The More We Learn

Leonard Arthur Herzenberg

At the award ceremony for the Kyoto Prize, I began by quoting an old Japanese proverb: "If you understand everything, you must be misinformed [Fig. 1]." In choosing this quote, I was not just being modest or diplomatic. I have always shied away from overly grand theories, all-encompassing ideologies, and the faith-based pursuit of some other-worldly revelation. My life's work as a scientist, teacher and political activist has been to find—and to help others find—facts that improve understanding, whether of the immune system, the genetic dangers of nuclear radiation, or the loudly proclaimed threat of weapons of mass destruction in some oil-rich country in the Middle East.

The Fluorescence Activated Cell Sorter (FACS), for which I am now being honored, grew out of this quest. I built it, and perfected it over the years, because my colleagues and I needed an accurate, factual way of describing the properties and functions of cells. Now, as many of you know, it is broadly used by medical and biological scientists to identify and characterize animal and plant cells. Others can speculate about the nature of life; my colleagues and I want—and need—hard data. Like Sgt. Joe Friday in the old television detective series *Dragnet*, our driving interest remains "Just the facts, Ma'am. Just the facts."

Theories such as Albert Einstein's monumental discovery of relativity, or Charles Darwin's equally powerful view of natural selection, play a vital role in guiding our search for these facts. But we repeatedly use the facts we find—the observable data from the real world—to test even our most cherished theories. And because, as sometimes happens, our old theories can no longer explain all the facts we have found, we must be prepared to adopt new theories that offer more productive and predictive explanations.

This is the way, the Tao, of science. A similar focus on hard data should also,

in my opinion, guide the choices we make in our personal and public lives. I still remember battling Cold Warriors like Herman Kahn, who insisted that fall-out shelters would protect us so we could defeat the Soviet Union in a nuclear clash. Ignoring basic genetics, these "deep thinkers" with loud public voices completely downplayed the threat that continuing radiation even quite distant from the bombsite would pose to anyone who survived the initial blast. They were simply wrong, and I said so. Later, I saw the proof of my argument in agonizing detail when I served on the Atomic Bomb Casualty Commission studying the survivors of Hiroshima and Nagasaki. Facts are facts, no matter what nonsense our political, business, and religious leaders try to sell us.

I came to this tough-minded approach early in life. I was born in Brooklyn, New York, a very hardheaded place. My parents were second-generation immigrants from Russia and Eastern Europe. My father worked in a clothing store, first as a salesman and then as a manager. My mother worked as a legal secretary. Hard times had forced her to leave high school in her first year, but she was a very bright woman and educated herself. From her, I gained a love of the schooling she never completed, and a passion for good books, which she never stopped reading.

I also learned to rely on myself and my own judgment. For a short while during World War II, my parents were both working long hours and they sent me to a boarding school in upstate New York. I was only 10 and 11 years old and still had weekly appointments with my orthodontist, who was in Brooklyn. So every Friday I took the train by myself from the school to Grand Central Station in Manhattan, found the right subway train to get to Brooklyn, got off the train at the right stop, and walked several blocks to the dentist's office. After the appointment, I walked to the clothing store where my father worked. Doing this—navigating the big city transportation systems without ever getting lost—gave me the kind of security in my own ability to do what is necessary, which later gave me the courage to embark on the FACS development project, even though I was not in any way an engineer.

I never really liked the boarding school, so after a while my parents put me back in my neighborhood school, P. S. 99, and hired a nanny to look after me when I

came home. I soon convinced my parents that I didn't need the nanny and that I was perfectly capable of taking care of myself. I made my own snack when I came home from school, and on most days I walked to the nearby public library, where I felt secure and happy reading every book that the librarian let me check out. She sometimes suggested books for me; other times, I picked them myself. Often, I checked out my limit of five or six books and took them home to read. My favorite books were non-fiction, which taught me about the world. My interest in biology and related fields grew from the science books I read and the way I used what they taught.

When I started high school, my friends and I formed a chemistry club, which met several days a week, mostly in my basement. Morris Meister, the father of one of the boys, had been a chemistry teacher before becoming a local high-school principal and going on later to become a well-known principal of the Bronx High School of Science. Dr. Meister gave us all kinds of interesting chemicals to work with, making it possible for us to learn to make perfumes and food smells by mixing various acetates, including isoamyl acetate, which smelled like bananas. He joined us for some of the meetings, but mostly we learned from experimenting ourselves with the chemicals we had and from reading chemistry books and manuals.

