Title
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Journal
Proceedings of the National Academy of Sciences of the United States of America, 110(52)

ISSN
0027-8424

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Publication Date
2013-12-01

DOI
10.1073/pnas.1321731111

Peer reviewed

RETROSPECTIVE

Founding father of FACS: Professor Leonard A. Herzenberg (1931–2013)

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The stunning ability to collect quantitative single-cell analyses of up to 20 parameters simultaneously (1), at rates of tens of thousands of cells per second, and then to divert and recover a specific single live cell, or a subpopulation of live cells, with desired characteristics is the genius of fluorescence-activated cell sorting (FACS). FACS applied in conjunction with fluorescently labeled monoclonal antibodies (Mabs) derived from immortalized hybridoma cell lines is a ubiquitous core technology in medicine and biomedical research, impacting all of immunology, cancer diagnosis, stem cell identification and transplantation, AIDS monitoring, and a variety of therapies, as well as plant and microbial biology. It is, in fact, very difficult to imagine the biomedical research landscape of the last 40 years without FACS.

The father of FACS, Leonard "Len" A. Herzenberg of the Department of Genetics at the Stanford University School of Medicine, died on Sunday, October 27, 2013 at the age of 81. Len will be remembered as a deeply innovative scientist, a biotechnology founding figure, a life-long mentor and gentle teacher, an early and effective proponent of diversity in science, and a committed social philanthropist. He is survived by his wife, Leonore "Lee" A. Herzenberg, also of the Stanford Department of Genetics, their children Berry, Jana, John, and Rick, four grandchildren, and an international extended family of former students and postdocs whose lives and careers he and Lee touched and enriched. Len and Lee were a unique team, their partnership transcending essentially all of the humdrum drudgeries of laboratory management and academic piffle for more than five decades. Len's contributions were all shared with Lee, and they were each other's staunchest champions and toughest insider critics (2).

FACS, in conjunction with fluorochrome-labeled Mabs detecting cell surface and intracellular antigens, is most often used to classify normal and neoplastic cells to distinguish functionally distinct subpopulations of immune system cell types, to identify alterations that occur in blood as immunological dysfunction progresses, and to quickly monitor changes during a variety of therapies. FACS can also be used to select specific cells internally expressing fluorescent proteins as a measure of gene activity over developmental and differentiation time frames. Perhaps surprisingly, and certainly less well known, are the important contributions made by FACS in the typing and analysis of bacteria associated with humans, animals, and plants and in understanding microbial diversity in environmental samples isolated from lakes, oceans, and mines. It is difficult to find an area of basic or applied biological research in which cell sorting and analysis with flow cytometry instruments is not a key technology. Not surprisingly, flow cytometry instruments are now produced by a variety of manufacturers, and tens of thousands of machines are currently in use around the world, making this technology a cornerstone of all of modern biology.

Len was born November 5, 1931, in Flatbush, Brooklyn, where he grew up, attended Brooklyn College, and met his future wife. Len fled to Pasadena and Cal Tech, where he received his PhD, and after a short period of separation and long-distance phone bills, he and Lee married. Lee became, in essence, the first female to earn an undergraduate degree at Cal Tech, acknowledged by signed letters from her various course instructors detailing her stellar academic performance. Len and Lee were then off to Paris, where Len was an American Cancer Society postdoc with Jacques Monod, studying the induction of Lac permease. After a short stint at the National Institutes of Health, Len was lured to Stanford as a founding member in Joshua Lederberg's new Department of Genetics in the newly opened Medical School, recently decamped from San Francisco to Palo Alto. Len never left, and his papers from that earliest era show a decided interest in the genetic control of immune cell function.

Len and Lee Herzenberg with a FACS, circa 1985.

Len quickly recognized the need for high-throughput, quantitative fluorescence measurements in the early 1960s. Frustrated with his own poor eyesight and the hours spent staring at cells under the fluorescent microscopes of the day, Len capitalized on advances in flow sorting of cell-sized particles made by Mack Fulwyler at the Los Alamos National Laboratory in conjunction with oscillatory ink-jet printing techniques developed by Richard (Dick) Sweet at the Stanford Electronics laboratory and later at Varian Associates and Xerox. Len seized on the visionary idea to build an instrument capable of identifying and rapidly sorting live cells with distinctive surface molecules detected by fluorescently tagged antibodies. Well before "interdisciplinarity" had achieved its current status as an abused buzzword, Len recognized that disrupting technological boundaries required innovative scientists from a variety of disciplines. He assembled and deployed a team of engineers, eventually including Dick Sweet, physicists, and computer scientists, and put the first FACS into

Author contributions: J.L.D. and L.L.L. wrote the paper.

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[2]J.L.D. was an undergrad and then doctoral student in the Herzenberg laboratory from 1978 to 1986 working on T-cell ontogeny and generation of chimeric immunoglobulins.

