

CYTOMETRY FOR LIFE



Changing Lives
Through Low-Cost Diagnostics
www.cytometryforlife.org

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What is the *Cytometry for Life* Program?

Across Sub-Saharan Africa millions of AIDS (Acquired Immune Deficiency Syndrome) victims are desperately in need of antiretroviral treatment. The measurement of the CD4 T-lymphocyte cell levels in the blood of AIDS patients is a critical step that enables AIDS patients to become eligible for therapy. Major gaps exist in the availability of affordable CD4 testing capabilities for the majority of HIV-positive Africans. The *Cytometry for Life (C4L)* program aims to mitigate this problem by providing low-cost CD4 T cell testing devices for HIV/AIDS patients in areas plagued by the AIDS epidemic that do not have sufficient access to affordable CD4 testing capabilities. Current costs for CD4 testing range from 10 to 12 USD; we propose to decrease this cost to 0.25 to 0.50 USD per test by using innovatively engineered and robust cytometry devices designed to measure only CD4 levels in terms of absolute count and percentage. Such an initiative is critical since approximately 30 million people are infected in Sub-Saharan Africa and almost 320 million Africans exist in extreme poverty, living on less than \$1 a day (http://devdata.worldbank.org/wdi2005/Section1_1_1.htm). Therefore, it is critical that the future of cytometry devices for CD4 measurement in Africa incorporate low-cost options.

Why *Cytometry for Life*?

The motivation for Purdue's *Cytometry for Life (C4L)* program grew out of a call to action by Mr. Stephen Lewis, the UN Secretary General's Special Envoy to HIV/AIDS Relief in Africa, when Mr. Lewis spoke at the 23rd Congress of the International Society for Analytical Cytology (ISAC) in Canada in May, 2006. Mr. Lewis indicated that one major problem (among many) in caring for infected patients in Africa was the lack of low-cost CD4 cell testing technologies.

Flow cytometers designed for highly industrialized health care environments are the same machines currently being used in developing countries. These instruments are multifaceted and were designed to perform 50 to 100 different tests. In addition to CD4 analysis, such cytometers have the capacity to perform complex multivariate analysis, link to advanced communication systems, and are often fully automated. However, in order to respond



Dr. J. Paul Robinson (left), *Cytometry for Life* Founder/President of the International Society for Analytical Cytology (ISAC) and **Mr. Stephen Lewis** (right), the UN Secretary General's Special Envoy to HIV/AIDS Relief in Africa, author of *Race Against Time*, during the 23rd Congress of the International Society for Analytical Cytology (ISAC), Québec City, Canada, May 2006

to the AIDS crisis across Africa, *Cytometry for Life* is introducing a new breed of cytometer that only measures CD4 T lymphocyte levels. In the *C4L* approach, the cytometer device functionality is targeted to one overarching objective: **AIDS epidemic mitigation via CD4 monitoring to facilitate antiretroviral treatment procedures**. This

targeted single-function approach is the primary means by which *Cytometry for Life* is able to offer such medical innovation with a significantly reduced cost while maintaining the highest standards of quality in terms of the CD4 testing procedure and result generation.

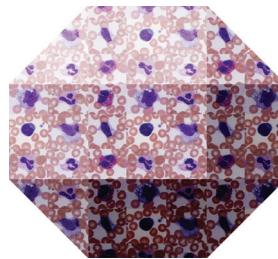
As HIV/AIDS ravages nations in Africa, Asia, Latin America, and the Caribbean, it is critical that scientists or supporters of international health and development initiatives contribute to the provision of innovative testing capabilities. Such initiatives must respond specifically to the need for accurate, low-cost CD4 measurement tools that can operate optimally in remote locations, in a timely fashion.

The *Cytometry for Life* CD4 Counting Device

The instrument for the *Cytometry for Life* program is designed solely to measure CD4 levels in absolute count and percentage of T cells. Cytometer instrument costs and individual test costs are minimized because the machine does not include complex automated fluidics or optics systems. For example, *Cytometry for Life* has designed an integrated module for the optical and fluidic assembly. The electronics are composed of minimal but very powerful chips. There is no separate computer attached or needed with this technology and therefore it does not require software integration. A USB port has been added to extract data should there be a need to do so. The instrument is low maintenance and does not require highly skilled technicians to operate. The *Cytometry for Life* CD4 counting instrument is portable and is

Lewis spoke of CD4 machines--which measure the number of CD4 immune cells and indicate whether someone with AIDS should be treated--that are bulky, difficult to use and, at about \$50,000 each, vastly out of reach for most of the continent.

