Concepts and Developments in Flow Cytometry and Cytomics at *Max-Planck-Institut für Biochemie, Martinsried (1960-2006)*

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- ***< Concepts in Cytomics***

1. Introduction

Cytophotometry (1) and electronic cell counting (2, 3) generated from early on significant interest amongst biomedically oriented scientists. It took, however, a certain time until eminent clinical hematologists were convinced of the usefulness of electronic red and white blood cell and platelet enumeration by *Coulter* counters (4, 5, 6) in comparison to the long established counting chamber methods.

To speed up the acceptance process, *Wallace Coulter* as globally thinking entrepreneur and instrument developper travelled around 1958/59 personnally with a Coulter Model A counter in his luggage to various institutions in Europe that he considered of strategic interest. One of these institutions was the Max-Planck-Institute für Biochemie in Munich (MPI-Biochemie), headed by nobel laureate *Adolf Butenandt* where he addressed *Gerhard Ruhenstroth-Bauer*, a hematology oriented scientist and director of the Department of Experimental Medicine. *Ruhenstroth* was interested, bought a Coulter A counter with a serial number around 550 and the particular interest to measure volume distribution curves of cells. This is possible because the signal amplitude of the counting pulses in electrical cell sizing is proportional to cell volume. He considered this feature of the instrumentat of particular interest for the better characterisation of cells from blood, leukaemias and cancers.

Thus *Klaus* and later *Odila Zang*, two young scientists of his laboratory, investigated volume distribution curves of various cell types (7) and recognized that Coulter cell volume distributions were right skewed for erythrocytes. This was considered of probably artefactual nature since from previous microscopic and electron microscopic evidence, symmetric Gaussian normal distribution curves were expected. *Wallace Coulter* being primarily interested in the use of electronic blood cell counting for clinical purposes was not enthousiastic about these unforeseen findings and considered legal action against the MPI-Biochemie for distributing non advantageous rumours about his counter.

2. Early instrument development

Ruhenstroth, remaining nevertheless highly interested in the determination of cell volume distribution curves, circumvented potential conflicts with *Coulter* by asking *Butenandt* for an equivalent of 250.000 Euro (countervalue of 10 high speed ultracentrifuges at this time, for example of Beckman Spinco L-50) to develop new instrumentation. *Butenandt* was hesitant but finally made these comparatively enormeous funds available. *Jürgen Gutmann* was hired as electronic engineer, an electronic as well as a workshop were equipped and the instrument building phase of the experimental medicine department began (8 --> **fig.1**, 9, 10, 11 --> **fig.2 left**) in its former location in central Munich, close to the main railway station (Goethestrasse 31) from where the institute moved to the newly built Martinsried facilities in 1972.

