30 Years DGfZ : A Personal View, History and Challenges

Günter Valet

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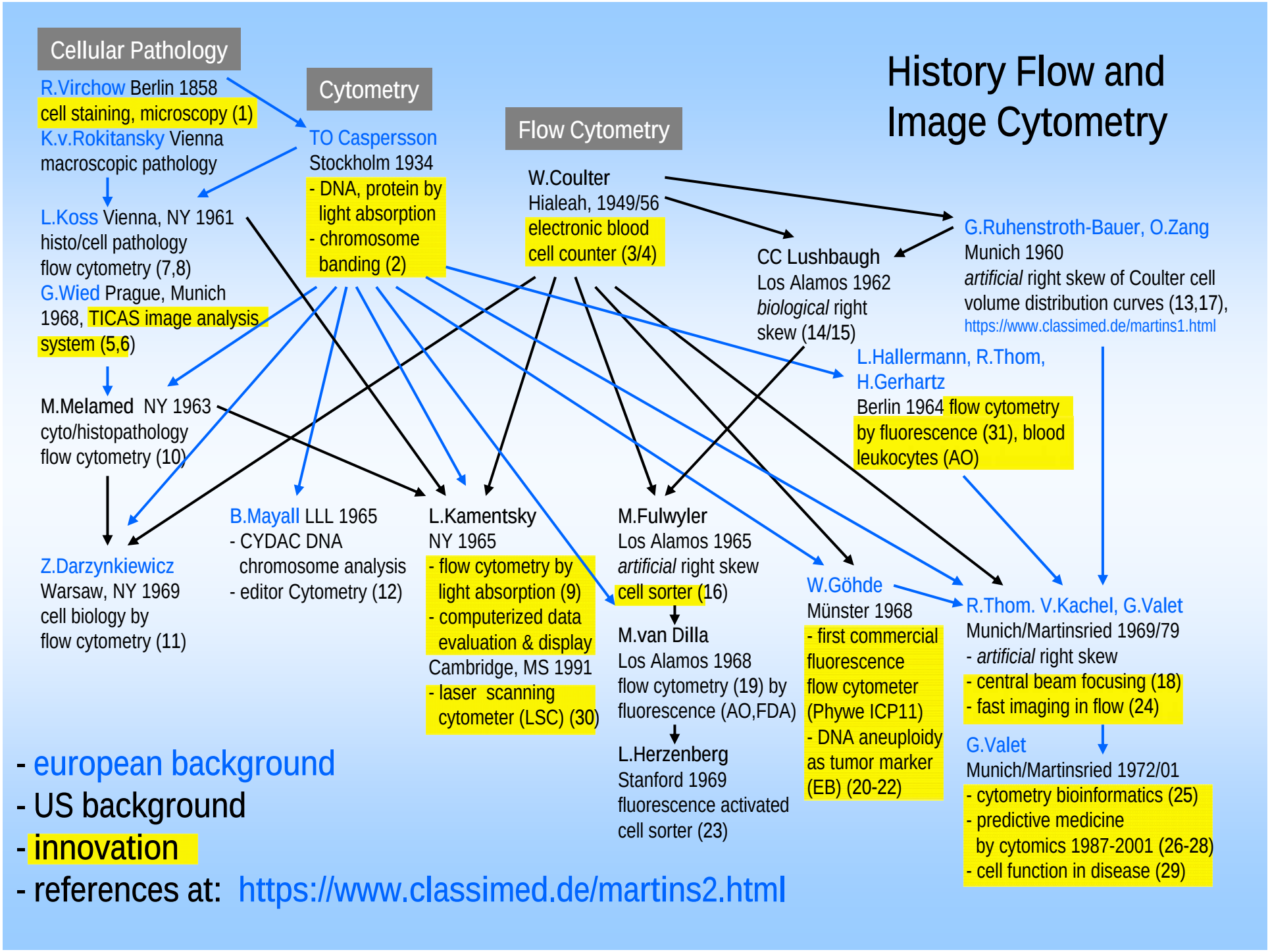
30th DGfZ Conference, Berlin, Oct 1-2, 2020

https://www.classimed.de/valdgz20.pdf

Major Phases of Early Flow Cytometry Development

- cytometer construction (arc lamp, laser, chip based, mass spectrum, imaging in flow…)
- DNA (cell cycle, DNA aneuploidy in cancers)
- antibodies (immunology: exploratory, clinical)
- cell functions like enzymes, pH, Ca2+, oxidation, reduction …)
- data analysis (display, information extraction, cluster calculations, predictive medicine by cytomics …)

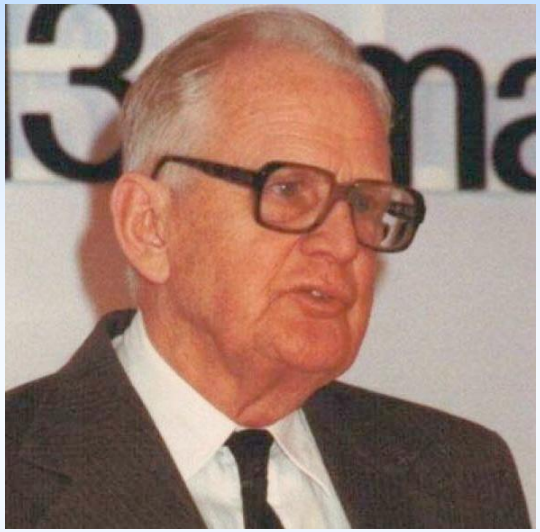
History Flow and Image Cytometry



- european background
- US background
- innovation
- references at: https://www.classimed.de/martins2.html