-Apoorva Mandavilli, *Nature Medicine* volume 12: number 10 , page 1107 October 2006



Significance of CD4 cell counts in HIV/AIDS

CD4-positive T helper cells are a sub-group of lymphocytes (a type of white blood cell or leukocyte). A CD4 count is used to monitor immune-system function in HIV-positive individuals. A declining CD4 T-cell count is associated with progression of HIV infection. In HIV-positive patients, AIDS is officially diagnosed when the count drops below 200 cells/ μL . Low CD4 counts in HIV-positive individuals (< 200 cells/ μL) is a strong indicator that antiretroviral therapy should be administered.

Antiretroviral Therapy (ART)

refers to the treatment of retroviruses, primarily HIV, through usage of certain therapeutic antiretroviral drugs that act at different stages of the virus life cycle.



housed in a small, solid carrying case that includes all necessary reagents and the battery power source. The *Cytometry for Life* low-cost CD4 testing device is designed to have the capacity to operate in remote, intemperate, and humid regions and not rely on a constant source of electricity.

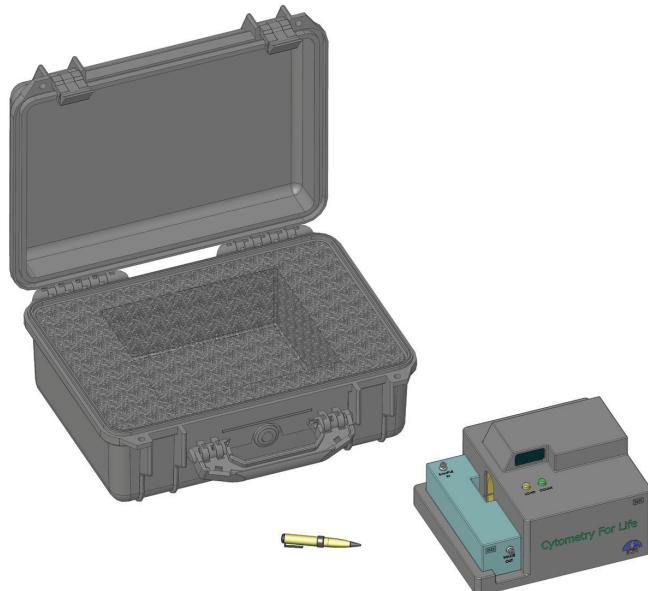
Cytometry for Life is collaborating with the Regenstrief Center for Health Care Engineering (RCHCE) and the Center for Advanced Manufacturing (CAM) at Purdue University to ensure that the *Cytometry for Life* low-cost CD4 testing device design can be manufactured in large quantities, at an economical price.

Cytometry for Life Program Approach

The *Cytometry for Life* program aims to achieve the following goals in two primary stages:

Stage 1:

- Identify appropriate CD4 device requirements for the African continent.
- Design CD4 measuring devices that are battery operated and computer independent so that they may be easily transported.
- Design a CD4 measuring device that will cost \$5000 USD or less per unit.
- Build these devices to be robust, very easy to use, and yet very high quality.
- Reduce the cost of a single test for CD4 to 0.50 USD or less.