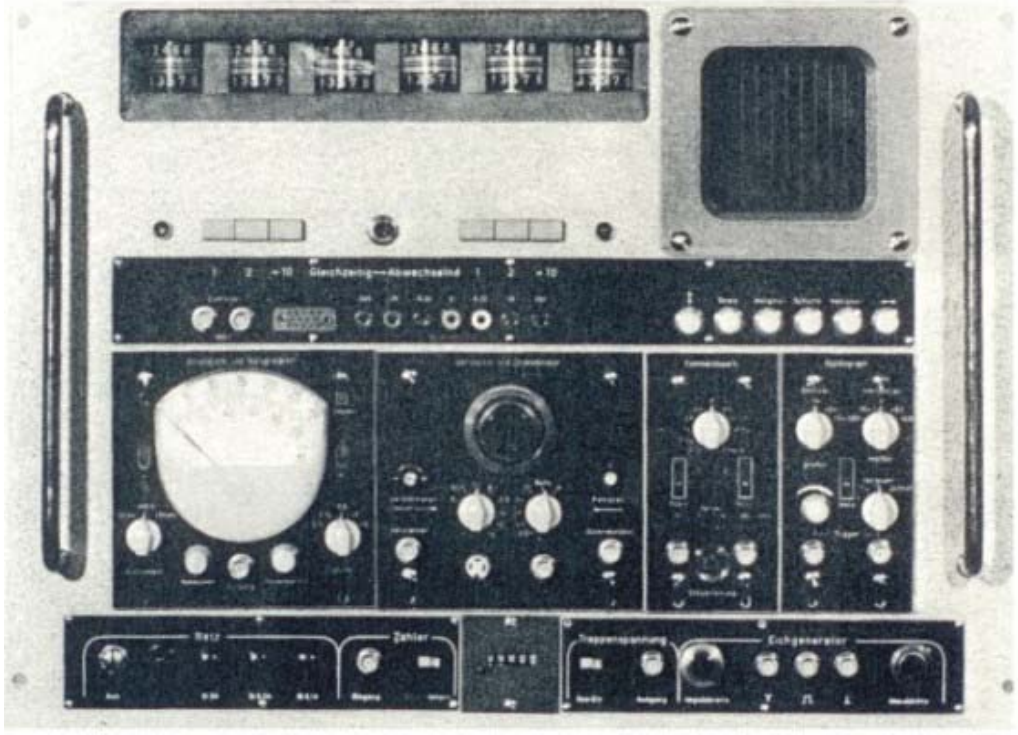

fig.1 Electrical impedance cell sizing unit, J Gutmann, Diplomarbeit Fachbereich Elektrotechnik TU-München 1963

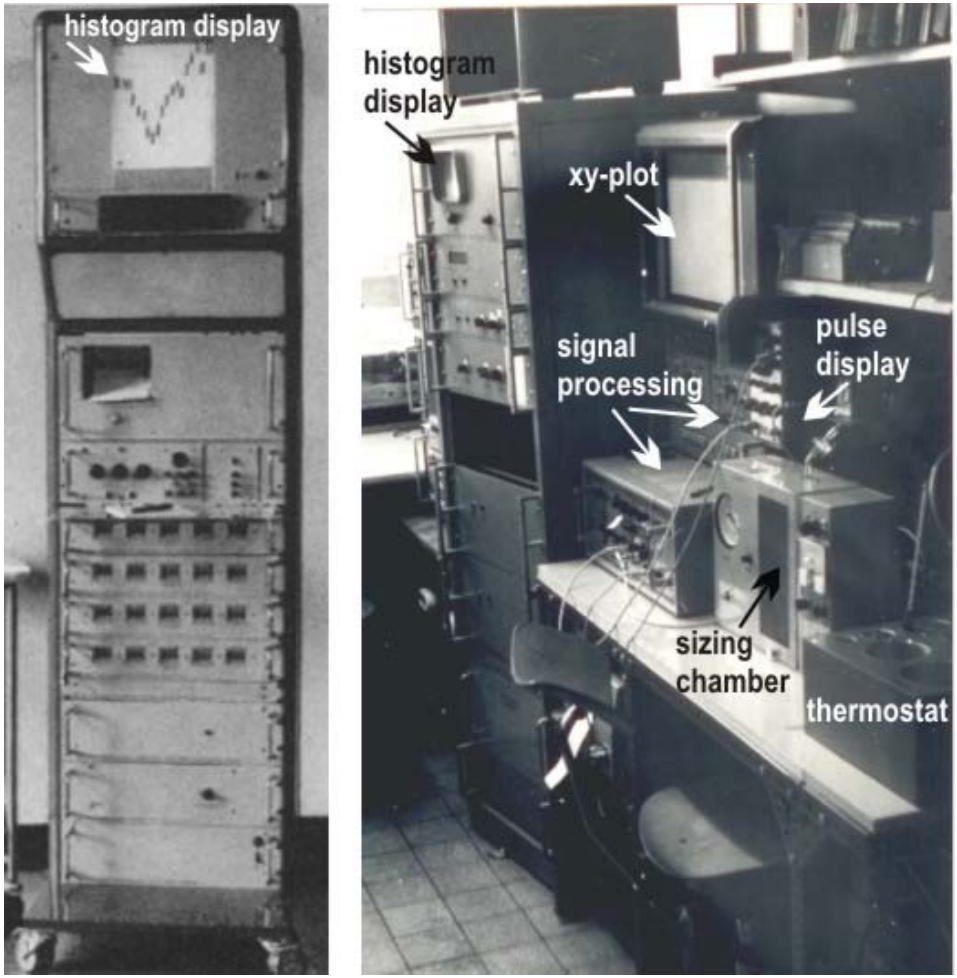

fig.2 *left:* Signal processing & histogram display unit from: J Gutmann, V Kachel, R Röttger, G Ruhenstroth-Bauer, Naturwiss 55:130(1968) (reproduction with kind permission of © Springer-Verlag Berlin Heidelberg), *right:* Metricell, Dec 1971

(image: © *Günter Valet*)

Klaus & *Odila Zang*, *Jürgen Gutmann*, *Mac Fulwyler* (12) and others observed the right skew of Coulter volume distribution curves, all considering it an artefact. *Gutmann* hypothesized that biconcave erythrocytes went through the sizing orifice in variable orientation like lengthwise, transverse or in intermediate position causing variable displacement of electrical field lines leading to increased signals for transversely passing erythrocytes. Right skew in nucleated cell volume distribution curves was interpreted as representing in part size difference during cell cycle.

