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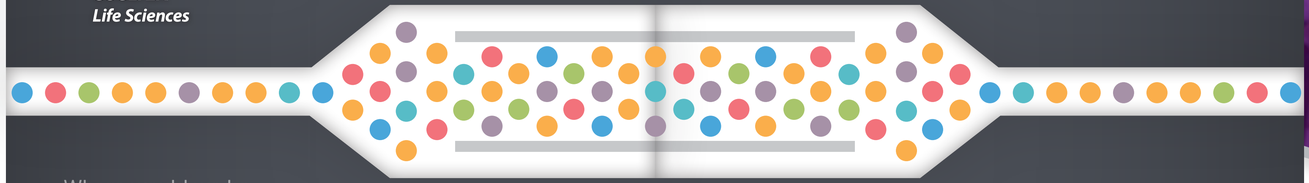

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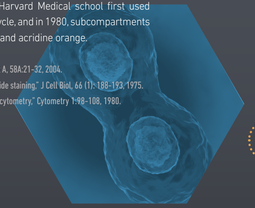
Flow cytometry has been responsible for a myriad of discoveries since its introduction into mainstream research labs in the 1970's. Here are some of the "aha!" moments that came about thanks to researchers having access to a flow cytometer at the right time.

Where would we be without flow cytometry?

75 A MATCH MADE IN HEAVEN: FLOW AND THE CELL CYCLE

Imagine the pains that researchers had to go through before they had flow cytometry at their disposal! Flow cytometry's introduction to cell biology labs permitted speeds of cell cycle analysis that were five orders of magnitude greater than microspectrophotometry. Instead of waiting days for results, the availability of flow cytometry meant data could be yielded instantaneously, which dramatically accelerated the field. Most notably, in 1975, Awtar Krishan of Harvard Medical school first used propidium iodide staining and flow cytometry to describe the cell cycle, and in 1980, subcompartments of the G1 phase of the cell cycle were found using flow cytometry and acridine orange.



Z. Darzynkiewicz et al., "Cytometry of the cell cycle: Cycling through history," Cytometry Part A, 58A:21-32, 2004.

A. Krishan, "Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining," J Cell Biol, 66 (1):188-193, 1975.

Z. Darzynkiewicz et al., "New cell cycle compartments identified by multiparameter flow cytometry," Cytometry 1:98-108, 1980.

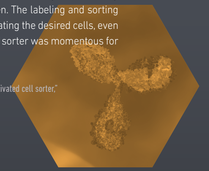
76 GOLD STANDARD: CLASSIFYING HEMATOLOGICAL MALIGNANCIES

Over four decades ago, in 1976, research undertaken by the French-American-British (FAB) Cooperative Group resulted in a morphologic and cytochemical criteria for subtyping acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML). At the end of their paper, they briefly mention that "Tests for B and T lymphocyte markers are now increasingly being applied to the investigation of leukemias and may become of value in classification as information accumulates." And they were right! This realization came to fruition because several research groups had access to flow cytometry. Within the following few years, the contribution from flow cytometry to diagnosing hematological malignancies was monumental, and flow cytometry using fluorochrome-labeled monoclonal antibodies is now the gold standard for immunophenotyping. Paradoxically, it was the AIDS pandemic of the 80's that moved flow cytometry into the mainstream as it transitioned from specialized research labs into common use.

J.M. Bennett et al., "Proposals for the Classification of the Acute Leukaemias French-American-British (FAB) Cooperative Group," Br J Haematol, 33(4): 451-458, 1976.

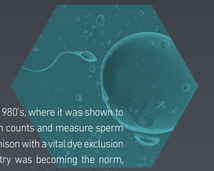
79 NOT LOST FOREVER: SAVING HYBRIDOMAS FROM NONPRODUCING CULTURE

Can you imagine performing modern research without monoclonal antibodies? We can't! But if it weren't for cell sorting, many a monoclonal-antibody producing hybridoma clone could have been forever lost in a cell-culture soup of undesirables. Traditionally, hybridoma antibody production was often screened for via a time-consuming approach of assaying culture supernates that could be overgrown with nonproducers or undesired clone variants. In 1979, a new method using cell sorting showed that this laborious task could be eliminated through selectively fluorescently labeling antibody-producing hybrid cells with fluorescent tagged antigen. The labeling and sorting combination allowed 10^7 cells to be screened per second, efficiently isolating the desired cells, even when they were just a fraction of the cell mixture. Having access to a cell sorter was momentous for this marvelous amalgamation.



D.R. Parks et al., "Antigen-specific identification and cloning of hybridomas with a fluorescence-activated cell sorter," Proc Natl Acad Sci USA, 76(4):1962-1966, 1979.