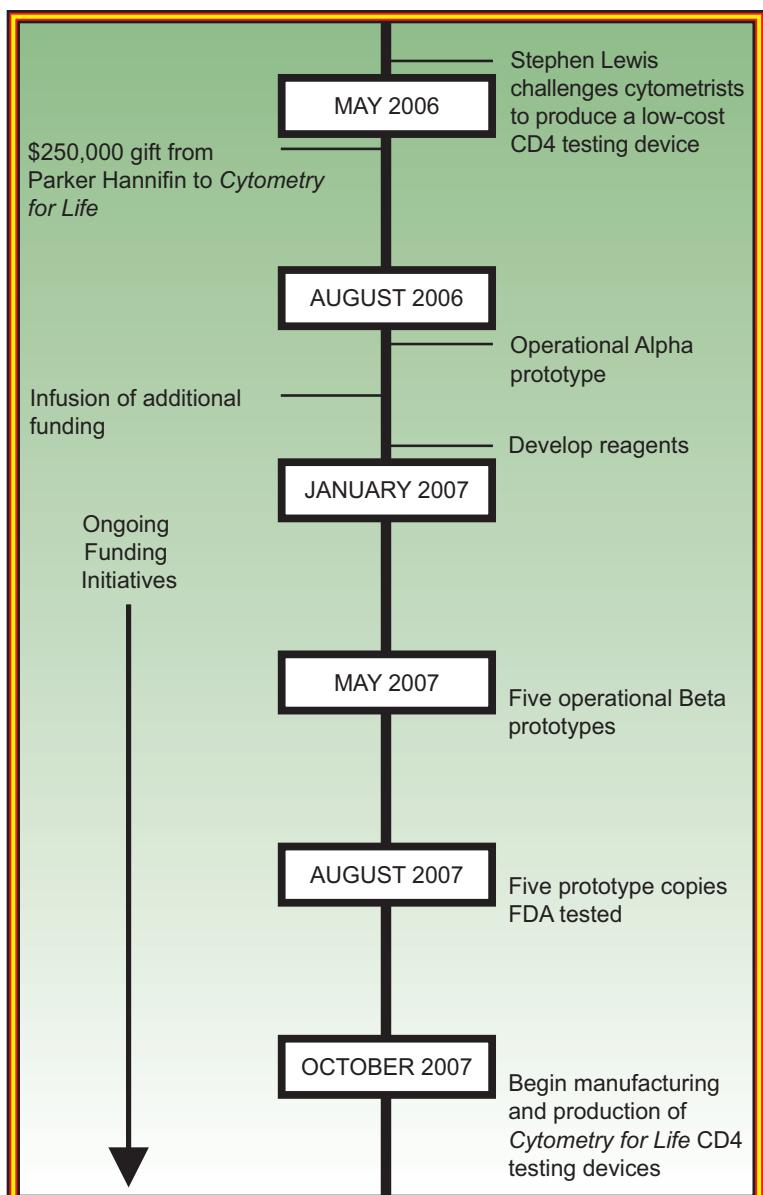


Stage 2

- Develop a manufacturing model for widespread distribution and management.
- Develop a reagent program that supports the instrumentation and functions in remote areas.
- Partner with manufacturing agents.
- Link with agencies to facilitate distribution to places of need and to provide long-term maintenance programs.

Lewis continues to speak against what he sees as “unconscionable neglect” and “reckless indifference” to AIDS. “I think the global community has responded lamentably,” he says, his voice rising. “We’re fighting for human lives, what in God’s name is wrong with these people?”

-Apoorva Mandavilli, *Nature Medicine*, volume 12: number 10, page 1107
October 2006



The Importance of Cytometry for HIV/AIDS in Africa

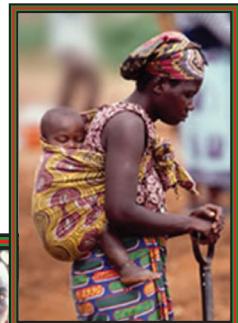
In Sub-Saharan Africa, there is a great need for antiretroviral therapy. Deficiencies in CD4 testing capacity remain a major barrier for the administration of antiretroviral therapy. The guidelines for antiretroviral therapy (ART) from the World Health Organization mandate therapeutic intervention for patients with Stage I or II HIV disease with CD4 cell counts less than 200/ μ l in Sub-Saharan Africa. This CD4 T-lymphocyte assessment is done by flow cytometry. Unfortunately, flow cytometry is a complex and expensive method in today’s high technology-focused world.

This problem can be solved. Cytometers can be made very inexpensively. With a 40-year history of technological development, cytometry has matured to a point that it can now be simplified for specific purposes, such as measurement of CD4 cells. While next-generation ideas are very important and have increased commercial opportunity for westernized healthcare networks, they are not the best solution for the immediate problem of evaluating AIDS patients in Africa today. The complexity of cytometers currently in use drives up the cost for the consumer, limits the number of instruments that can be purchased, and also increases the need for high-cost maintenance and highly skilled operators. Introduction of low-cost, robust, and portable machines through the *Cytometry for Life* program seeks to close gaps in CD4 testing coverage, particularly in rural African settings which demand innovative solutions in order to best service this target population.

Every day in Africa, . . .

HIV/AIDS kills about 6,600 people of whom approximately 1,400 are children

Almost 9,000 more are infected with the HIV virus (<http://www.data.org/whyafrica/checkthefacts/>)

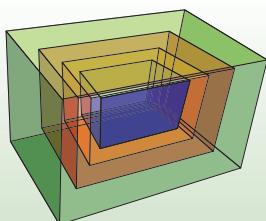


RURAL vs URBAN: What are the challenges of implementing CD4 testing capabilities in rural Africa?

UNAIDS estimates that over half the approximately 30 million people living with HIV/AIDS in Sub-Saharan Africa reside in rural areas. Poverty in Africa

is particularly onerous in rural areas. More than 70% of the continent's poor people live in rural areas. The *Cytometry for Life* program responds to a fundamental and urgent need for the introduction of low-cost, robust, and portable CD4 testing devices. This is especially critical in rural areas where CD4 testing capacity must still contend with exorbitant costs, inaccessibility, and a lack of trained personnel to operate and maintain equipment. While some current cytometry instruments are suitable for urban areas with sufficient infrastructure, rural/remote areas with moderate to poor infrastructure are still urgently in need of affordable, environment-appropriate CD4 testing solutions.