In those days, we could go to any of a number of chemical supply stores to buy whatever we didn't have. We bought solid sodium in oil, which we knew would burn and explode in water. We cut the sodium into little pieces with a kitchen knife, put them into a dry bottle with a loose cap, and tossed it into the storm sewer. As we expected, the water got into the bottle and created a nice little explosion. We then got braver—or more foolish—and put bigger pieces of sodium into the bottle. Those explosions sent water gushing up from the sewer on the opposite side of the street. Fortunately, no one got injured, but our parents yelled at us and told us to stick to less dangerous chemicals.

Most of my formal education came from the New York City public schools, which were truly excellent. Throughout high school, I took all the math and science courses I could, but I have to admit that I was never a top student. It took the more independent and challenging college curriculum to get me going. To attend any public

university in New York City, I needed only to do well in the College Board examinations, which I did. I then decided to go to Brooklyn College, which was essentially free and just across the street from my high school.

The Brooklyn College faculty was outstanding. I found their math and science courses much more interesting and got straight A's in them. The biology and chemistry professors also sponsored extracurricular student organizations. In my first semester, I joined the chemistry club and the Society of Biology and Medicine, whose members were mostly World War II veterans using the G.I. Bill of Rights to study pre-med, intending to become medical doctors. They paid dues, but rarely came to meetings. Those of us who did attend were more interested in biology than medicine.

Brooklyn College had no dormitories; the students were all commuters. Some came on buses or subways, while I rode my bicycle to college every day, carrying all my books along with a raincoat or heavy winter clothing. Happily, the Biology Department had given our Society of Biology and Medicine a greenhouse full of ornamental plants and flowers for the college's beautifully maintained gardens. So I had a warm place to meet my friends and park my belongings when I went to class, either during the day or in the evenings.

Early on, I got elected to head the Society's program committee, which allowed me to choose speakers for our weekly meetings. I kept this position for the entire four years of college and was able to invite a long list of excellent, well—known, and sometimes famous scientists from the many fine universities in the NYC area. As program chairman, I got to introduce the speakers before their talks and, very important for me, to take them to lunch with our faculty members. I was really thrilled to be able to spend time talking to these famous scientists, and to listen to them talking to our faculty—all paid for by the club dues.

The Society offered other advantages as well. We sponsored parties to which we invited potential recruits, including girls! At the end of my senior year, just as I was getting ready to leave Brooklyn and become a graduate student at Caltech I met Leonore (Lee) Adlerstein, who was just starting her first year at the college [Fig. 2]. A

year and a half later, I married Lee, changed her name to Herzenberg, and over the years watched her—even helped her—become a leading immunologist. We have happily and productively collaborated in science and life for some 53 years, and she has participated fully in most of the work for which I am now being honored. I am sorry that the Kyoto Prize committee could not include her as winner along with me.

At Brooklyn College I had many good teachers, but two were particularly important to me. Professor Seymour Fogel [Fig. 3] had studied the genetics of corn (maize) at the University of Missouri, and was continuing his research in the heart of Brooklyn, in an urban cornfield next to the college. Early in my freshman year, I asked him if I could do some experimental research with him. He had his own collection of dry corn seeds, and suggested that I study the rate at which different seeds took up water.

This was a good undergraduate project because I would not need any fancy equipment: just a few small dishes; some filter paper I could moisten; a balance on which I could weigh the seeds; and some graph paper on which I could plot a series of curves to show how much water each of the seeds absorbed over time. As I pursued the work, I learned the importance of measuring, recording and analyzing quantitative data. I also saw that there was no correlation between visible characteristics of the seeds, such as color, and the amount of water they took up.

In addition, in doing this project, I learned how experimental research often leads in completely unexpected directions. In the moist environment of the dishes, my seeds naturally became contaminated with bacteria. Since I was taking a biology course at the time with a professor who had been a co-discoverer of the antibiotic streptomycin, I asked him if it might inhibit the bacterial contamination. He gave me some and it worked to kill the bacteria.

This was interesting, but the surprise came when I completed the experiment and planted the seeds in our college greenhouse. Growing up in the big city, I had never seen how corn seedlings grew and I was curious to find out. The shoots came up green, but a number of the seedlings had white stripes. I showed them to Dr. Fogel and he

agreed that they were highly unusual. Looking under the microscope, I saw white chloroplasts along with the normal green ones. I also found that the white stripes grew from individual cells whose chloroplasts the streptomycin had bleached.