[3]L.L.L. began his collaboration with the Herzenbergs as a postdoctoral fellow at the University of New Mexico and subsequently began his independent career as a Principal Investigator at the Becton Dickinson Monoclonal Center in Mountain View, CA.

www.pnas.org/cgi/doi/10.1073/pnas.1321731111

action just before 1970 (3, 4). One of these earliest FACS machines is now in the Smithsonian Museum.

However, Len remained frustrated by the capriciousness of comparing the results of immunofluorescence studies performed with one polyclonal antisera to those resulting from the use of another antisera, because there was never any guarantee that these reagents would detect the same epitopes with the same affinity. Furthermore, polyclonal antisera are finite—when the rabbit, goat, or horse serum is gone, that's it—you start from scratch with a new batch from a different source. Len, Lee, and graduate student and subsequent long-time collaborator, Vernon Oi, spent an auspicious sabbatical in Cambridge with Cesar Milstein in 1976, where they were among the first to reproduce the then brand new technique of fusing a single antibody-producing B cell to an immortal, nonproducing myeloma cell to create "hybridoma" cells capable of producing exquisitely specific Mabs forever.

Fluorochrome-coupled Mabs removed a key roadblock to widespread adoption of FACS because hybridomas are an inexhaustible supply of reproducible, highly specific reagents. This was combined with constantly evolving FACS tricks like dual, tunable dye lasers for excitation and single-cell cloning (5). Len and his team eventually extended the capabilities of FACS to encompass the simultaneous measurement of 11 different fluorescent markers and 13 total parameters, including light scattering properties, all at flow rates of several thousand cells per second (6).

Len and colleagues demonstrated the burgeoning utility of the FACS in landmark studies of cell biology and genetics of the mammalian immune system throughout the 1970s and 1980s. This began by using FACS and allotype-specific Mabs to define genetic differences in immune markers (7). Their early results included the first demonstration that membrane-bound and secreted immnuoglobulins were serologically and structurally distinct and likely encoded by alternatively spliced exons (8). Concurrently, many of the key cell surface markers that defined functional subsets or neoplasias of T and B lymphocytes were first identified using xenogeneic monoclonal antibodies developed by Len, Jeff Ledbetter, Robert Good, and other colleagues (9, 10). As parents of a son with Down syndrome, Len and Lee were driven, together with Diana Bianchi, to develop methods to isolate rare fetal cells from maternal peripheral blood, ushering in a safe method to detect a variety of fetal chromosomal abnormalities (11).

Today, the fruits of these efforts are visible in a wide variety of clinical applications, including $CD4^+$ T-cell monitoring in HIV patients, classification of leukemias and other tumors, tumor staging by cell cycle analysis or surface marker expression, isolation of stem cells for transplantation, and monitoring the progression of bone marrow transplants. The current therapeutic use of Mabs to treat autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and psoriasis, organ transplantation rejection, and cancer owes much to both FACS technology and to Len's pioneering role, with Vernon Oi and in collaboration with Sherie Morrison and Paul Berg, of molecular methods for producing the "humanized" chimeric Mabs that are central to many successful therapies (12).

Equally important was Len's visionary leadership of a thriving sector of the biotechnology industry. Len recognized very early that neither the FACS nor monoclonal antibodies would be useful for basic research or clinical applications unless both the FACS instruments and the reagents were produced commercially by companies capable of scaling up to meet demand. Together with Bernie Shoor, Len stimulated and oversaw the development of a competitive flow cytometry industry—being a founder of the Becton Dickinson Monoclonal Center—including commercial sources capable of delivering high-quality monoclonal reagents labeled with numerous fluorochromes for FACS use. His contributions in this area, begun in 1970, made Len one of the early pioneers in the modern biotech industry and, most importantly, marked him as a practical scientist whose work enabled fruitful research in countless laboratories throughout the world. Subsequent clinical studies that used these methodologies rested on Len's career-long attention, along with the Stanford FACS engineering team, to the development and deployment of FACS in the service of science and medicine. It is not hyperbole to suggest that Len's research has saved tens of thousands of lives and touched many more than that.

Len's career forever altered the landscape of biomedical science research and application, and he was duly awarded many honors, including election to the NAS in 1982 and culminating in the 2006 Kyoto Prize. The continuing development of FACS technology, including recent development of sophisticated software for storage and analysis of 20-parameter cell biology data, has not slowed, despite 45 years of constant progress. This reflects Len's magical ability to remain curious and engaged and to his talent for sparking curiosity and engagement in his team of engineers, physicists, and computer scientists. In a remarkable testament to that courage and creativity, many members of the FACS development team spent their entire careers in his laboratory. This loyalty to purpose and dedication to a long-term vision to change the world for the better is unique in modern biomedical science.

Len's career was marked by a gentle and refreshing outlook toward discovery and innovation. Len pursued knowledge with the joy of a child; each breakthrough was celebrated as if it were his first and therefore somehow miraculous. Len established a long line of former students, postdocs, and collaborators, ourselves included, who carry that joy into our own careers and lives. Len's desire to improve humanity through science, technology, and judicious reasoning set a high bar for all of us, and we try daily to honor Len by practicing what we learned from him.

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