High-end options



Typical high-end flow cytometer used in clinical laboratory to analyze a variety of hematological conditions

Cost: \$100,000-150,000 USD

Analysis: Broad spectrum

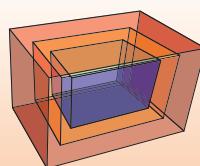
Not portable

High maintenance required

Highly trained technician needed for operation

Requires high-level infrastructure

Mid-Range Options



Mid-range flow cytometer used to analyze a variety of hematological conditions

Cost: \$30,000-\$50,000 USD

Analysis: Immunological (CD3, CD45, CD8, CD4, etc.)

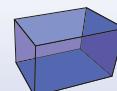
Portable

Moderate maintenance required

Highly trained technician needed for operation

Functions in infrastructure-poor areas with sufficient technical support

The *Cytometry for Life* "Low-Cost" option



Cytometry for Life single-task cytometer for monitoring CD4 levels in AIDS patients

Cost: \$4,000-\$5,000 USD

Analysis: Immunological (CD4 only)

Portable /enclosed in carrying case

Low maintenance required

Minimal training required

No infrastructure required

RURAL

- Low-throughput technology
- Nominal infrastructure
 - Unreliable electricity
 - No external quality control
- Lack of skilled technologists
- Patients are difficult to contact and follow up

URBAN

- High-throughput technology
- Relatively good infrastructure
 - Electricity
 - Filtered water
 - External quality control available
 - Secure laboratory
- A pool of skilled technologists

History of Cytometry

Cytometry is about the measurement of cells and their various environments. In AIDS, a virus takes over a very important cell, the CD4 lymphocyte. The cell is destroyed, the immune system is compromised and eventually, the patient cannot survive.

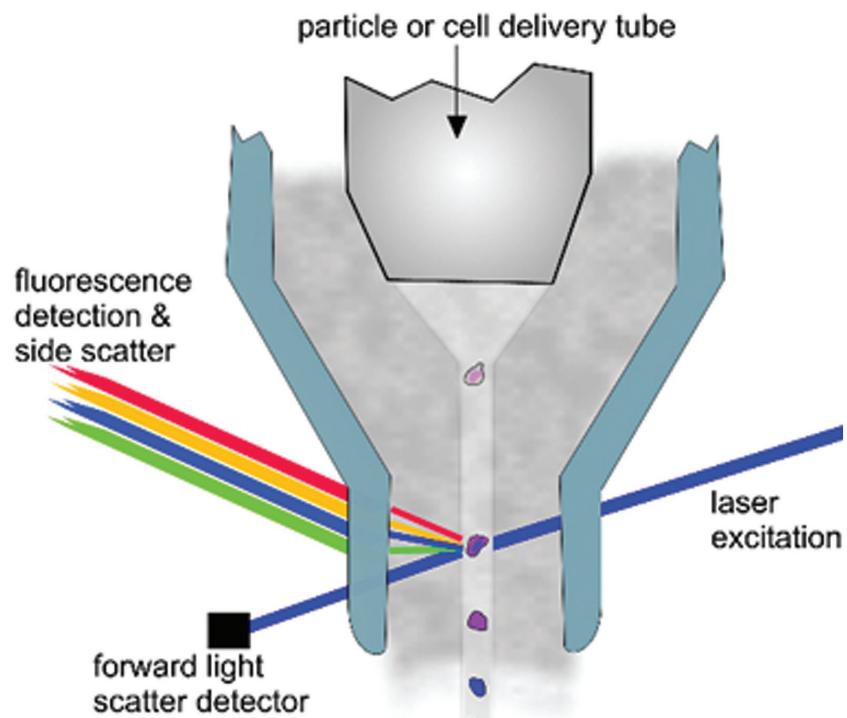
Cytometry is the field of science that began approximately 50 years ago with Wallace Coulter's cell counter invention, which is basically an instrument that counts cells or particles. Expansion of the original Coulter blood cell counter resulted in an instrument called the *Cell Sorter* that could count blood cells as well as sort them according to their physical and chemical characteristics. The *Cell Sorter* was invented by Mack Fulwyler in 1965. In the 40 years since the invention of the *Cell Sorter*, a revolution has occurred within the world of immunology, driven primarily by the innovations of Leonard Herzenberg, an immunologist and

recipient of the 2006 internationally renowned Kyoto Prize for his significant contributions to cytometry. Herzenberg's work catalyzed the maturation of cytometry as a powerful technology in medical research and clinical practice. Present-day expansions of Fulwyler's work, known as **Flow Cytometry**, are techniques for counting, analyzing, and sorting microscopic particles or cells that have been suspended in reagent or streams of fluid. This technology allows concurrent multiparametric analysis of chemical and/or physical characteristics of single cells flowing through an optical and/or electronic detection apparatus.

This technology is now used in all kinds of clinical applications that involve hematological abnormalities such as leukemias and lymphomas, as well as monitoring the status of patients with AIDS.