90 A NEW CONVENTION: FERTILITY INVESTIGATIONS



Flow cytometry as a tool in semen analysis was first reported in the early 1980's, where it was shown to be a powerful, fast technique that had not only the ability to perform sperm counts and measure sperm motility but was also able to measure mitochondrial activity and viability in unison with a vital dye exclusion assay. By the late 1990's, automated semen analysis using flow cytometry was becoming the norm, thanks to its high precision, accuracy, and low costs. And later, flow cytometry methods to examine DNA integrity – involved in postfertilization failure and embryo toxicity – were developed, which complemented new genomics and proteomics methods. Flow cytometry is becoming a much-valued non-invasive tool in infertility investigations that helps in the identification of individuals that may be suitable for interventional treatment. If it weren't for research labs and clinics having access to flow, in-house fertility investigations may still be performed by light microscopy, which misses crucial information about sperm abnormalities.

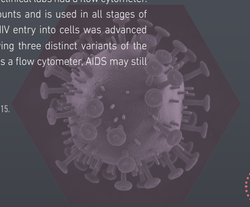
D.P. Evenson et al., "Simultaneous measurement by flow cytometry of sperm cell viability and mitochondrial membrane potential related to cell motility," J Histochem Cytochem, 30: 279, 1982.

D.P. Evenson et al., "Flow cytometric evaluation of sperm from patients with testicular carcinoma," J Urol, 132(6): 1220-1225, 1984.

F. Ferrara et al., "Automation of human sperm cell analysis by flow cytometry," Clin Chem, 43(5):801-807, 1997.

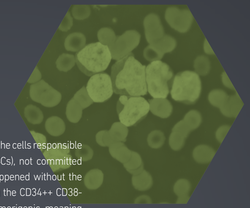
90 UNRAVELING A PANDEMIC: FLOW IN AIDS RESEARCH

The onset of the AIDS pandemic in the mid 1980's was a puzzling phenomenon. But flow cytometry was able to provide some of the first clues about the disease; before the AIDS-causing virus was even discovered, flow cytometry measurements showed CD4+ T-cell deficiencies. The technique quickly became key in AIDS diagnosis and by the late 1990's most clinical labs had a flow cytometer. Now, flow cytometry is the gold standard for measuring CD4 counts and is used in all stages of diagnosing AIDS. Furthermore, the underlying mechanism for HIV entry into cells was advanced thanks to flow cytometry with antigenic marker profiling, allowing three distinct variants of the virus to be identified. Without researchers' ability to readily access a flow cytometer, AIDS may still be a disease that is shrouded in mystery.



I.C. Clift, "Flow cytometry and the AIDS pandemic," Laboratory Medicine, 46(3):e59-e64, 2015.

97 A NEW FRAMEWORK: CANCER STEM CELLS (CSCs)



In 1997, it was discovered by University of Toronto researcher John Edgar Dick that the cells responsible for initiating acute myeloid leukemia (AML) were in fact cancer stem cells (CSCs), not committed progenitor cells as was initially hypothesized. This discovery could not have happened without the lab's flow cytometer, which was crucial for separating, phenotyping, and sorting the CD34++ CD38- cells. Cancer stem cells have since revolutionized cancer research. CSCs are tumorigenic, meaning they have the ability to give rise to all cell types found within a particular tumor, and are thought to be responsible for cancer relapse and metastasis. Targeted therapy to CSCs are hoped to improve survival for cancer patients, particularly those living with metastatic disease.

D. Bonnet and J.E. Dick, "Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell," Nat Med, 3(7): 730-737, 1997.

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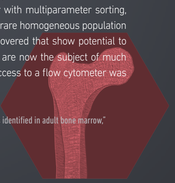
90 A DIFFERENT WAY OF THINKING: VERY-SMALL EMBRYONIC-LIKE (VSEL) STEM CELLS

In the early 2000's, the stem cell research community was divided. Initial exciting results that showed hematopoeitic stem cells (HSC) from bone marrow could be employed as precursors for other stem cells for the regeneration of solid organs was waning, after several groups could not reproduce this finding. However, researcher M.Z. Ratajczak, at the University of Louisville, had a thought: What if bone marrow had heterogeneous populations of stem cells? Several other groups were thinking along the same lines and consequently endothelial-, bone-, hepatic-, neural-tissue and many other types of committed stem cells were discovered, but none were characterized well. Using a flow cytometer with multiparameter sorting, Ratajczak's group delved deeper into bone marrow stem cells. Accordingly, a rare homogeneous population of stem cells termed very small embryonic-like (VSEL) stem cells was discovered that show potential to have the ability to differentiate into cells from all three germ-layers. VSELs are now the subject of much excitement – as well as huge debate – in the stem cell community. Having access to a flow cytometer was pivotal for Ratajczak's research.



M. Kucia et al., "A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow," Leukemia, 20:857-869, 2006.