Fathers of Cytometry

Rudolf Virchow
(1821-1902)
-cellular pathology

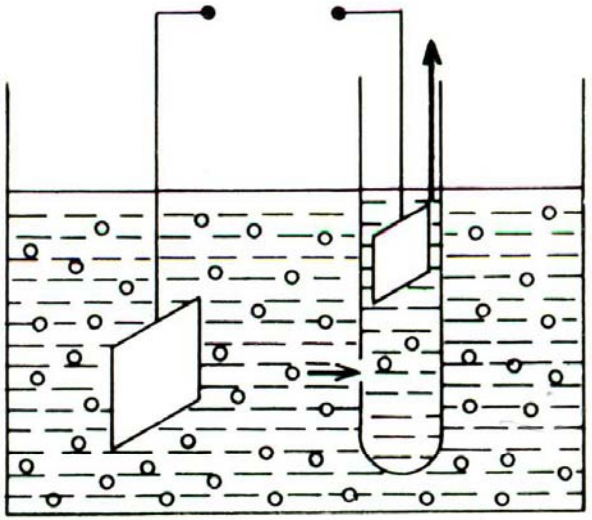


Torbjörn Caspersson
(1910-1997)
-cell DNA determination
by *image* cytometry
-chromosome banding

Wallace Coulter
(1913-1998)
-Coulter counter
-flow cytometry

Coulter Cell Counting and Sizing

Coulter model A counter 1956



$I = konst.$

$\Delta u = \Delta R \cdot I$

$\Delta R \sim$ Volumen des Partikels

$\Delta u \sim$ Volumen des Partikels

Coulter Cell Counting and Sizing

- undisputed counting (Ruhenstroth, Zang 1960)
- *right skew* of Coulter volume distribution curves:
  real or *artefact* ?

- *real:* C.Lusbaugh 1962 (reticulocytes)
- *artefact:*
  - M.Fulwyler 1965: *cell sorter* but *no* explanation
  - R.Thom, V.Kachel 1970: central beam *hydrodynamic focusing*, *fast imaging* in flow, explanation of right skew

Electronic Measurement of Cellular Volumes. II. Frequency Distribution of Erythrocyte Volumes

By C. C. Lushbaugh, N. J. Basmann and B. Glascock

Blood, Vol. 20, No. 2 (August), 1962

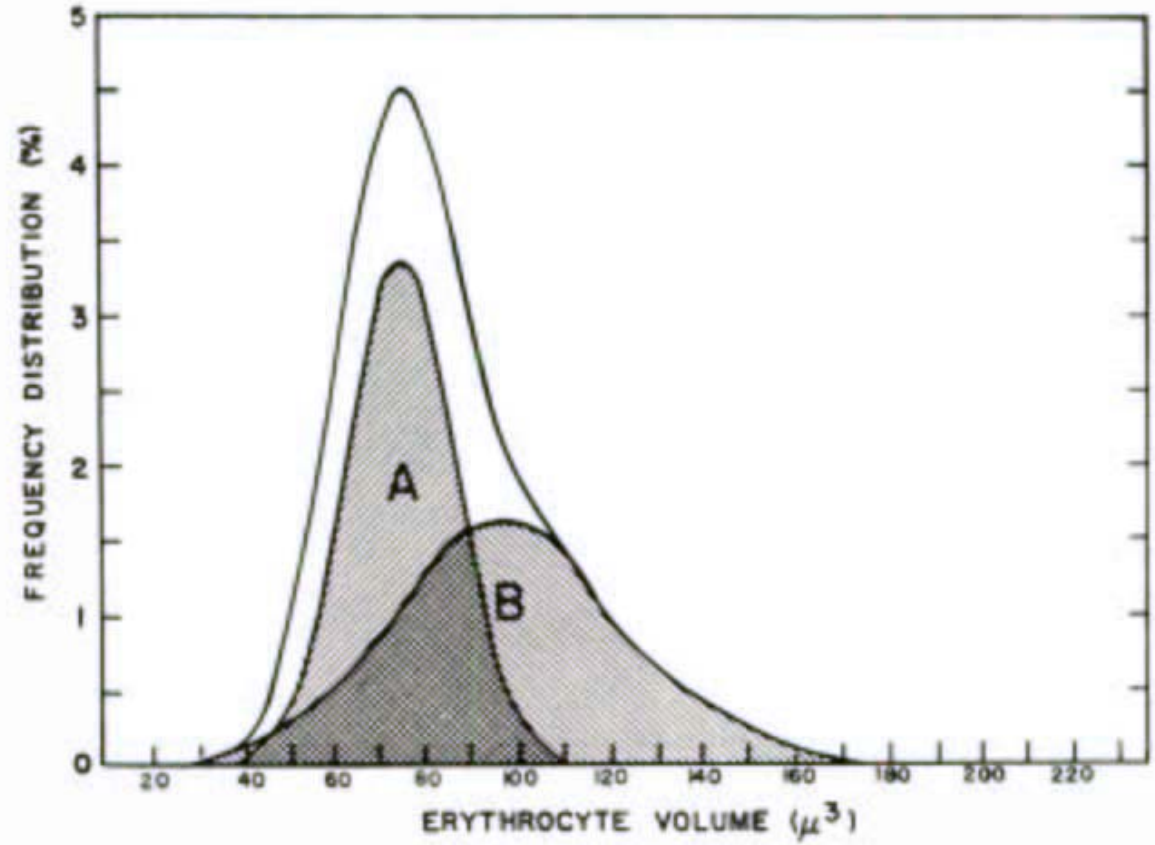
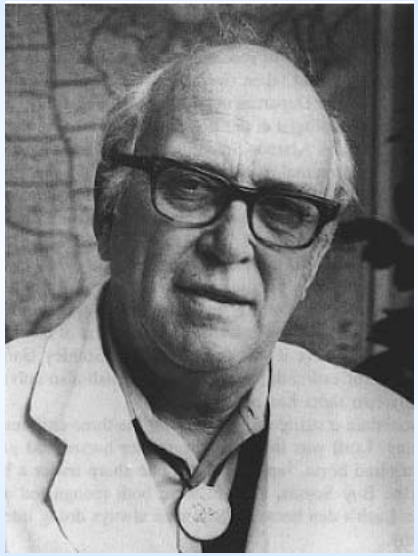


Fig. 2.—Frequency distribution curve of erythrocyte volumes of human blood and its representation by two cell populations (A and B), each with a normal Gaussian distribution.