How does the flow cytometer work?

A laser beam (the blue line) passes through a flow chamber where a stream of fluid is flowing. Within the stream, the cells are flowing single file. These cells have different fluorescent labels on them. When a cell with an attached fluorescent molecule passes through the laser beam, photons are emitted. These photons are measured on detectors (see the different colors going out the back of the chamber). Using light scatter signals, it is also possible to determine morphological characteristics (shape, size, etc.) of each cell.



This is the key part of a flow cytometer-- you can see cells in the middle stream of fluid--the blue line shows the laser excitation; where it strikes the cells, a signal is sent out to several detectors that allows us to determine the identity of the cells based on their physical characteristics.

The Science of CD4 Testing

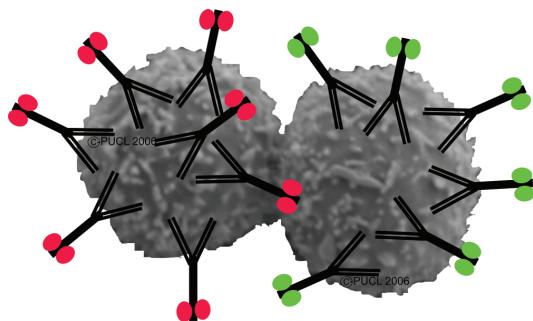
CD4 testing is a normal practice in the clinical laboratory, and is accomplished through the use of flow cytometry. *Flow cytometry* is a standard technique used in virtually every hospital and research institution around the world. While flow cytometry techniques are straightforward, they are not necessarily simple, particularly when applied to clinical practice. A great deal of automated technology is employed to gather measurements, analyze data, and produce reports. Consequently, the cost of performing these tests is relatively high.

How is a CD4 test performed today?

CD4 testing is performed by a flow cytometer. In this process, a small tube of blood cells is treated with a reagent which is specific to the CD4 lymphocyte (a type of white blood cell).



The reagents used for identification are called monoclonal antibodies (MABs). These antibodies can specifically attach to a certain type of cell. In this case, the antibody will attach to a CD4 T cell. Certain molecules (called fluorochromes) are attached to the antibody (such as the green dots below) to aid in the identification process.



Example of how a monoclonal antibody (MAB) binds to specific receptors on lymphocytes - these antibodies can have colored tags that emit light in specific colors when the tag is excited.

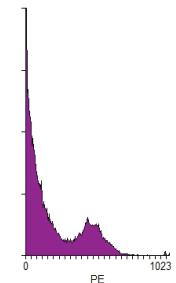
When a laser beam is fired at the cells, those cells with the attached molecules emit a signal identifying themselves. This signal is called *fluorescence* and can easily be measured with sensitive detectors.

The colors of fluorescence can also be changed by adding “red” colors to some antibodies and “green” colors to other antibodies. This allows us to make two measurements at the same time. If hundreds or thousands of cells are forced past the laser very quickly, an accurate count can be obtained of all cells that are present.

CD4 cells are measured in this manner. *CD* is an acronym for “cluster of differentiation,” and that simply means that this molecule can be classified into a well defined group of cells. In this case, it is cluster of differentiation 4, or a CD4 helper T lymphocyte cell.

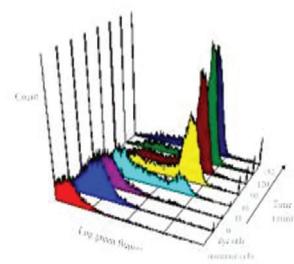
How are the results interpreted?

First, the data are collected in the flow cytometer. Each cell is plotted in a histogram display, which is a frequency distribution. In a typical histogram as shown to the right, two peaks appear.



The first peak represents cells that are negative, and thus have no specific binding to the probe. The second peak represents cells that are “positive” for that particular marker, or monoclonal antibody (MAB). Thus, if this were a CD4 MAB, these cells would be considered $CD4^+$ positive (cluster of differentiation positive). From this histogram we can determine the percentage of cells positive for this MAB.

Using additional monoclonal antibodies it is possible to classify each type of cell (or subset of cells) present in the blood. In the field of cytometry, this is called multiple labeling or multi-color fluorescence, as shown below.