3. Metricell

Gutmann's concept was not confirmed by the subsequent investigations initiated by *Reinhard Thom*, a clinician from Berlin (Klinikum Westend, Freie Universität Berlin). He modified a standard Coulter orifice by a hydrodynamically focused cell influx capillary in front of the sizing orifice while being a guest scientist at the MPI-Biochemie (13, 14, 15). The available Metricell sizing instrumentation, as developed by *Volker Kachel* (16) (**fig.2 right**) in continuation of *Gutmann's* earlier work was used for the measurements.

The interest in path, shape and orientation of the cells on the way through the orifice required their photographic visualization, using a special observation chamber in combination with ultrashort flashlight (40nsec) pulses (16 -> **fig.4a**). A quartz observation chamber had been fabricated earlier by Zeiss (Oberkochem) during a collaborative effort 1966/67 that had been stimulated by the development of the first optical flow cytometer in a modern sense by *Kamentsky* (17). In this cytometer, cell DNA was determined by optical absorption at 253.7nm simultaneously with a cell light scatter measurement at 410.0nm. The project idea with Zeiss was to simultaneously measure DNA at 253.7nm and protein content at 280nm together with the electricaly determined cell volume. Zeiss provided a tuneable monochromator with HBO200 mercury arc lamp in conjunction with a sophisticated UV-microscope. The measurements were technically possible with this setup but it became soon clear that the observed signals were mostly light scatter signals and therefore not sufficiently specific for the separation between DNA and protein of unstained cells in flow. The project was abandoned but the quartz chamber, kindly donated by Zeiss, proved very useful for the subsequent extensive high speed photographic investigations of cells passing through electrical sizing orifices.

The photographs showed that cells travelled always lenghthwise in the quickly accelerating fluid stream through the orifice. Cell passing over the edges of the orifice entrance, as possible in the typical Coulter counter, passed, however, through zones of elevated electrical field strength, resulting in higher electrical pulse signals than cells passing through the center of the orifice. Focusing particles on restricted pathways through the orifice provided similar electrical field strength conditions for all measured cells, resulting in the postulated symmetrical volume distribution curves for erythrocytes (14, 18) or monodisperse microbeads. *Spielman & Goren* (19) had equally observed a narrowing of Coulter volume distribution curves by hydrodynamic focusing around the same time but did not provide experimental explanations for their observation.

Conclusion: The extensive experimental work connected with the proof of the hypothesis that the right skew of volume distribution curves in normal Coulter counters at unrestricted particle path through the sizing orifice was an artefact has lead to the development of the *cell sorter* (12) as well as to *fast imaging in flow* (20)

4. Data analysis

A major bottleneck in cytometric investigations concerned from the beginning on *data display* and *data analysis*, given the comparatively high data acquisition rates between

typically 1.000 and 5.000cells/sec. The initial hardware solution at MPI-Biochemie consisted of a set of around 20 relays having little weights attached to strings running over small step wheels at each relay. Each wheel was advanced by its relay after a given number of counted cells within its window of pulse amplitudes (channel) that is within a certain cell size range. This provided "negative cell size histograms" (**fig.2 left**). The absolute cell count for each histogram channel was separately printed on paper. The use of an oscilloscope as histogram display and of an xy-plotter to permanently visualize the measured histograms (**fig.2 right**) improved record keeping but the quantitative information for histogram comparison and further analysis remained still printed on paper.

The increased resolution of hydrodynamically focused measurements of cell volume distribution curves lead to the discovery of discrete erythrocyte populations by size during the first trimenon of life (21) in various mammalian organisms or in adults after x-irradiation (22) or strong bleeding. Depending on species, these populations showed in addition different hemoglobins, antigen expression or electrophoretic mobility as consequence of switched gene expressions patterns.

Having shown that the volume distribution curves had the potential to monitor *gene activation patterns* in the hematopoietic system, a more detailed data analysis was required to understand the sequence of events. The printed cell contents of the various channels of the volume distribution histograms were analysed in a first approximation on probability paper (21, 22) to obtain a model of the sequence of cell populations, thus concentrating the many initial histogram channel counts into a sequence of means, standard deviations and % contribution of the various cell populations over time.