Clarence C.Lusbaugh
(1916-2000)

Electronic separation of biological cells by volume
MJ Fulwyler
Science 150:910-911(1965)

Mac Fulwyler

Cell Sorter

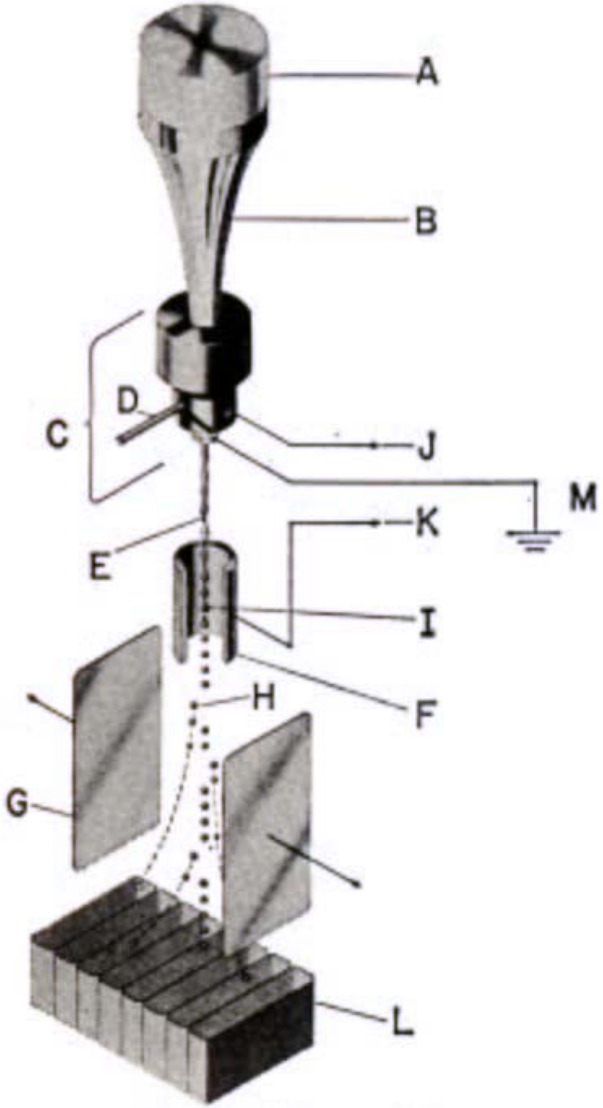


Fig. 1. Cell separator.

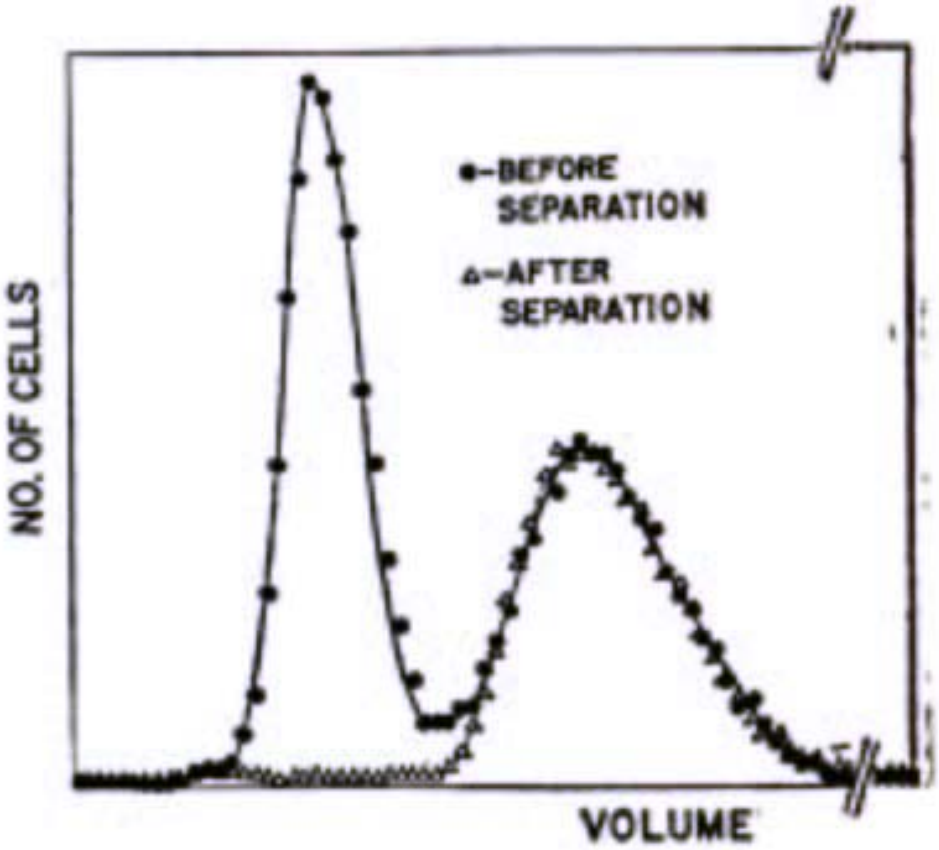


Fig. 2. Distribution by volume of mouse and human erythrocytes before and after separation.

