Fathers of Cytometry. In Pursuit of Instruments and Methods to Measure Constituents of Individual Cells

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Stages in Development of Cytometry

- Microspectrophotometry, microinterferometry, microfluorometry
- Era of autoradiography
- Flow cytometry
- Image-assisted cytometry

My adventures/contributions/doodling along this path
- application in field of cell biology
Torbjörn Oskar Caspersson (1910-1997)

Developed and skillfully used the first cytometry instruments: microspectrophotometer and microinterferometer

1934-36, Nucleic acids content in nucleus and nucleolus
Feulgen reaction – DNA in nucleus

1937-39, Role of RNA in synthesis of protein
(with Jean Brachet)

1969 - Chromosomes banding
(with Lore Zech)

1955 – considered for Nobel Prize competing with
(Hugo Thorell- physiology)
Era of autoradiography

Measurement of individual cells or cell constituents with radioisotopes; detector - nuclear emulsion
Can be classified as one of cytometry branches

1943 - Leblond JC, *J. Anat.*, 77: 149. Incorporation of $^{131}$Iodide by thyroid cells

Monographs
1969 - Baserga R & Malamud D, Autoradiography. Techniques and Applications  
*Meth. Exp. Path.* Vol. 1
1973 - Rogers AW, Techniques of Autoradiography. Elsevier, Amsterdam,
Attempts were made to count silver grains in emulsion using absorption or reflection (dark field) microscopy. Tracks count (number of disintegrations) provided the most accurate quantification of targeted molecules.
Autoradiography: Milestone Discoveries

Beginnings of Flow Cytometry
First attempts to measure cells in flow

Wallace H Coulter (1913 – 1998)

  Cell counter (“Coulter counter”, “Coulter principle”,
  “Electrical sensing zone method”)
  The first flow cytometer – wide applications
Sizing paint particles
Subsequent upgrades: Cell volume; cell differential
1960 – John Scott Award for Scientific Achievement
  (T. Edison, Marie Curie, J. Salk, G. Marconi)
1998 - National Academy of Engineering
2004 – (posthumously) National Inventors’ Hall of Fame
82 patents
Wallace H Coulter Foundation: “Science serving humanity”
Louis A. Kamentsky


1970 - Commercially available instruments - Cytofluorograph (BioPhysics Inc., Ortho Instruments)


J. Mendelsohn, M. Melamed and L. Kamentsky at the ISAC Congress in Colorado Springs, 1998
Dittrich W & Göhde W


PARTEC Inc., series of flow cytometers

Manufactures inexpensive instruments for CD4 cells count to fight HIV/AIDS in underdeveloped countries
Marvin Van Dilla & Mack Fulwyler


1969- Hullet HR et al., Science 166:747 – Cell sorting based on fluorescence

FACS® instruments commercialized & sequentially upgraded by Becton Dickinson
Advances in Methodology

Cell cycle, univariate DNA content analysis
Cell cycle, multivariate analysis, proliferation markers
Cell proliferation – kinetics assays
Chromosomes identification and sorting
Immunophenotyping
Assessment of cell viability, probes of apoptosis
DNA damage response
**Cell cycle – Univariate DNA Content Analysis**

1977 – Stöhr M *et al.*, *Histochemistry*, DAPI
1983 – Vindeløv LL *et al.*, *Cytometry* 3:323, Propidium iodide, detergent & proteolysis
1983 – Hedley DW *et al.*, *J Histochem Cytochem* 31:1333 – Paraffin block isolated nuclei
1983 – Galbraith DW, *Science* 220:1040, Analysis of the cell cycle in plant cells
1999 – Smith PJ *et al.*, *J Immun Meth* 229:131, DRAQ5, supravital staining
Deconvolution of DNA Content Histograms


ModFit LT Verity Software, Inc

Our Contribution to DNA Staining Methodology

Accessibility of DNA In Situ to Various Fluorochromes: Relationship to Chromatin Changes During Erythroid Differentiation of Friend Leukemia Cells

