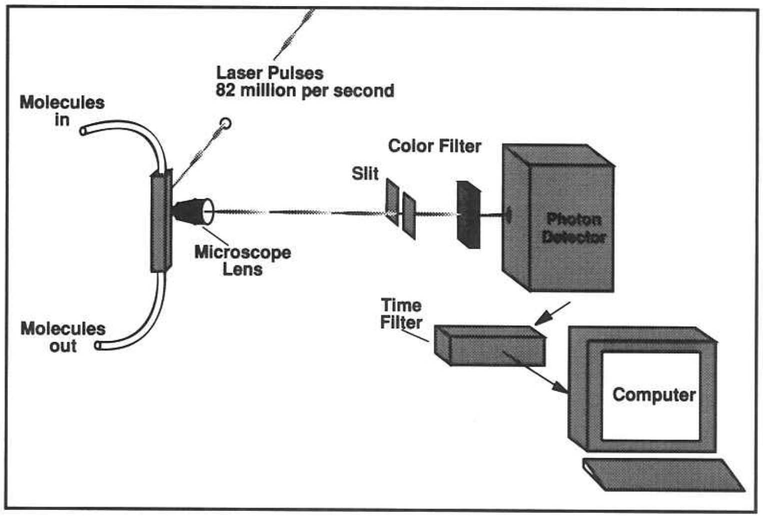
Single Molecule Detector

Inventors: E. Brooks Shera, Physics Division; Newton K. Seitzinger;* Lloyd M.Davis;† Richard A. Keller, Chemical and Laser Sciences Division; and Steven A. Soper‡



Fluorescent molecules in liquid stream past a fast-pulsed laser beam. The molecules absorb energy from the beam and emit a burst of dim light. A photon detector and a computer identify each molecule by counting the photons in the burst.

Researchers have long sought to develop instruments sensitive enough to detect and study small numbers of molecules of a given substance. Los Alamos National Laboratory scientists have achieved the ultimate goal of that pursuit, an instrument that identifies a single molecule in a liquid. Although scientists have for some time observed single molecules trapped in a vacuum, where they are isolated and relatively easy to detect, until now they have not been able to detect them in a liquid, where they are surrounded by billions of other molecules. Because important biological and chemical processes occur in liquids, the ability to track single molecules in such an environment provides a tool of enormous potential for scientists.

The achievement means that with the expected development of supporting technology, researchers may soon greatly speed DNA sequencing for the Human Genome Project. They may be able to analyze biomedical samples, detecting and quantifying minute amounts of chemicals, enzymes or viruses in blood, for example. The instrument may also be used to detect minute quantities of environmental contaminants; to identify specific enzymes or proteins in or on the surface of cells, viruses, or other particles; and to study the interaction of viruses and their binding sites. The detector should thus have a major impact on medical research and treatment.

An expected application will be in basic research on chemical or biochemical reactions and kinetics. The detector will make it possible to study the biochemistry of single molecules interacting one-on-one. Using the Single Molecule Detector, scientists expect to learn things that are not apparent when only large ensembles of molecules are studied. These basic research uses as well as the other important applications of the Single Molecule Detector brought the inventors a 1991 R&D 100 Award; the awards are given annually by *Research and Development Magazine* for the one hundred most significant technical innovations of the year.

The Invention—How It Works

The motivation for developing the Single Molecule Detector was to find a way to do extremely rapid DNA sequencing for the Human Genome Project. After studying a variety of novel approaches to DNA sequencing, a group of Los Alamos scientists—James Jett and John Martin of the Life Sciences Division, Richard Keller, and Brooks Shera—concluded that the most promising was an enzymatic cleavage approach that required, among other new technologies, a single molecule detection capability. The detector that was subsequently developed uses a highly focused laser beam. A liquid containing molecules of a fluorescent dye streams through a thin glass tube past the beam. When a fluorescent molecule passes through the laser beam, it absorbs energy and emits a burst of dim light. A light detector identifies the molecule by counting the photons it emits. The technique registers over 85 percent of the fluorescent molecules that pass through the beam. Individual molecules were first successfully detected with a prototype instrument in 1989. The efficiency has been continually improved, and individual dye molecules of various colors can now be efficiently detected.

In the uses envisioned for the detector, molecules of compounds not naturally fluorescent would be made so by attaching a fluorescent dye or selectively binding a fluorescent tagging molecule to them. The system is similar to a flow cytometer, which identifies cells or cell components in liquid by detecting the cells' fluorescence as they flow through a laser beam. But even the best commercial flow cytometers fail to detect cells whose brightness is less than that of a

**Newton Seitzinger worked on the Single Molecule Detector while he was a postdoctoral fellow at the Laboratory.*

†Lloyd Davis collaborated on the work on single molecule detection during three summers at Los Alamos.