Hydrodynamic Focusing

Blut, Band XXI, Seite 48–50 (1970)

KURZE MITTEILUNG

Aus der Med. Klinik und Poliklinik der Freien Universität Berlin im Städt. Krankenhaus Westend, und dem Max-Planck-Institut für Biochemie München

Fortschritte für die elektronische Größenbestimmung von Blutkörperchen

Von R. Thom und V. Kachel

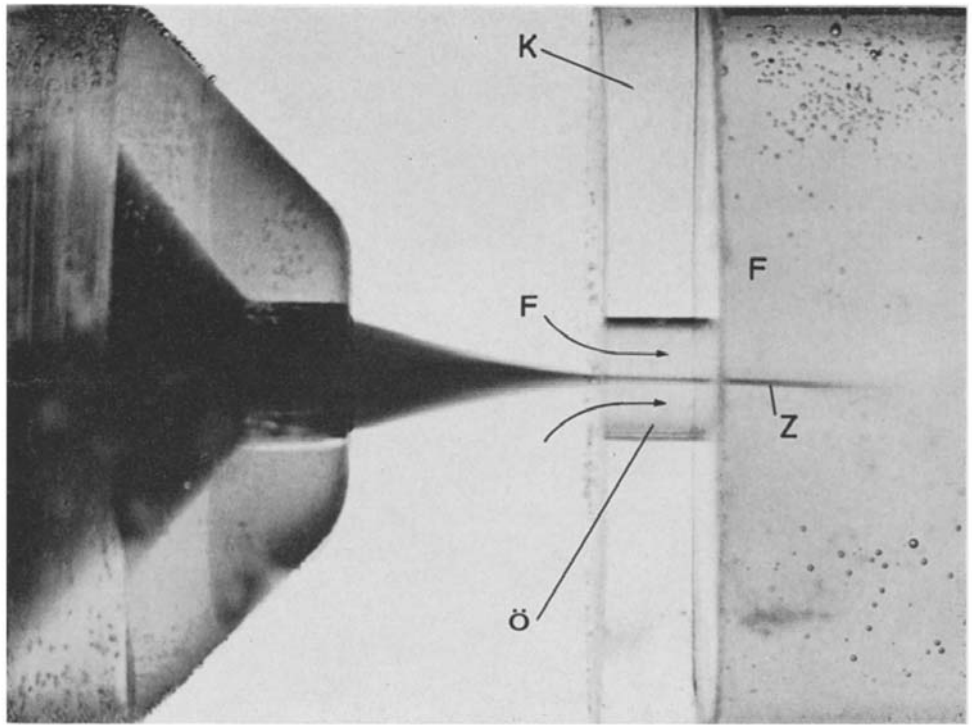


Abb. 1: Meßanordnung K = Kapillarwand; Z = Zentralstrahl, F = Ummantelnde Flüssigkeit; ö = Meßöffnung.

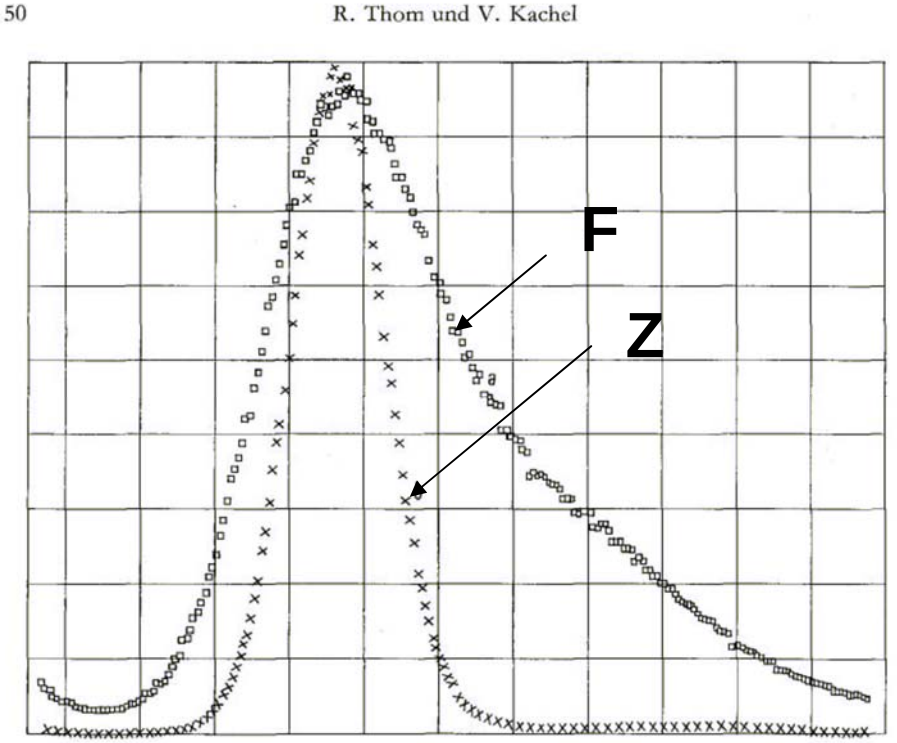


Abb. 3: Volumensverteilungskurven von nativen Erythrozyten. Messung in einer Coulter-Kapillare (100 μ) (a) und in der Zentralstrahlkapillare (b).