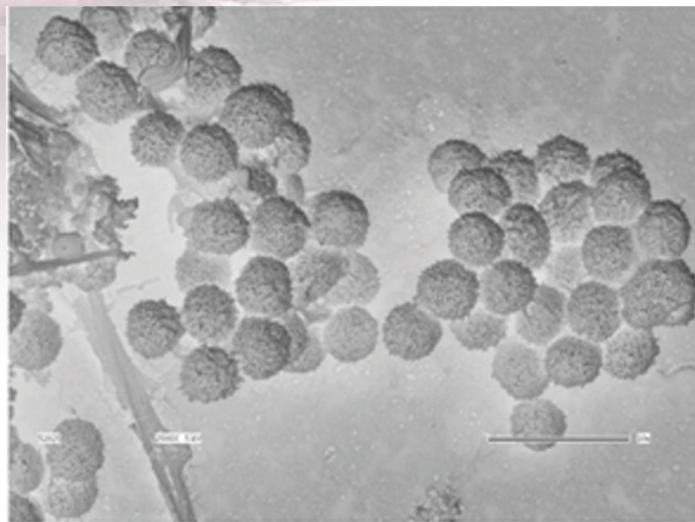


A multipanel display of monoclonal antibodies (MABs) binding to cells. Each histogram identifies a different cluster of cells in the sample. If we integrate the histogram we can determine exactly how many cells are present in each histogram.

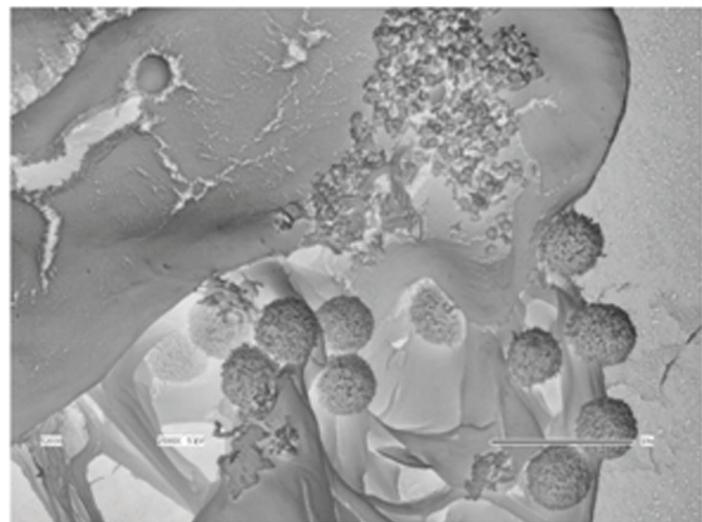
Where do the reagents come from?

The reagents used to identify CD4 cells and other cells types are called monoclonal antibodies (MABs). These MABs are conjugated (labeled) with fluorescent molecules that can easily be identified

using a flow cytometer. These MABs require precise and careful manufacturing protocols. They are quite expensive. In many cases, in addition to the cost of the instrument, the cost of reagents contributes greatly to the overall expense of CD4 determination.