Zbigniew Darzynkiewicz, Frank Traganos, Jan Kapuscinski, Lisa Staiano-Coico, and Myron R. Melamed
Memorial Sloan-Kettering Cancer Center, Walker Laboratory, Rye, New York 10580
Received for publication October 3, 1983; accepted November 9, 1983

Not all DNA is accessible to fluorochromes – role of chromatin structure in restricting the accessibility. Caution while defining aneuploidy
Cell Cycle – Multivariate Analysis

The “magic” fluorochrome acridine orange; DNA vs RNA

1979 - Darzynkiewicz et al., PNAS, 76: 358, Rate of S-phase progression/ RNA content
1981 – Shapiro HM, Cytometry 2:143, Combination of Hoechst 33342 and pyronin Y

The first marker of quiescent – G0 cells
More of the “Magic” Acridine Orange

Susceptibility of DNA in situ to denaturation


The first marker of mitotic cells in flow cytometry
There Are More Than Four Phases of the Cell Cycle

New Cell Cycle Compartment Identified by Multiparameter Flow Cytometry\textsuperscript{1,2}

Zbigniew Darzynkiewicz, Frank Traganos and Myron R. Melamed
Memorial Sloan Kettering Cancer Center, New York, New York
Received for publication March 26, 1980, accepted May 30, 1980

12 compartments were distinguished based on differences in DNA/RNA content, DNA denaturability and BrdU incorporation.
Still More of Acridine Orange “Magic”

1980 – Evenson DP, Darzynkiewicz Z, Melamed MR
Science 210:1131
Sperm Chromatin Structure Assay (SCSU)

DNA strand breaks in abnormal sperm cells

One of the most commonly used male fertility assays
Pioneering Application of Cytometry in Studies of Cancer Biology

1977 – Andreeff M, Doctoral Dissertation

Still More of Acridine Orange Applications

Flow Cytometry of leukemia

- Norm.Lymph
- ALL
- AML

DNA
RNA

Analyzing the number of leukemia blast cells, identified by aneuploid DNA values, correlates well with conventional microscopy counts and could be followed during the course of treatment. Thus, acridine orange flow cytometry can be used to discriminate subtypes of human leukemias, to determine cell cycle stages, and to detect and monitor aneuploid leukemia stemlines.
Cell Cycle, Cell Proliferation Markers

1987 - Gerdes J et al., Int J Cancer, 31:13, Ki-67 Ab (MIB-1, prognostic marker in oncology)

1987 - Tan EM et al., J Rheumatol 14 Suppl 14: 89; Celis JE et al., Leukemia Res., 10:237 PCNA

Cell Cycle, Other Markers

1998 - Juan G et al., Cytometry 32:71, identification of mitotic cells based on histone H3 Ser10 phosphorylation
DNA Replication - Cell Proliferation Assays

Assessing DNA Replication Without Denaturing DNA

1996 – Li X. et al., Exp Cell Res 222:28, DNA photolysis (SBIP)

1997 – Juan G et al., Cancer Res, 57:803, Correlation cyclinA cyclin B and DNA replication

Cell Kinetics Assays

1985 - Begg AC et al., *Cytometry* 6, 620, Cell kinetics – Tpot from single measurement of BrdU incorporation (Tpot = λT_s/LI)

Chromosomes Identification and Sorting
Flow Karyotyping

1975 – Gray JW et al., *PNAS* 72:1231, Univariate DNA content analysis (Munjack, CHO cells, human)

1982 – Langlois RG et al., *PNAS* 79:7876, Bivariate chromomycin A3 vs Hoechst 33342 staining


Cell Surface Immunofluorescence – Immunophenotyping Immunoassays


1977 – Loken MR *et al.*, *J Histochem, Cytochem* 25:899, Two color immunofluorescence

1979 – Reinherz EL *et al.*, *PNAS* 76:4061 – Subclassification of T cells


No One Contributed More in Area of Cytometry in Immunology than Leonard Herzenberg