Reinhard Thom Volker Kachel

Fast Imaging in Flow (1979)

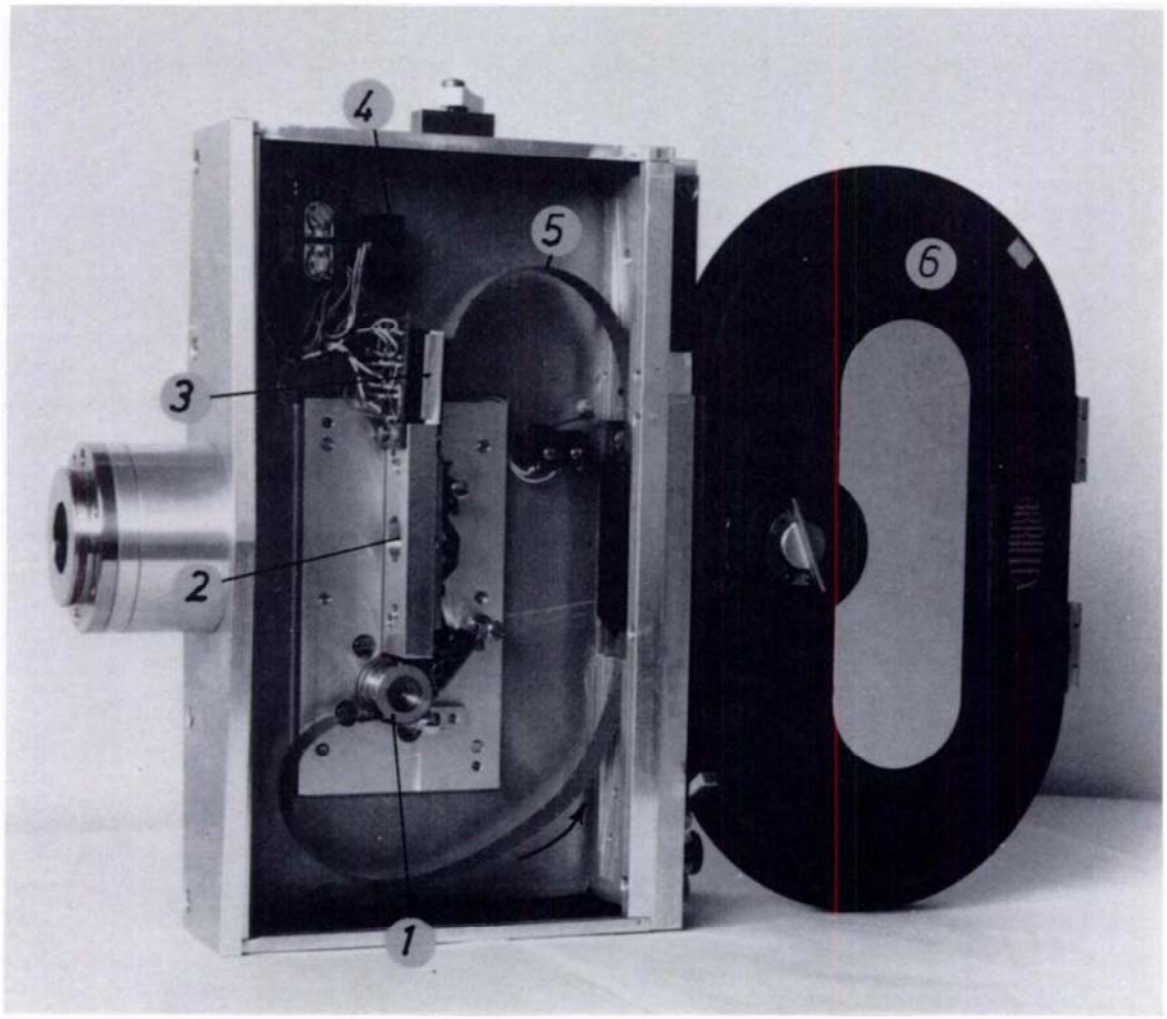


FIG. 4. The camera with Arriflex cassette. 1, sprocket, driven by the step motor; 2, picture gate; 3, three digit hexadecimal film marker; 4, infrared light barrier for controlling the film loop; 5, film loop; 6, Arriflex cassette; the direction of the film motion is indicated by the arrow.

V.Kachel, G.Benker, K.Lichtnau, G.Valet, E.Glossner. J.Histochem. 27:335-341 (1979)

Fast Imaging in Flow (1979)