Computers were at this time far too expensive to purchase them for such an analysis. The move of the institute to Martinsried in 1972 provided, however, access to a Siemens 4004 main frame computer that had been purchased by the Max-Planck Gesellschaft for the equivalent of around 2 million Euros to advance the evaluation of electron microscope and x-ray crystallography data. The computer had a core memory of around 1Mbyte. From now on, cell volume distribution curves could be iteratively fitted by standard Gaussian normal distributions or by other functions with substantially more information and knowledge being extracted (23, 24) than by the visual inspection of histograms. It was also easier to evaluate cell aggregation for example of erythrocytes as cause of the clinically observed erythrocyte sedimentation rate (ESR) in anticoagulated blood samples (23).

5. FLUVO-Metricell

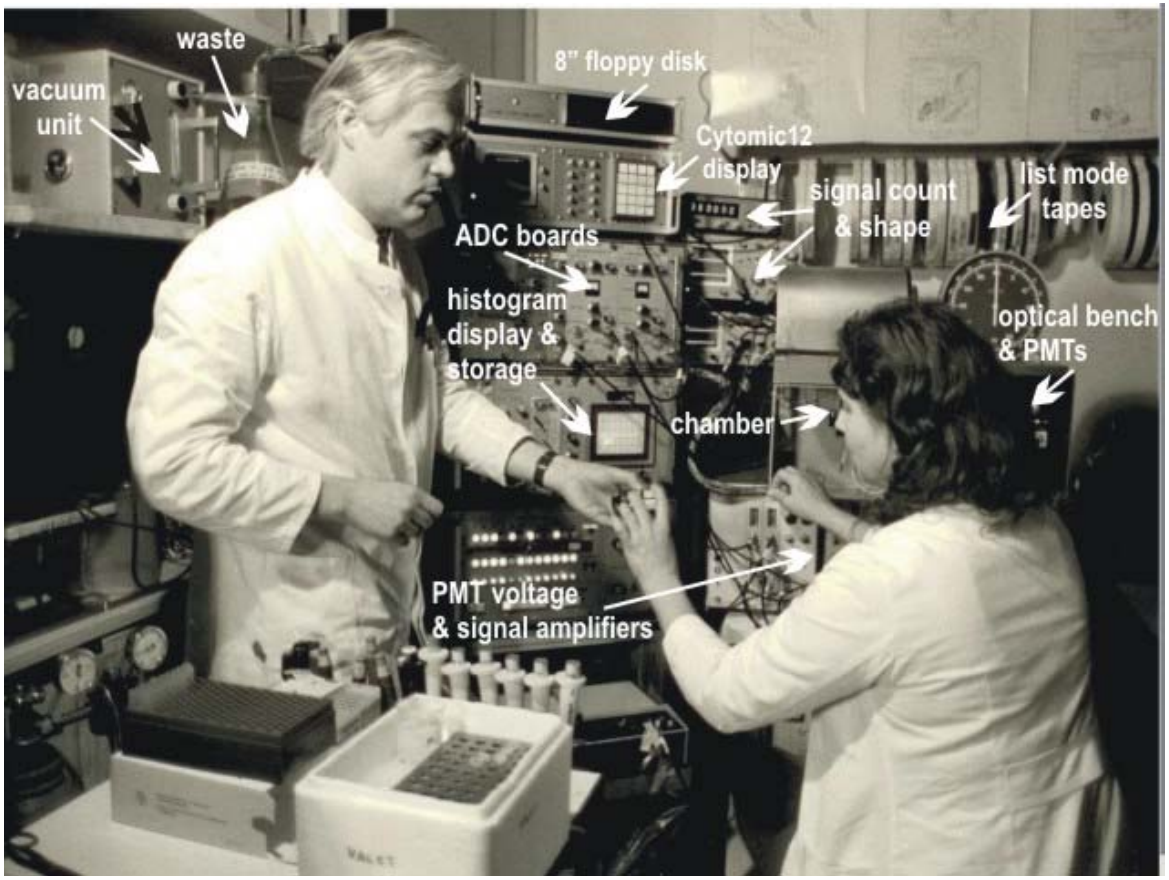

fig.3 Fluvo Metricell 1982, *Günter Valet, Hanna Kahle*, (reproduction with kind permission of © KNA Bild, Bonn)

With the introduction of *fluorescence* into flow cytometry by *Wolfgang Göhde* (25, 26) and into cell sorting in *Len Herzenberg's* laboratory (27), flow cytometry gained access to an essentially unlimited number of specific molecular stains. It seemed especially important for medicine oriented cell biochemistry to develop into this direction since work prior to the development of fluorescence flow cytometry had shown the importance of relating biochemical changes in tissues to the cellular level in order to better understand their biological and physiological significance (28). Phywe company (Göttingen) commercialized *Göhde's* instrumentation but difficulties existed in purchasing only the optical part of the instrument since the intention at MPI-Biochemie was to use own electronics for signal amplification and list mode data acquisition as well as a performant computer for data evaluation. A member of the Phywe board of directors happened to be senator of the Max-Planck-Gesellschaft. He arranged the purchase of the optical part of the first commercially produced Phywe ICP11 instrument (*W Göhde* personal communication) in 1969.