What a discovery I had made! Streptomycin caused mutations in the chloroplasts. I went to a scientific meeting to present my results, only to discover that a Swedish Nobel laureate had just published "my finding." I was disappointed that I was not the first to publish, but I had reproduced what a famous scientist had just found. That wasn't so bad for an undergraduate! It really turned me on. I worked all through college in Dr. Fogel's cornfield and with his seeds. That's how I learned to do independent research.

The other teacher who greatly influenced my college career was a very bright and interesting woman, Professor Priscilla Pollister [Fig. 5]. Every spring semester, she taught invertebrate zoology and allowed me to be her teaching assistant. The course consisted of examining and learning about all kinds of animals without backbones. I will never forget how colorfully she told us how she had gone to Monterey, California, where she saw the specimens that the famous American writer John Steinbeck had collected for Doc Ricketts, a real character whom Steinbeck immortalized in the novel *Cannery Row*. The glass cabinets in her classroom housed lots of specimens she had brought back from that visit. They were housed in jars with labels written by hand by John Steinbeck.

I stayed as Dr. Pollister's teaching assistant for the four years I was at Brooklyn College. In the last year, when I was considering where I should go for a Ph.D., she urged that I apply to Caltech in California, which would be ideal for my interest in the developing field of molecular biology. My parents and their friends would have preferred that I remain on the East Coast and go to Harvard, which they knew much better and which was closer to home. My family also worried about the earthquakes in California, fearing that I might fall off the end of the earth. Dr. Pollister, Dr. Fogel and others gave me very strong recommendations. Caltech accepted me immediately, as did Harvard. To my parents chagrin, I chose Caltech.

At Caltech [Fig. 5], I was thrilled to have the opportunity to talk with great scientists such as Linus Pauling, George W. Beadle and Alfred H. Sturtevant. I was assigned a desk in the laboratory of Dr. Herschel Mitchell, who talked to me about genetic studies with *Neurospora* (a common bread mold that grew on very simple salt media with only one vitamin, biotin, and an energy source like a sugar.) Mitchell suggested I start by studying a maternally inherited *Neurospora* mutant called "poky" because it grew slowly. This work led eventually to my thesis, "Studies on a cytochrome-destroying system in *Neurospora*", which I completed in 1955.

Next, I went to do my first postdoc at the Pasteur Institute in Paris, where I worked with the molecular biologist Jacques Monod [Fig. 6]. Some of my work there contributed in a small way to a fundamental discovery in gene regulation for which Monod, François Jacob and Andre Lwoff later won the Nobel Prize.

After about a year and a half in Paris, I got my draft notice, which ordered me to report for two years of compulsory military service. With the Cold War escalating, the United States Army wanted me to carry a rifle for my country. I preferred to carry a pipette by finding a position in the United States Public Health Service. I ended up working for two years with Dr. Harry Eagle at the National Institutes of Health (N.I.H.) near Washington D.C.

Eagle and his group at the N.I.H. had developed methods and media for growing mammalian cells in culture. I decided to use this technology to study the genetics of mammalian somatic cells, much the way I had been studying *Neurospora* and bacterial genetics. Although I initially used drug resistance markers in these studies, the work soon led me to explore the use of H−2 antigens and other cell surface markers. This, in turn, took me directly to the studies that led to the invention of the Fluorescence Activated Cell Sorter.

My experiments with mammalian cells were largely inspired by the work on bacteria for which Joshua Lederberg had won the Nobel Prize. So, I was doubly delighted when Dr. Lederberg took an interest in my work and invited me to join the new genetics department he was founding at the Stanford University School of

Medicine. The medical school was moving from San Francisco to the Stanford campus in Palo Alto, and was attracting a stellar faculty, making it the most exciting research and teaching school in the country. My new colleagues would include Joshua and Esther Lederberg, the Nobel laureate Arthur Kornberg and his entire department from Washington University in St. Louis, some of the top young Ph.D.s in the life sciences, and an exciting stream of visiting professors in Immunogenetics and other hot areas of genetics [Fig. 7].