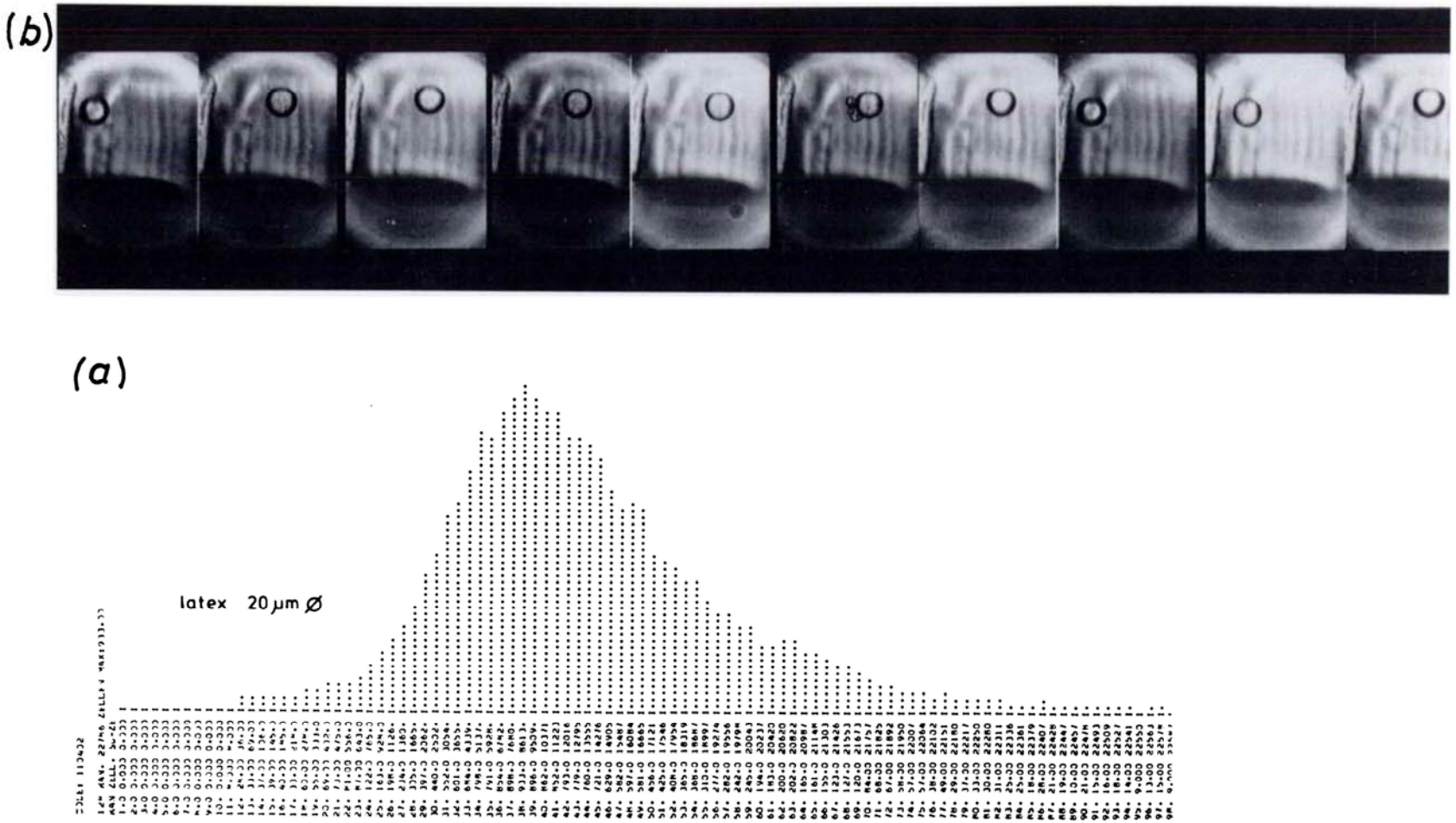
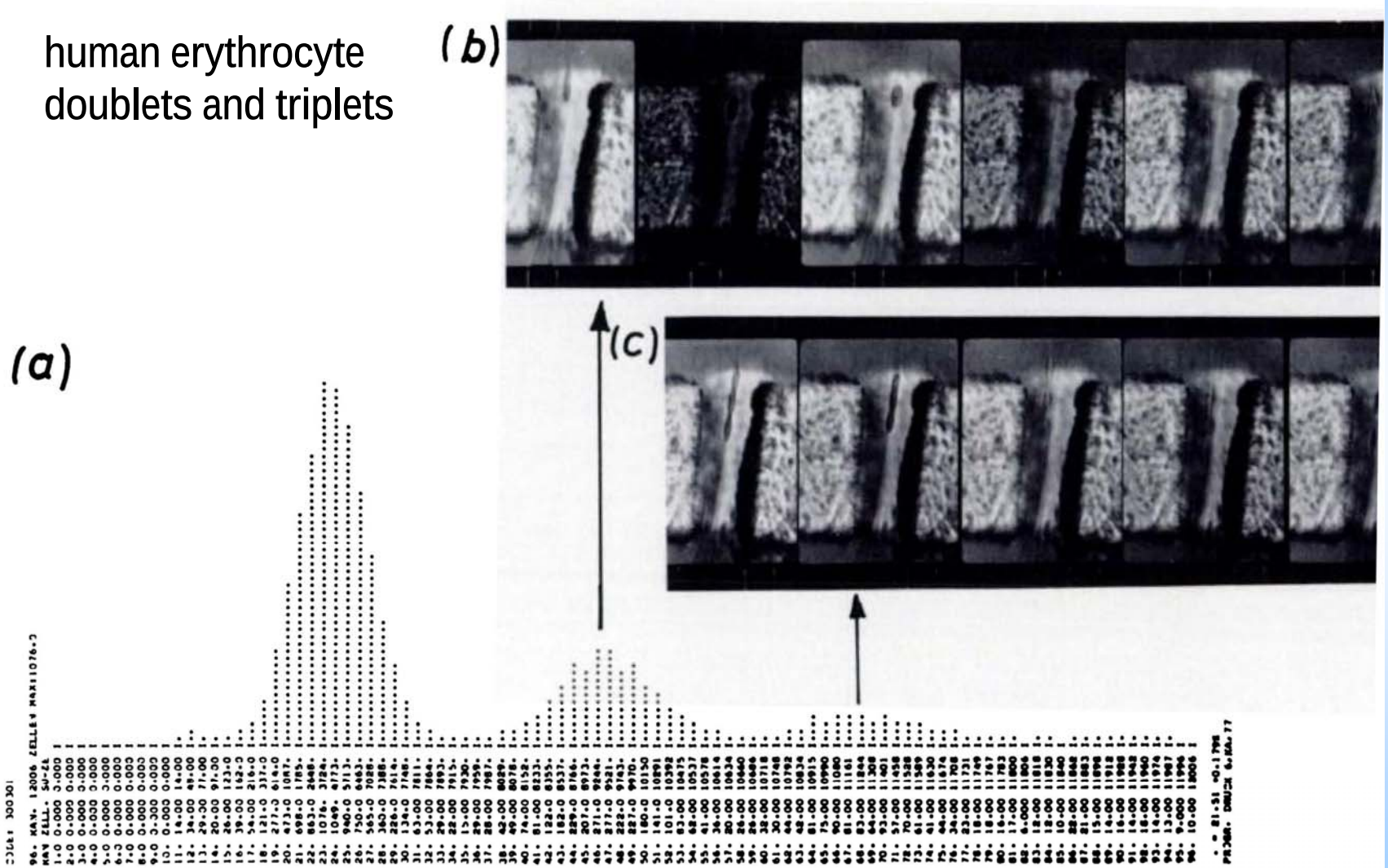


FIG. 7. (*a*), Volume distribution curve of 20 μm mean diameter latex particles measured with the imaging chamber. Channel width 120 μm. (*b*), Sequence of particles selected from the right slope of the distribution curve (large particles). Flow direction from bottom to top.