Despite high interest and intensive development, it took until 1977 to accomplish the full functionality of the devised FLUVO-Metricell flow cytometer (29) (**fig.3**). The instrument measured hydrodynamically focused electrical cell volume and two fluorescences with the Phywe ICP11 optics. Signals were amplified by 3-decade logarithmic amplifieres and visualized on hardware display units. The measured pulse heights of cells or particles in each of the data acquisition channel were simultaneously transferred in list mode format on-line to a Perkin Elmer INTERDATA 7/32 computer, equipped with a 220Mbyte hard disk drive and a 9-track tape unit for data storage. This provided an essentially unlimited list mode storage capacity as well as a significant list mode evaluation potential. The total unit was especially with regard to data processing for years in advance of commercially available instrumentation. During the FLUVO-Metricell instrument development, cell volume studies as well as software developments were further advanced (30, 31). The use of electrical sizing instead of light scatter as in laser instruments complicated the hardware but was considered essential in a cell biochemistry environment to be able to calculate relative substance concentrations in cells as well as average molecule packing densities on cell surfaces.

The concentration on *cell functions* (functional flow cytometry, functional cytomics) as fast indicators of disease activity in patient cells was a main strategic goal at MPI-Biochemie. Stains and assays for the flow cytometric determination of intracellular pH, Ca2+, free radicals, glutathione, proteases, esterases and phosphatases or for

transmembrane and mitochondrial potentials in single viable cells were developed, using propidium iodide to monitor simultaneously the DNA of dead cells at UV or blue light excitation (for details refer to **32**, **33**). Other institutions followed different goals. The efforts of a significant number of hospitals and research institutions using the standard mercury arc lamp equipped Phywe ICP11 flow cytometer, mostly in Germany, concerned preferentially *clinical DNA analysis* in malignant disease. The Los Alamos and Livermore laboratories with their laser flow cytometers and cell sorters were substantially centered on *cell cycle* and *chromosome* research, the Sloane Kettering group on *DNA conformation* in the cell cycle of normal and abnormal cells while *immunological* and *immunogenetic mechanisms* were of prime interest for the Stanford laboratory.

6. Mildred-Scheel Laboratory for Cancer Cell Research (1981-1989)

Dr.Mildred Scheel, the wife of former German Bundespräsident *Walter Scheel* had founded the *Deutsche Krebshilfe* as well as the *Mildred-Scheel Foundation* to provide better care for cancer patients and to advance cancer research in Germany. Following significant funding of clinical institutions, certain criticisms emerged that not enough was done for the research sector. This prompted the Mildred-Scheel Foundation together with the Max-Planck Gesellschaft zur Förderung der Wissenschaften to internationally announce a 5 year research project in the area of medicine oriented basic cancer research. *Günter Valet* applied as did more than 50 other scientists and was finally appointed head of the independent Mildred-Scheel Laboratory for Cancer Cell Research (**fig.4**) after a thorough two year selection process by a search committee, consisting of around 20 eminent scientists from the Deutsche Krebshilfe, the Max-Planck-Gesellschaft as well as from several universities and cancer research institutes in Germany.

fig.4 Opening of the Mildred-Scheel Laboratory for Cancer Cell Research at Max-Planck Institut für Biochemie, Martinsried on July 15, 1981, *Dr. Mildred Scheel (President Deutsche Krebshilfe), Prof. Peter Hans Hofschneider* (Institute Director) (reproduction with kind permission of © MPG-Pressestelle, Munich)

The submitted project proposal aimed at the simultaneous multiparameter analysis of single cells by flow cytometry as a sensitive approach for the automated detection and molecular characterization of cancer cells in patients as well as at the development of suitable benchtop instrumentation for this purpose. The Mildred-Scheel Foundation provided the equivalent of 1.5 Mio Euro for salaries while the Max-Planck Gesellschaft contributed the laboratories and running costs in the order of 0.5 Mio Euro. *Dr.Scheel* as former radiologist, followed the project with close attention but died in 1985 of cancer.