Bringing my mammalian cell cultures to Stanford in 1959, I set out to pursue further studies of the H—2 antigens. I asked my wife Lee to make antibodies that would detect these antigens on our cells, thus solidifying our life-long collaboration. Among our first publications was an important paper by Lee showing the presence of anti—H—2 antibodies in female mice whose earlier litters had different H—2 antigens. This was the first finding of a human Rh—like system in mice. The medical significance was obvious. In humans, an Rh—negative woman who carried Rh—positive babies would become immunized to the Rh antigen. If in a subsequent pregnancy she had another Rh—positive child, her immunity would trigger a reaction against her baby's Rh—positive cells, causing a profound anemia known at the time as "blue baby syndrome." Lee's paper opened the possibility of using mice to do further research on the problem.

In other early studies at Stanford, we focused on the chemistry of the H—2 antigens and their location on the cell membrane, correcting the view of Nobel laureate Peter Medawar that H—2 was a DNA antigen. As part of this work, Lee and I attached a fluorescent molecule to antibodies that would stick to H—2 antigens and used these antibodies to make cells with surface H—2 antigens visible with a fluorescence microscope. This was a technique pioneered by Albert Coons at Harvard in the early 1940s. Straining my weak eyes to see the fluorescent cells one by one, I realized that it would be a lot quicker and easier to identify and sort millions of cells with a machine [Fig. 8, 9].

My search for such a time-and-eye-saver took me to the Los Alamos National Laboratory, where my country had developed the atomic bombs that we dropped on Hiroshima and Nagasaki and was (belatedly) working on determining the effects of

radioactive fallout. I had heard that scientists at Los Alamos had created a machine to measure and sort particles in the lungs of animals they had sent up in balloons into the mushroom clouds created by above-ground atomic bomb testing. Indeed, when I arrived at Los Alamos, I found that Mack Fulwyler and his colleagues had developed an efficient volume analyzer and sorter for such particles, many of which were in the size range of mammalian cells.

I was delighted that such a machine existed and asked Mack and his colleagues if they would add fluorescence detection to the machine, so that it could also make useful and interesting biological measurements on cells. They replied that this would be beyond their "mission." Finally, after a full day of discussion, they agreed to let me take plans of their machine back to Stanford where I could get some engineers in our department to do whatever I wanted with it.

Adding a fluorescence capability and making several other necessary changes to the Los Alamos machine turned out to be substantially more difficult and more expensive that I had hoped. But eventually my colleagues and I succeeded in turning this machine, which had been built as part of the world's most destructive enterprise, into a powerful force for healing [Fig. 10, 11]. If science requires independent thinking, it also depends on some very strange collaborations.

Much of my work in science, in fact, has involved collaborations with colleagues whose interests, sometimes quite different from mine, have opened the way to studies that neither they nor I could have done independently. This, too, is part of the Tao of science, and is very much a part of the fabric of my life as a scientist.

Looking back at my list of publications over more than a half century, I am reminded of the many collaborators with whom I have had the privilege of working, starting with Lee, who began working with me at Caltech and has worked with me—or I with her—ever since [Fig. 12]. Although we have separate research interests, our approach to these is usually so interactive that more often than not we wind up as coauthors on each other's papers. Similarly, in teaching, in collaborations and in doing the day-to-day work of running a laboratory, we complement (and compliment) each

other much of the time.

Our life, of course, has been enriched by hundreds of co-workers, from graduate students and medical students to postdoctoral fellows, engineers, computer scientists and physicians. Teachers are best remembered by the accomplishments of their children, their students and the students of their students. Lee and I take enormous delight in the accomplishments of more than three intellectual generations, and enjoy many of them and their families as friends [Fig. 13, 14].

Of course, we also take great pride and enjoyment from the accomplishments of our four children [Fig. 15]: Berri, who runs a bicycle shop and education project of bicycle maintenance and safety; Jana, a singer/songwriter who runs a small but prestigious jazz record company; and our youngest, Eric, who is and apprentice carpenter and an accomplished potter. Michael is our special Down syndrome child who lives in a group home and does simple tasks in our lab. Megan Phillips, an adopted granddaughter, who was raised in our home, now works at the lab and is on her way to becoming a scientist. In addition, as with our students, fellows and children, we take great pleasure in the successes of the Fluorescent Activated Cell Sorter, or FACS as we and many others call it, that have made this instrument central to so many aspects of modern science and medicine [Fig. 16].