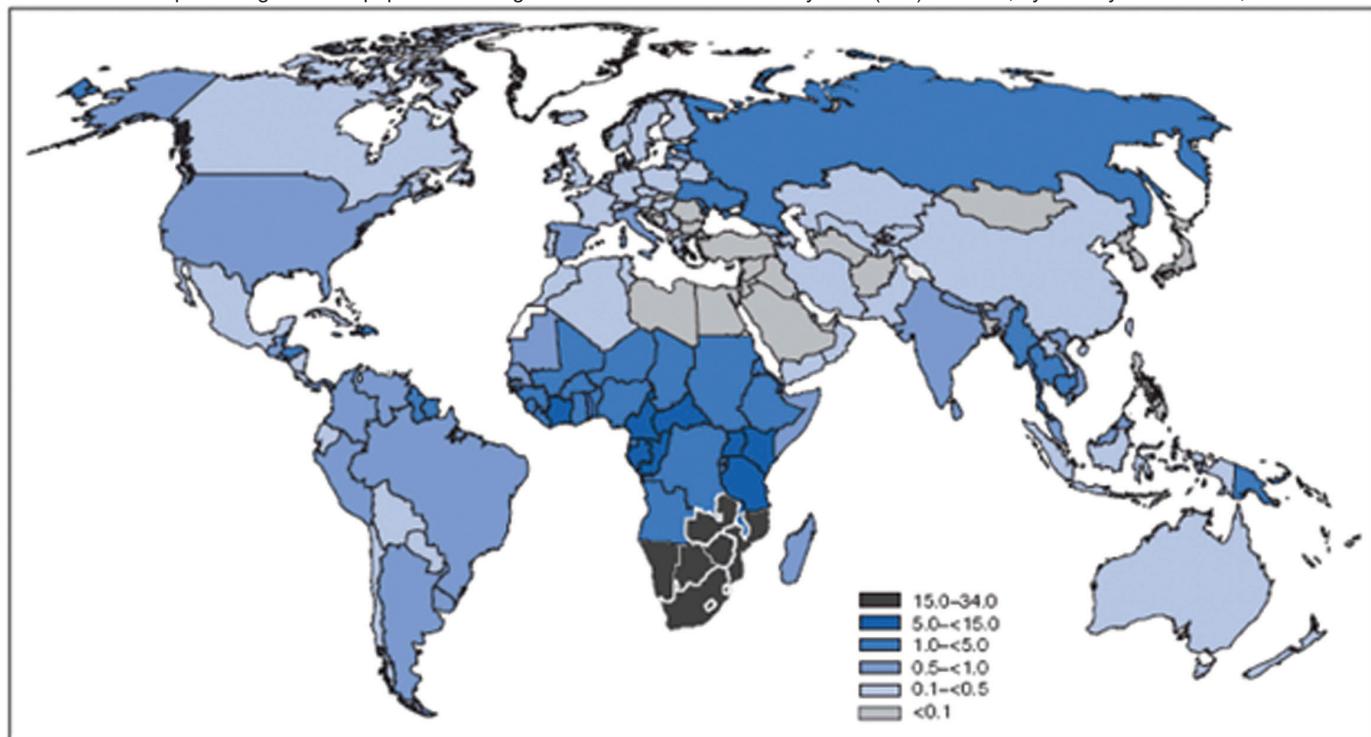
CD4 Lymphocytes



CD8 Lymphocytes

Normal human lymphocytes, T cells are indistinguishable from other lymphocytes using normal visual or size evaluation. To tell the difference, we must add a monoclonal antibody that binds to a specific receptor on either the CD4 or the CD8 cells. We also add a fluorescent molecule to the antibodies and then use flow cytometry to measure the percentage of each type of lymphocyte present.

Estimated percentage of adult population* living with human immunodeficiency virus (HIV) infection, by country -- worldwide, 2005**



SOURCE: Joint United Nations Programme on HIV/AIDS (UNAIDS). 2006 report on the global AIDS epidemic. Geneva, Switzerland: UNAIDS; 2006. Available at http://www.unaids.org/en/hiv_data/2006globalreport/default.asp.

*Aged 15-49

**The worldwide estimate of the number of persons living with HIV is 38.6 million.

SPONSORS

The *Cytometry for Life* program is an extension of the Purdue University Cytometry Laboratories within the Bindley Bioscience Center at Purdue University's Discovery Park. The program is entirely driven to change the lives of people in areas most impacted by HIV/AIDS, particularly on the African continent.

Current Major Sponsors:

Purdue University Cytometry Laboratories

iCyt Visionary Bioscience

Parker Life Sciences



Current Partnerships:

The *Cytometry for Life* program was initiated by academic and industry experts in the field of cytometry who want to make a difference today. Their drive is to create a program that is focused on changing lives through the usage of current-day technology at the most effective level and at the lowest possible cost.

The *Cytometry for Life* consortium has already successfully generated over \$250,000 in funds. Two funding goals for the program have been set: (1) to raise \$1 million to design robust technology and reagents; and (2) to raise another \$4 million to deliver the technology across nations in need. This project is entirely driven to mitigate the AIDS epidemic in Africa and respond to the need for low-cost and sustainable CD4 testing capabilities that function efficiently within rural African environments with minimal healthcare infrastructure. *Cytometry for Life* has the potential to positively impact the lives of millions of people in Africa and other nations severely affected by HIV/AIDS.

Participate in the *Cytometry for Life*

Campaign

What you can do to help

The *Cytometry for Life* program needs your help to achieve the goal of providing inexpensive and life-changing tests for AIDS victims in Africa.

This program is currently open for sponsorship.

Your corporate or personal gift will make an important contribution toward directly impacting the lives of millions of AIDS patients who would greatly benefit from affordable and sustainable CD4 measuring capabilities.

If an individual or organization would like to become a partner or major sponsor of this program, please contact the *Cytometry for Life* team. We would be happy to visit your institution and give a presentation on the *Cytometry for Life* program.

Cytometry for Life is a 501(c)(3) non-profit organization. As such, all donations are tax deductible. Donations can be made to *Cytometry for Life* at Purdue University. Please contact us for more information.

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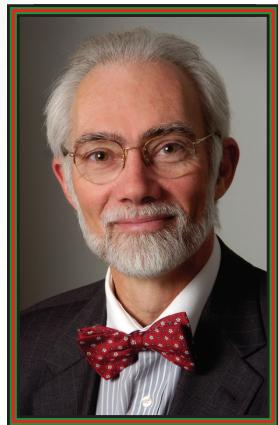
About the *Cytometry for Life* team

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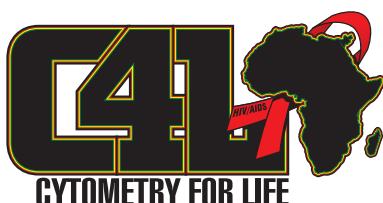
Gary Durack, MS

Gary Durack is the founder, President and Chief Technical Officer of iCyt Visionary Bioscience. He also currently holds an appointment with the University of Illinois at Urbana-Champaign as the Associate Director for Technology in the university's Biotechnology Center. He is the author of numerous publications, has edited a book on advanced technologies, and is recognized internationally as an expert in the field of cytometry. He currently serves as a Technical Councilor for the International Society for Analytical Cytology. He received a BS in electrical engineering from Purdue University.