2006 Kyoto Prize in Technology
“LEONARD HERZENBERG WINS KYOTO PRIZE FOR DEVELOPING REVOLUTIONARY CELL-SORTING TECHNOLOGY”
**Physical Attributes of the Cell**


1970’s - Contribution of T. Sharpless towards development of the data presentation software
Viability Tests, Assessment of Apoptosis

1980 - Hamori E et al., Cytometry 1:132, Hoechst 33342 and fluorescein diacetate (FDA)
1980 – Umansky SR et al., Biochim Biophys Acta 655, 5462., “Sub-G₁“ cells, apoptosis
Detection of Apoptosis

1994 - Hotz MA et al., Cytometry 15, 237, Acridine orange

1993 - Gorczyca et al., Cancer Res, 53:1945, 3186, TUNEL approach (>1300 citations)
Another Probe to Detect Apoptosis

RAPID COMMUNICATION

Activation of Caspases Measured in Situ by Binding of Fluorochrome-Labeled Inhibitors of Caspases (FLICA): Correlation with DNA Fragmentation

Elzbieta Bedner,* † Piotr Smolewski, * Paul Amstad, ‡ and Zbigniew Darzynkiewicz* †

Collaboration with Intergen Co

BINDING OF FLICA

Activated caspase

FLICA

RAPID COMMUNICATION

Interactions of Fluorochrome-Labeled Caspase Inhibitors With Apoptotic Cells: A Caution in Data Interpretation

P. Pozarowski, † 2 X. Huang, † D. H. Halicka, † B. Lee, † G. Johnson, † and Z. Darzynkiewicz

A Novel Assay to Measure Loss of Plasma Membrane Asymmetry During Apoptosis of Adherent Cells in Culture

Manon van Engeland, Frans C.S. Ramaekers, Bert Schutte, and Chris P.M. Reutelingsperger

Annexin V binding – exclusion of propidium
Detection and Sorting of Stem Cells


2000 – Keeney M, Sutherland DR, *Cytotherapy* 2:395, Excellent review, includes their earlier contributions

Cytometry of DNA Damage Response

Activation of Ataxia Telangiectasia Mutated (ATM) (Ser-1981 phosphorylation)
Histone H2AX (Ser-139 phosphorylation)
Chk2 (Thr-68 phosphorylation)
p53 (Ser-15 phosphorylation)

2003 - Huang X, et al., Cell Cycle, 2:614, γH2AX, Top1 and top2 inhibitors – apoptosis
2005 - Kurose A, et al., Cytometry A 68 A, 1-9, ATM-P and γH2AX, top1 and top2 inhibitors
Other Great Contributors

Richard P Haugland

Developing new fluorescent probes
The catalog – the “bible” for cytometrists

2002 – Molecular Bioanalytics Award,
Roche Diagnostics Munich
(L. Stryer and R Haugland)
Alan S Wagonner
Carnegie-Mellon University, Pittsburgh Pa
Series of cyanine dyes.
Howard M Shapiro

Practical Flow Cytometry
Third Edition

Howard M. Shapiro

The “living encyclopedia” of cytometry
Paul J Robinson

Purdue Cytometry Internet Board

Magnificent contribution to the field of cytometry
First Comprehensive Monographs and Proceedings

1979, 719 pages, 41 chapters
MR Melamed, PF Mullaney, T Lindmo & ML Mendelsohn

1990, 824, 39 chapters

1975, CAM Haanen, HFP Hillen, JMC Wessels
Nijmegen, The Netherlands
Clinical Cytometry Monograph

1993 - KD Bauer, RE Duque & TV Shankey
Cytometry journals

Brian H Mayall
Jan WM Visser
Charles L Goolsby
Attila Tarnok

1975, 1976, 1979

Cytometry 1980 -

Clinical Cytometry
The Nobel Prize in Chemistry 2008
“for the Discovery and Development of
Green Fluorescent Protein (GFP)”

Osamu Shimomura
Martin Chalfie
Roger Y. Tsien

Immense contribution to cytometry by developing the “rainbow” colors fluorescent proteins
My apology to many contributors in different areas of cytometry whom I was unable to present