research Center in Seattle, Washington, and their colleagues reported progress in isolating human stem cells. They found that a protein on the surface of 1% to 4% of bone marrow cells appears to be a marker for a stem cell population in both humans and baboons. "This CD34 antigen is expressed by cells that will establish hematopoeisis [blood cell formation] in vivo," says Bernstein. When the researchers inject 15 to 19 million of bone marrow cells enriched for $CD34^+$ cells into lethally irradiated baboons, the animals regain their ability to form all blood cell types.

A major difference between this experiment in primates and those done by the Stanford group in mice is that the human stem cells are clearly not a homogeneous population. As part of a new collaboration, the Stanford and Seattle researchers hope to purify the human stem cells. Then they may be able to test whether the cells can generate all the different blood cell types in an experimental mouse just developed by Weissman's group.

The new information should answer some basic questions about the development and differentiation of hematopoietic cells. For instance, a running debate has been whether bone marrow stem cells are already programmed to become a certain kind of cell—a T lymphocyte for example—or whether the biological environment dictates the cell's final differentiated state.

Weissman's data support the latter hypothesis. The stem cells are obviously capable of producing all lineages of blood cells when injected intravenously, a procedure that exposes them to many different biological environments in the body. But if they are injected directly into the thymus gland, they differentiate only into T lymphocytes. This result implies that something about the microenvironment of the thymus gland directs their differentiation into T cells.

■ DEBORAH M. BARNES

Near-Field Microscopes Beat the Wavelength Limit

Normal optical microscopes are limited to resolutions no better than the wavelength of visible light, but a new technique gives a tenfold improvement in detail and should open new vistas to viewing with visible light

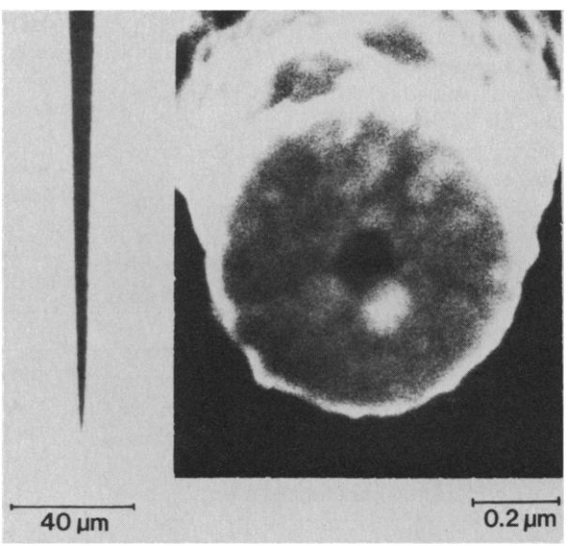
THE QUEST TO BUILD microscopes that see the world in smaller and smaller detail eventually runs into a Catch-22. Better resolution requires using probe particles of increasingly smaller wavelengths, which means increasingly higher energies, but higher energy particles damage the object under view and often require special preparation of the sample.

X-ray microscopes, for example, give up to 50 times better resolution than visible light microscopes, but their ionizing x-ray radiation harms a sample considerably. Electron microscopes are yet another 25 times more powerful than x-ray microscopes, but they work only on samples put in vacuum and they too damage the sample. Scientists would like a microscope as benign to samples as a light microscope but with as much resolving power as an x-ray microscope.

Enter the near-field scanning optical microscope. Cornell University physicist Michael Isaacson says this new microscope, which he developed with Aaron Lewis, now at Hebrew University in Jerusalem, can make out details as small as one-tenth the wavelength of visible light. Eventually its resolution might be pushed to one-fiftieth of a wavelength, Isaacson says, which would put it on a par with current x-ray microscopes.

The laws of physics make such resolution impossible with normal lens-based light microscopes. No matter how carefully such a microscope is designed or built, the diffraction of light restricts its resolution to no better than the wavelength of the light used by the microscope. (Visible light has wavelengths between about 400 and 700 nanometers, or 400 to 700 billionths of a meter.) By using x-rays, which have much shorter wavelengths, microscopists have achieved resolutions as good as 10 to 20 nanometers, and electron microscopes can make out details as small as 0.2 nanometer. (With electron microscopes, the limiting factor is the focusing ability of the electromagnetic lenses and not the wavelength of the electrons, which can be made very small by increasing the energy.)

Isaacson has developed a lensless light microscope that sidesteps this wavelength resolution limit. The basic idea is to use a tiny probe and bring it right up next to the sample, so that the probe is seeing only a very small part (less than a wavelength across) of the sample at any given time. Then by scanning the probe over the sample, the microscope can piece together an entire image.