In our own laboratory, we have ventured into a number of medical areas where FACS is important. In HIV/AIDS, where FACS is used to chart the progress of the disease by measuring the loss of helper T cells, we have helped characterize the loss of these cells and the oxidative stress that occurs as the disease progresses. In cancer, where the use of FACS to distinguish and monitor tumors has enabled cures in diseases that used be death sentences, we have largely done pre-clinical work but have collaborated in bone marrow transplantation studies. With cystic fibrosis, we have identified a major cause of lung damage and are currently carrying out clinical trials that promise to extend life expectancy and improve quality of life. And, by continuing to develop and improve the FACS, we have made it possible to characterize and isolate the human embryonic stem cells that may one day alleviate so much disease and suffering—provided, of course, that this productive line of research is allowed to continue

unimpeded by the faith-based politicians who would kill it.

As many of you know, President Bush and some of his supporters have declared that scientists no longer need to take stem cells from aborted human embryos, but can rely instead on strains already collected. Mr. Bush is simply wrong. Almost all of the existing strains are damaged, and none of them can be transplanted. Facts are facts, and no amount of religious certainty can change them. Either we take stem cells from human embryos, or we condemn untold numbers of us to remain paralyzed for life, endure terrible pain, or die preventable deaths. Mr. Bush and those who agree with him can refuse for themselves any treatment that comes from stem cell research, if this is what their God demands. But they have no God-given right to condemn the rest of us to needless suffering!

When I was a young student blowing things up with my chemistry set or learning the mysteries of corn seeds and fruit flies at Brooklyn College, I might have indulged the fantasy that science would, in time, lead us to understand everything. But now I hope I know better. As our knowledge expands, and as we develop new technologies that we cannot now imagine, our students and their students will always find more questions than they can answer. As scientists and teachers, ours is an unending, ever—shifting, existential quest, with none of the cosmic certainty that religious zealots seem to crave. We will never understand everything, but we must never stop trying to come as close as we can.

This is the joy and the strength of science, which we must once again—and forever—defend against the irrational attacks of those who think they already know the Ultimate Truth.

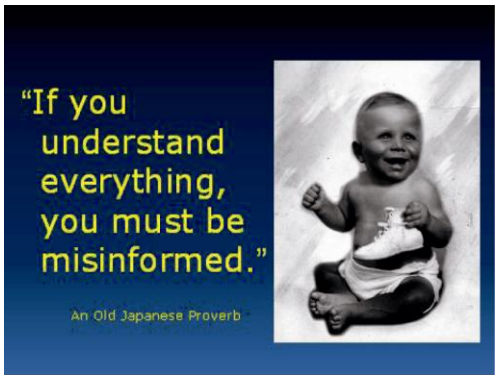
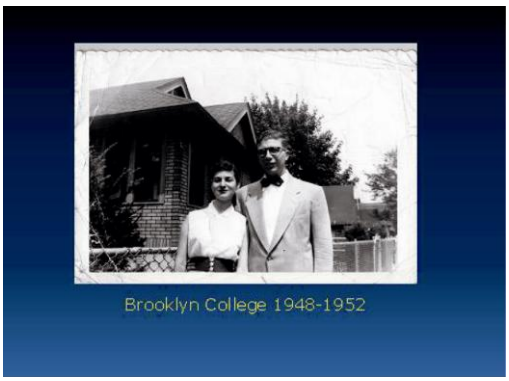


Fig. 1



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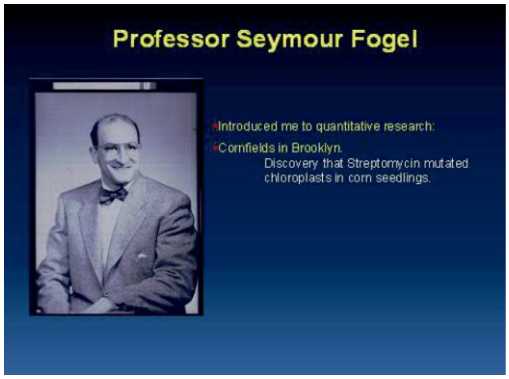
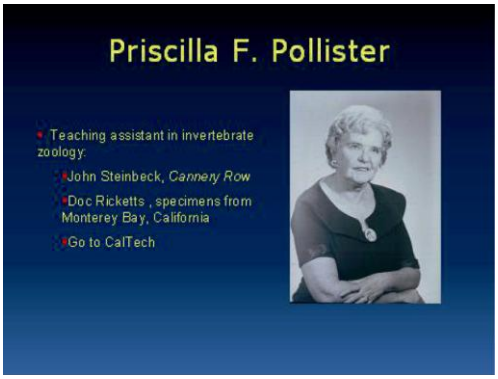