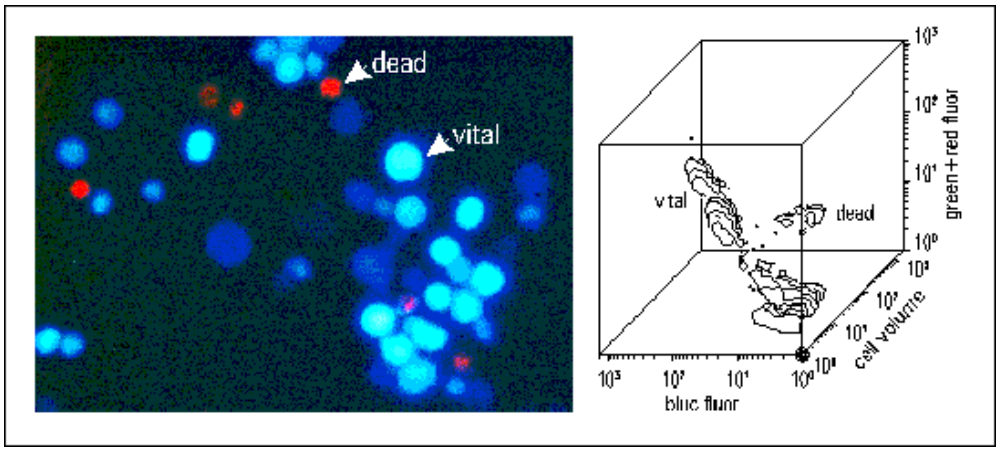

fig.5 Cell function in cytomics: **left:** intracellular pH & esterase staining (ADB/DCH) of viable rat bone marrow cells (*blue*) and of the DNA in dead cells (PI) (*red*). **right:** contour line display of the flow cytometric measurement of around 6.000 cells of the stained cell preparation from the left panel.

Major focus points of the scientific project work concerned the:
- establishment of sensitive cell function assays for cancer cell detection by flow cytometry (**fig.5**)
- development of individualized cytostatic drug assays for patient cells
- development of data pattern analysis for knowledge extraction from complex multiparameter data
- application of this new potential to patient studies in collaboration with a variety of clinical institutions
- development of benchtop instrumentation (FLUVO-Metricell II, Cytomic123, **34**, **35**, **36**, **37**) by *Volker Kachel*

for further details of the cell biochemistry group see literature references 1981-1990.

After 5 years, the project was prolonged for another 3 years until 1989, amounting to a total funding of around 3.2 Mio Euro. It was at this time the largest project, the foundation had ever funded. About 10 FLUVO-Metricell II instruments, data recording or display modules were produced by the technical group and sold to scientific institutions between 1984-1989 with reinvestment of the income into further research. The reasons for the termination of the project in 1989 were that flow cytometry had become a routine technology and research in the earlier context was not considered of primary interest any more.

7. Cell Biochemistry Group (1990-2006)

Following the termination of the Mildred-Scheel Laboratory project, the cell biochemistry part of the Martinsried flow cytometry group remained as Cell Biochemistry Group scientifically independent and continued the work with funds from European research projects, through Sonderforschungsbereiche of the Deutsche Forschungsgemeinschaft (DFG) and basic funding by the Max-Planck-Gesellschaft.

Given the fast technological progress, commercial instrumentation such as the PASIII cell analysis and closed piezo sorter system (Partec, Münster, Germany) met increasingly the needs of basic cell biochemistry research and did not require further in-house technological development. Likewise, personal computers provided enough computing power for cytometric list mode data analysis. Decreasing support in combination with an increasing accumulation of multiparametric data sets in many clinical environments generated a gradual shift from the production of own data to processing other groups data with the advantage that it would have been experimentally not easily possible by own investigations to generate the amount of experimental & clinical data required for the gradually developed concepts.

The limited interest for flow cytometric cell biochemistry at the national level (see frequency of terms like *Zytomik* or *Humanzytomprojekt / Humanzytom-Projekt* on the Internet) favoured this increased concentration onto the intellectual core competence of *knowledge extraction* from complex multiparameter data at the international level. Essential transit points in this effort over time were:

- the automated diagnosis from flow cytometric list mode data (1987)
- the view that cytometry and later cytomics constitute a biomedical key discipline where the explicit analysis of the *heterogeneity* of cell systems in form of system cytometry (1997) represents a comparatively efficient top-down strategy for the systematic resolution of the cellular and molecular biocomplexity of higher organisms with one of the important advantages being that complex disease mechanisms can be efficiently investigated without necessity for extensive a-priori knowledge and molecular pathway modeling.
- the individual patient disease course prediction by SMDC (predictive medicine by SMDC) (2000) (**fig.6**)
- the definition of cell systems as cytomes (2001) in combination with
- the introduction and redefinition of the plant science term cytomics for cell biochemical purposes
- the predictive medicine by cytomics concept (2001) as well as
- the elaboration of concepts for a human cytome project (2004) and for a periodic system of cells (2005)