The tip of the probe. *Electron micrographs show the profile of a pipette (left) and its tip, which has a 100-nanometer-diameter hole.*

The most obvious way to do this is to illuminate only a small part of the sample at a time. By putting a subwavelength hole in a mask, say, and shining a light through the hole, one gets a spot of light less than a wavelength across. Shining this light through the sample one point at a time eventually images the whole sample.

The key to this technique is keeping the sample within the so-called "near field" of

the source of light—no farther from the hole than about half the diameter of the hole. The reason is that once the light passes through the hole it rapidly spreads out—like water leaving a garden hose, as Isaacson puts it—and no longer illuminates just one small part of the sample. But very close to the hole the spot of light will remain much less than a wavelength across.

The microscope built by Isaacson and Lewis works in a slightly different way. Instead of restricting the illumination to a subwavelength spot, it relies on a detector that can see only a subwavelength bit of the sample at a time.

The detector is a hollow, needle-shaped tube with a tiny opening at its tip. This probe is made by drawing down a glass pipette until its tip is as small as 150 nanometers across and the inner hole has a diameter of just 50 nanometers. The outside of the pipette is then covered with a thin coat of aluminum so that light can enter only through the hole. Finally, the pipette is hooked up to a sensitive light detector that measures the amount of light coming through the tube.

By illuminating the sample and moving the tip of the pipette over the sample's surface in 15-nanometer steps, the microscope builds an image bit by bit. The microscope, which Isaacson now operates with graduate student Eric Betzig, has achieved resolution as good as 50 nanometers, or about one-tenth the wavelength of visible light.

The theoretical limit of the resolution should be around 10 nanometers, Isaacson says. Past that point, the small amount of light that leaks through the aluminum coating on the pipette becomes a problem.

So far, Isaacson has looked mostly at test samples to study the microscope's abilities, but he foresees a number of applications. Because visible light does little or no damage to samples and because it can be used in air or water, the near-field optical microscope should be useful in examining biological structures, such as viruses or chromosomes, in vivo. Since the microscope is sensitive to visible light, it might be used in conjunction with testing techniques that induce fluorescence in biological samples.

The microscope could also be used to examine the surfaces of integrated circuits without damaging the circuits as scanning electron microscopes do. And its sensitivity to visible light might be useful in measuring directly the output of laser diodes.

Since the near-field scanning optical microscope detects visible light with much greater resolution than possible before, it opens up a new world to human sight.

■ **ROBERT POOL**

Pattern and Process in Extinctions

The subject of extinction has in recent years become the focus of a great deal of interest and research activity, not least because the occasional mass die-offs that punctuate Earth history demand explanation. More generally, however, the pattern of extinction is likely to reflect something about the processes underlying the evolutionary histories of the myriad groups of organisms that constitute the biota. With this in mind, University of Chicago paleontologists David Raup and George Boyajian posed the following question: "Does the fossil record of extinction in the Phanerozoic show a consistent, repeatable pattern, or is it a confused amalgam of signals produced by many independent evolutionary histories?" The answer is that the extinction pattern is surprisingly uniform, at least among the nine groups of marine organisms they studied (see diagram below).

The result is a surprise, because there is considerable evidence that extinction can be a selective process, depending upon a variety of ecological, geographical, and physiological variables. Such selectivity would be expected to affect different groups in different ways, yielding a random, not uniform, response to extinctions. And, as Raup and Boyajian note, this expectation is apparently supported by the oft-repeated assertion that "My group was not affected by [this or that] extinction."

The Chicago researchers are not suggesting that their results imply a complete absence of selectivity, merely that the strongest component of the pattern is uniformity of response across different groups. "We have extinction events that cut across functional, physiological, and ecological lines," say Raup and Boyajian, "and this suggests common external causes. In other words, the results suggest that extinction is physically rather than biologically driven." The key issue is that, whatever the mechanism, "major pulses of extinction result from geographically pervasive environmental disturbances."

Norman Newell, of the American Museum of Natural History, New York, was promulgating this very same message more than 30 years ago, note Raup and Boyajian, but it became lost amid a suspicion of inferring large-scale patterns from necessarily incomplete data, and a collective concentration on particular cases. Now, with a growing interest in pattern and process, the Chicago researchers were able to reexamine the issue by using the data set of some 20,000 marine genera heroically compiled by John Sepkowski.

■ **ROGER LEWIN**

ADDITIONAL READING

D. M. Raup and G. E. Boyajian, "Patterns of generic extinction," *Paleobiology* **14**, 109 (1988).