‡ Steven Soper worked on the Single Molecule Detector while he was a postdoctoral fellow at the Laboratory.

For more information about the Single Molecule Detector, please contact Dr. Ronald E. Barks, Industrial Applications Office, Los Alamos National Laboratory, P.O. Box 1663, Mail Stop M899, Los Alamos, NM 87545, Telephone (505) 667-3839.

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November 1991

thousand fluorescent molecules. The new system is thus 1,000 times more sensitive than a conventional flow cytometer.

The key to the detector's sensitivity was to find a way to sort out the molecule's glow from the scattered laser light in the background. The researchers accomplished that by using extremely short pulses of laser light; the laser pulses 82 million times a second. Because the dye molecule takes a few billionths of a second to react, the flash of the laser fades before the molecule glows. To take advantage of this characteristic, the photodetector is "time-gated"; that is, its "gate" closes when the laser turns on and opens when it turns off. Because the molecule's fluorescence lifetime is shorter than the time it spends in the laser beam, the process is repeated many times, so that each molecule identifies itself in a brief burst of fluorescent photons as it passes through the beam. The photon detector's computer uses a new signal-processing algorithm to isolate these weak signal bursts.

Applications

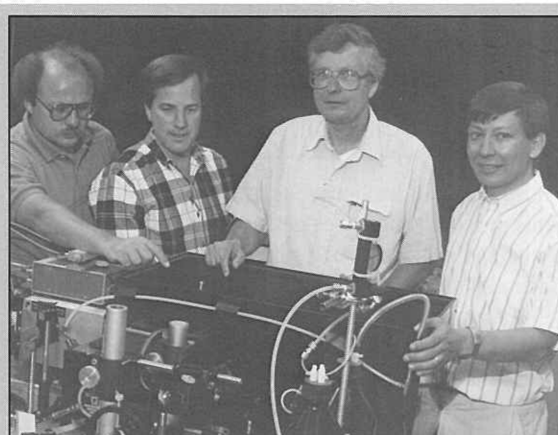
A Los Alamos group now holds a patent for applying the technology to DNA sequencing for the Human Genome Project. Life Technologies Inc., a Maryland biotechnology company, has signed a Cooperative Research and Development Agreement with the Laboratory to pursue the DNA sequencing application.

Some applications of the detector will require the development of new fluorescent tagging dyes and chemistries. But the inventors believe the availability of the Single Molecule Detector will stimulate such developments, which will in turn expand the detector's versatility.

E. Brooks Shera, of the biophysics group in the Physics Division, began developing numerical simulations to model the Single Molecule Detector in 1989. When the measurements matched the simulations, the researchers were confident they were on the right track. Shera has long been interested in sensitive measuring devices. For 10 years he was principal investigator for a program in which muonic atoms were used to obtain precise measurements of the shapes and sizes of nuclei. Before that he studied nuclear level structures and made precision energy measurements of high-energy gamma rays. He earned his Ph.D. in physics at Case Western Reserve University. After a two-year postdoctoral appointment at Argonne National Laboratory, he came to Los Alamos in 1964.

Also contributing to the development of the Single Molecule Detector was Newton K. Seitzinger, a postdoctoral fellow in chemical and laser sciences from 1987 to 1989. His Ph.D. in chemistry was awarded by Purdue University. He is now with the U.S. Pharmacopeial Convention, Inc., a private, nonprofit organization that sets standards for pharmaceuticals.

Lloyd Davis, professor at the University of Tennessee, spent the summers of 1989, 1990, and 1991 collaborating on the single molecule detection work. Davis earned his degrees, including the Ph.D. in physics, at Auckland University in New Zealand. In 1985 he came to the University of Tennessee Space Institute as a research associate and in 1987 became a professor.



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Showing the Single Molecule Detector are (from left) Steve Soper, technician Mark Peters, Brooks Shera, and Lloyd Davis.

Richard A. Keller, of the Chemical and Laser Sciences Division, earned his Ph.D. in physical chemistry at the University of California at Berkeley in 1961. After a stint as assistant professor of chemistry at the University of Oregon, he joined the physical chemistry division of the National Bureau of Standards in 1963. He has been a staff member at Los Alamos since 1976, working in the areas of laser-induced chemistry, laser-induced isotope enrichment, and laser-based analytical techniques.

Steve Soper worked on the Single Molecule Detector for the Human Genome Project from 1989 to 1991 as a postdoctoral fellow in chemical and laser sciences. He is now a chemistry professor at Louisiana State University. Soper earned his Ph.D. at the University of Kansas.