From 1979 through 1989 he worked for Coulter Electronics Inc. (now Beckman Coulter Inc.). During his tenure at the company, Mr. Durack served as a field applications specialist, director of their customer support operations, and manager of the research cytometry instrument development program.

In 1989 he moved to Purdue University to manage the Purdue University Cytometry Laboratories. In 1993 he was appointed Director of the University of Illinois Biotechnology Center Cytometry Facility. In his seven years as director he secured substantial grant and research contract support to modernize instrumentation and to develop new technologies at the UIUC Cytometry Facility. Among these developments was the first DSP-based flow cytometer capable of performing fluorescent lifetime measurement. During this period he collaborated with numerous UIUC faculty, taught a graduate course in flow cytometry, authored several papers, edited a book, and wrote several book chapters.

Since 2000 he has been engaged in numerous cytometry-related design and development projects for Fortune 500 companies, as well as products now marketed through iCyt Mission Technology. These products include the X-Cyt™ Software for cytometry information management, the Lyt 200™ solid state laser, and the Reflection® multi-channel, high-speed cell-sorting system.



Kathryn L. Beaver, RN, MS

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**Kathryn L. Beaver, RN, MS**

Kathryn L. Beaver is Assistant Director of Bindley Bioscience Center in Discovery Park at Purdue University. With over 20 years of health care program management experience, her most recent management experiences include university-based biotechnology research, in-patient hospital, multi-specialty physician group practice, and social services venues in which Ms. Beaver functioned as a catalyst to implement positive change, ignite new operations, and resolve organizational challenges. She earned an Associate Degree in Nursing and a Master's Degree in Health Promotion and Education from Purdue University, during which time she trained over 1,000 undergraduate and graduate American Red Cross emergency first aid students and instructors annually. In 1992, she was awarded an "Excellence in Teaching Award" from Purdue University and received a research award to study emergency providers' response to AIDS/HIV. Publications include journal articles related to AIDS research and emergency care services, as well as teaching manuals for first-aid students and instructors. She has worked as a regional specialist for the national American Red Cross, functioned in various clinical nursing roles, and served as a community advisor to Eli Lilly and Company. Currently Ms. Beaver is working toward a Ph.D. in Health Promotion/Disease Prevention, with a research focus on personalized medicine, emerging technologies, and the improvement of health care.

Hildred Sarah Rochon, MPH, MS

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**Hildred Sarah Rochon, MPH, MS**

Hildred Sarah Rochon holds a Master of Public Health (MPH) degree in International Health Systems Management from

Tulane's School of Public Health and Tropical Medicine, a Masters degree in Biology from Indiana University-Purdue University, Indianapolis, and a BS degree in Public Health, summa cum laude, from Dillard University in New Orleans. She was a 2004-2005 Fulbright Fellow in adolescent reproductive health research and a 2001 University of Michigan Population Fellows Program MSI Intern with John Snow, Inc., both in Madagascar. She also received a University of Michigan mini-grant in 2004 for service in Sénégal, supporting reproductive health/family planning education. She is proficient in French, having studied at the Université de Paris IV-Sorbonne. She held internships with the United States Environmental Protection Agency in Cincinnati, addressing water-borne pathogens, and with the Seattle Biomedical Research Institute for research on African sleeping sickness, specifically related to *Trypanosoma brucei*, presenting at IEEE IGARSS in Toronto, Canada. In fall, 2004, she was a research assistant for a cervical cancer project at Purdue University. In March 2006, she facilitated conference organizing and co-authored a presentation at the Global Summit on HIV/AIDS, Traditional Medicine & Indigenous Knowledge held in Accra, Ghana.

Lova N. Rakotomalala

- Research Associate, Cytometry for Life Program

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**Lova N. Rakotomalala**

Lova N. Rakotomalala is a research assistant in the department of Basic Medical Sciences at Purdue University. Born in Nice, France, Lova grew up in Antananarivo, Madagascar. After finishing high school at lycée Stanislas (Paris, France), he attended Tulane University where he received a BS degree in Cell and Molecular Biology with a minor in Business Management. He also assisted in teaching sustainable development courses at the Payson Center for International Development, including translation of course materials for French-speaking African nations. He is currently completing a Ph.D. investigating the mechanism that leads to liver cancer caused by the hepatitis B virus, utilizing various flow cytometric and cell-imaging approaches.



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