Nr.	classification category	category abbreviation	mask coinc. factor	disease classification masks
1	normal	N	1.00	0 0 0 0 0 - - - - -
2	infarction risk	R	1.00	+ + + + +

Nr.	clinical classification (TH5LEARN)		CLASSIF1 classification	mask coinc. factor	patient classification masks (.=no value)
1	#102	N	N	1.00	0 0 - 0 0 ←
2	#103	N	N	0.60	+ + 0 0 0
3	#104	N	N	0.80	0 + 0 0 0
4	#106	N	N	0.80	0 0 + 0 0
5	#107	N	N	0.80	0 0 0 + 0
6	#108	N	N	1.00	0 0 0 - 0 ←
7	#109	N	N	1.00	0 0 0 0 - ←
8	#111	N	N	0.80	0 0 0 0 +
9	#112	N	N	0.60	0 0 0 + +
10	#113	N	N	1.00	0 0 0 0 0
14	#137	R	R	1.00	+ + + + +
15	#138	R	R	0.80	+ + 0 + +
16	#139	R	R	1.00	+ + + + +
17	#141	R	R	1.00	+ + + + +
18	#142	R	R	1.00	+ + + + +
19	#143	R	R	1.00	+ + + + +
20	#144	R	R	0.80	+ + 0 + +
21	#146	R	R	0.80	+ + 0 + +
22	#147	R	R	0.80	+ + 0 + +
23	#148	R	R	1.00	+ + + + + val

fig.6 CLASSIF1 data pattern analysis: Discriminatory *disease classification masks* (top of rightmost column) consisting in this case of the 5 most discriminatory out of 44 measured thrombocyte parameters, permit to correctly distinguish between risk and non-risk patients for myocardial infarction from molecular properties of peripheral blood thrombocytes. The CLASSIF1 algorithm (*CLASSIF1 classification* column) correctly recognizes the two types of patients (*clinical classification* column) from the analysed flow cytometric data (details, **38**)

8. Collaborators, Clinicians, Concepts

Excellent collaborators in the cell biochemistry and technology areas as well as a significant number of highly interested clinicians have very much contributed to the success of the various scientific projects. I want to thank them all for their continued enthousiasm and their committment to the goals of this highly transdisciplinary work. I am furthermore very grateful to several scientists at the national and international level for their openness to the conceptual aspects of this work. They have by numerous discussions and a number of joint publications very significantly contributed to the elaboration and dissemination of the cytomics, predictive medicine by cytomics and human cytome project concepts

Cell Biochemistry	Technology	Clinicians	Concepts
Gregor **Rothe**	Volker **Kachel**	Friedrich Otto **Scheyffarth**	Attila **Tarnok**
Sven **Klingel**	Eberhard **Menke**	Rainer **Wirsching**	Paul **Robinson**
Andreas **Oser**	Gerhard **Benker**	Florian **Liewald**	Enrique **O'Connor**
Michael **Collasius**	Karl **Schedler**	Hans Heinrich **Warnecke**	Andreas **Radbruch**
Christoph **Zirkelbach**	Heinrich **Schneider**	Wolfgang **Kellermann**	Peter **van Osta**
Jeanette **Malin-Berdel**	Ernst **Kordwig**	Thomas P.U. **Wustrow**	Bob **Murphy**
Alexander **Raffael**	Ewald **Glossner**	Rolf **Lamerz**	Andres **Kriete**
Lorenz **Rüssmann**		Hansjörg **Sauer**	Gerd **Schmitz**
Vincentiu **Manta**		Diethelm **Tschöpe**	Gero **Brockhoff**
Max **Hasmann**		Heinz Gerd **Höffkes**	Susann **Müller**
Gerburg **Wulf**		Andreas **Neubauer**	
Susanne **Burow**		Herbert **Leyh**	
Jürgen **Treumer**		Rainer **Repp**	
Hella **Horst**		Thomas **Dörner**	
Ganesh **Shankar**		Luc **Kestens**	
Ken **Trevorrow**		Jan **Gratama**	
Tarek **Elsherif**		Elisabeth **Bräutigam**	
Hanna **Kahle**			