V.Kachel, G.Benker, K.Lichtnau, G.Valet, E.Glossner. J.Histochem. 27:335-341 (1979)

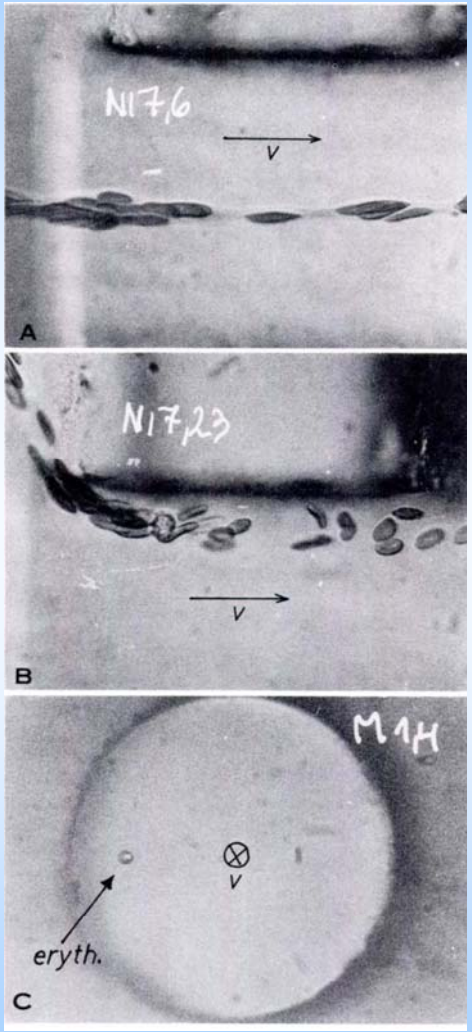
Fast Imaging in Flow (1979)

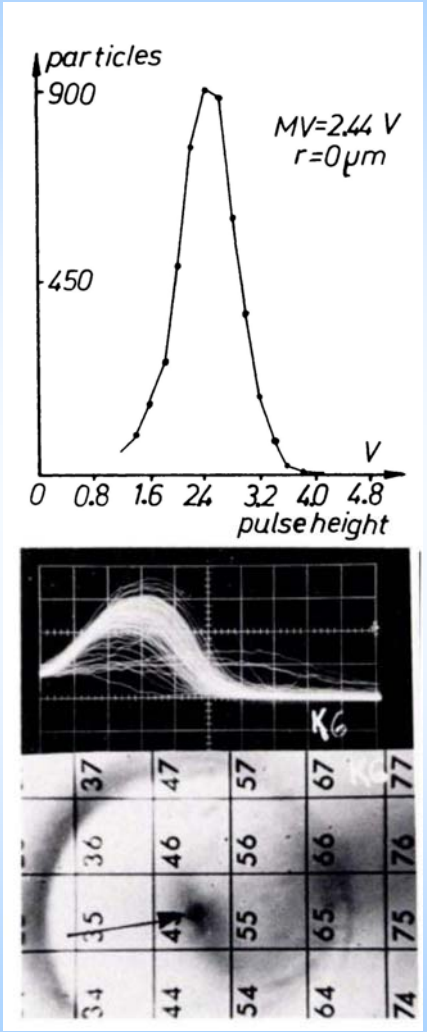
human erythrocyte
doublets and triplets

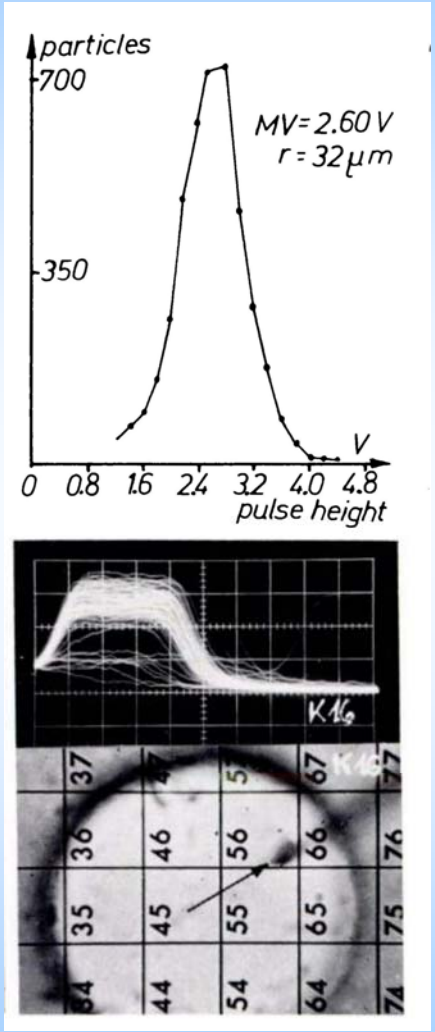


V.Kachel, G.Benker, K.Lichtnau, G.Valet, E.Glossner. J.Histochem. 27:335-341 (1979)

Fast Imaging in Flow (1979)







- **hydrodynamic focusing**
- **particles remain in *constant* electric field**
- **result: *symmetric* volume distribution curves (right)**
- **elongation of native erythrocytes to cigars in the orifice center (left a, c) but tumble over the orifice edges (b)**