Fig. 3



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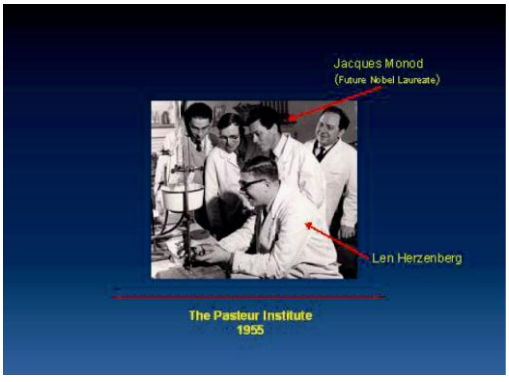

Fig. 6

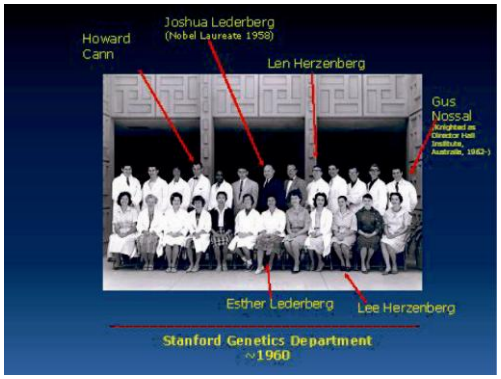

Fig. 7

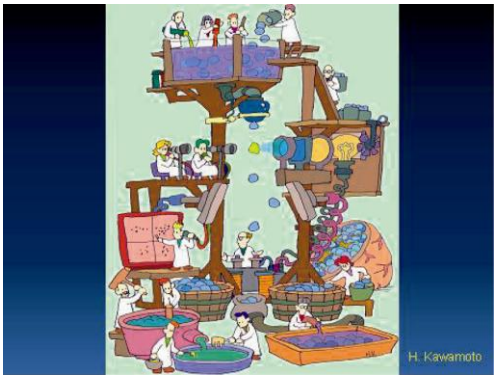

Fig. 8

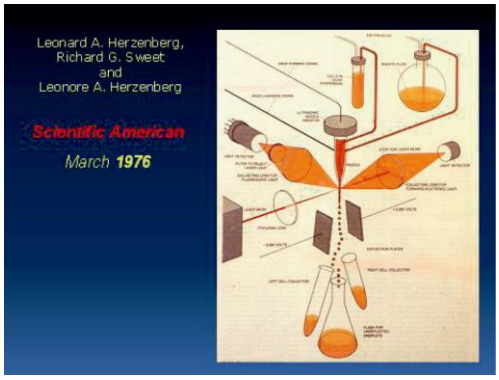

Fig. 9

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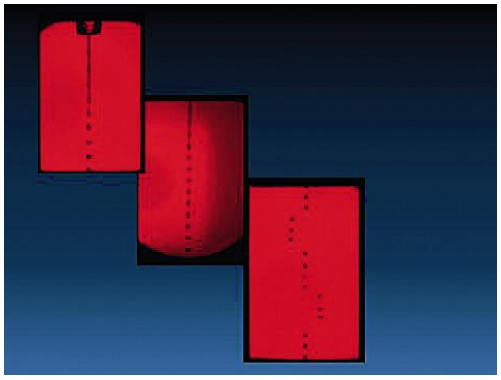

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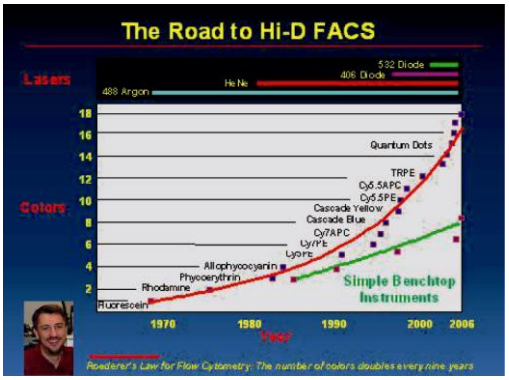

Fig. 16