9. Overall Impact (1960-2006)

The Martinsried flow cytometry group in its cell biochemistry and technology branches, has contributed to several developments and concepts that have significantly shaped the cytometry field over time:
1. The early instrument development terminated the long going controversy about the right skewedness of cell volume distribution curves by electrical sizing by recognizing the importance of hydrodynamic focusing. It furthermore provided early computerized list mode data acquisition as an important prerequisite for the efficient extraction of information and knowledge from multiparameter flow cytometry measurements.
2. The development of flow cytometric cell function assays enabled the fast molecular evaluation of disease states as well as predictions about the future disease progress in individual patients. The flow cytometric cell function area has advanced on its own into a steadily growing field in medicine and cell biochemistry but also in the

pharmaceutical industry in form of high-throughput and high-content assays for drug discovery, both by flow cytometry and image cytometry.
3. The development of data pattern analysis (data sieving) by the CLASSIF1 algorithm permits the exhaustive and standardized knowledge extraction from flow cytometric list mode files as well as from multitudes of other multiparameter data.
4. Data pattern analysis enabled amongst others the development of the concepts of individual patient disease course prediction by SMDC (standardized multiparameter data classification) and system cytometry as well as the cytome & cytomics definitions (Omics-glossary (2001)), leading to the concepts of predictive medicine by cytomics, of a human cytome project and of a periodic system of cells by cytomics.

10. External feedback

The experimental and conceptual work of the Martinsried Cell Biochemistry Group has led to diverse forms of external interest such as the election into leading positions of international scientific societies, membership in various editorial boards, associate editor & editor of the "cytomics" editorial column in Cytometry A, invitation to more than 20 review articles in the cytomics area since 2001 and to more than 200 invitations for presentations of the groups cell biochemistry work at scientific meetings or in many institutions worldwide since 1981, furthermore to awards, membership in scientific advisory boards and collaboration with the pharmaceutical industry in the area of predictive medicine by cytomics (-> personalized medicine, individualized medicine). The presently widespread international interest is also reflected by inclusion of various definitions and concepts into on-line glossaries like Omes & Omics or enzyclopedias like Wikipedia (cell biochemistry, predictive medicine, cytomics, human cytome project) or in the ranking of the groups Internet pages by search engines like Google (date: *position/total number of hits*):

cell function, "cell function"	cell biochemistry, "cell biochemistry"	predictive medicine, "predictive medicine"	cytome(s)	cytomics	human cytome project
21.10.07: *10/186mio, "2/2.0mio"* 06.04.08: *2/20.5mio, "2/1.2mio"*	18.11.04: *5/75.400* 14.04.07: *4/42.4mio,"3/161.000"* 06.04.08: *3/3.5mio,"2/60.400"*	02.08.01: *32/43.000* 26.02.03: *5/75.400* 14.04.07: *4/1.69mio,"4/88.100"* 06.04.08: *5/388.00,"5/42.100"*	02.08.01: *--/-- (0/0)* 10.08.01: *--/-- (1/3)* 26.02.03: *--/-- (1/18)* 14.04.07: *3/88.900 (1/337)* 06.04.08: *4/28.100 (8/542)*	02.08.01: *--/81* 05.04.02: *1/150* 07.09.05: *6/9.730* 14.04.07: *3/40.600* 06.04.08: *3/20.000*	12.12.03: *--/3* 16.12.03: *3/10* 06.04.05: *1/139* 14.04.07: *3/14.000* 06.04.08: *3/10.400*

11. Outlook

The fields of cytometry and cytomics have been fascinating over many years by the collaboration of scientists being interested in trans-disciplinary (cross-disciplinary) concepts and their potential to provide entirely new insights into cellular biomechanisms. Flow cytometry and image cytometry instrument developpers were initially (1965-1985) rather competing against each other for supremacy in the sensitive detection of cancer cells but the efforts have become more and more collaborative and mutually complementing in recent years. Flow cytometry with its early on fluorescence orientation has substantially enhanced the development of new stains, proving equally useful for molecular biology as for single cell oriented image cytometry like in confocal or laser scanning microscopy.

The cytometric field continues to be fascinating through its potential to unfold the organismal biocomplexity (organismic biocomplexity) top-down by single cell molecular

analysis in-situ that is with all molecules in place, lending itself to the reverse engineering of the assembled molecular machinery as well as to the investigation of the natural heterogeneity of cells in tissues and in complex disease mechanisms as one of the bases for the adaptivity of organisms in variable environments. The top-down approach seems particularly promising since the knowledge of the entire set of biomolecules as derived at the genome level does by itself so far not provide enough information for their molecular reassembly in form of living cells, tissues, organs or organisms. The fascination for the cell biochemistry and cytomics fields will therefore in all likelihood not only continue but further increase, seen the overall potential and challenges of this approach.

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