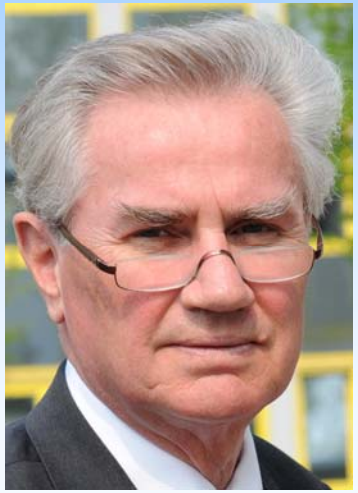
V.Kachel J.Histochem.Cytochem 24:211-230 (1976)

Flow Cytometer Development in Germany

- Five *commercial* flow cytometers were developed in German *scientific institutions* (1969-84).
- Only Partec (Münster), since 2013 as Sysmex-Partec (Görlitz) survived

Phywe (Göttingen) ICP-11 (1969)

High pressure mercury arc lamp, 2 fluorescence channels



Wolfgang Göhde,
Universität Münster

-first commercial fluorescence flow cytometer

-acquired by Ortho Diagnostics (Raritan, NJ)

-market removal

Continued Activities with Partec (1985)

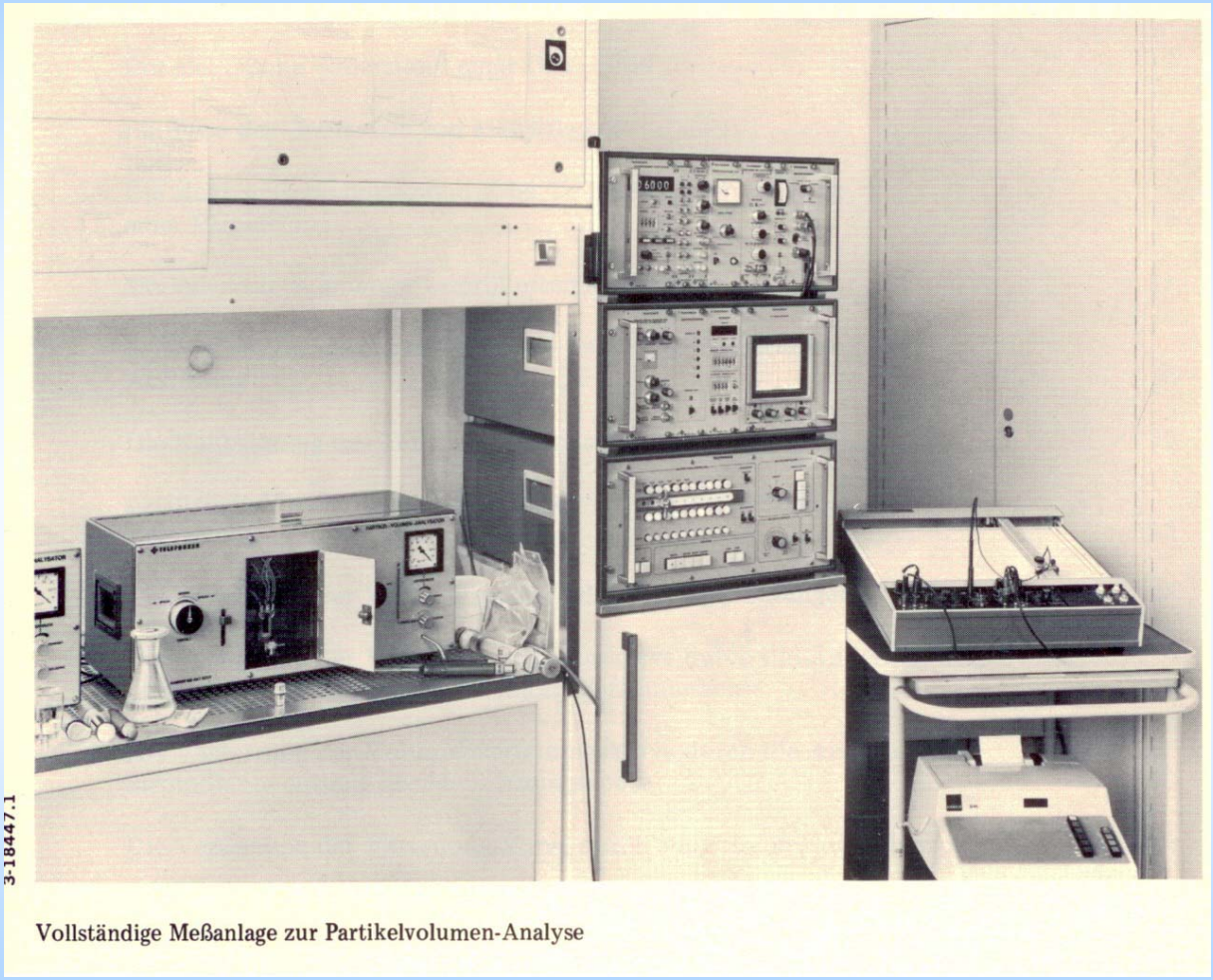
PAS-III flow cytometer

- Ortho had also acquired all Phywe flow cytometry patents, being internationally valid until 1988, with the exception of *Switzerland* where *Wolfgang Göhde* continued flow cytometer development (PAS-I, PAS-II) with his own Partec company, moving upon patent expiration to *Münster* (PAS-III) after 1988 and in part to *Görlitz* following the German reunification in 1989.

- Partec was acquired 2013 by Sysmex (Kobe, JP) and continues activities as Sysmex-Partec

Hydrodynamically focused Coulter cell volume, laser FSC, SSC,
2 or 3 fluorescence channels, piezo driven fluidic cell sorting chamber

AEG-Telefunken (Berlin) MPV-1 (1982)



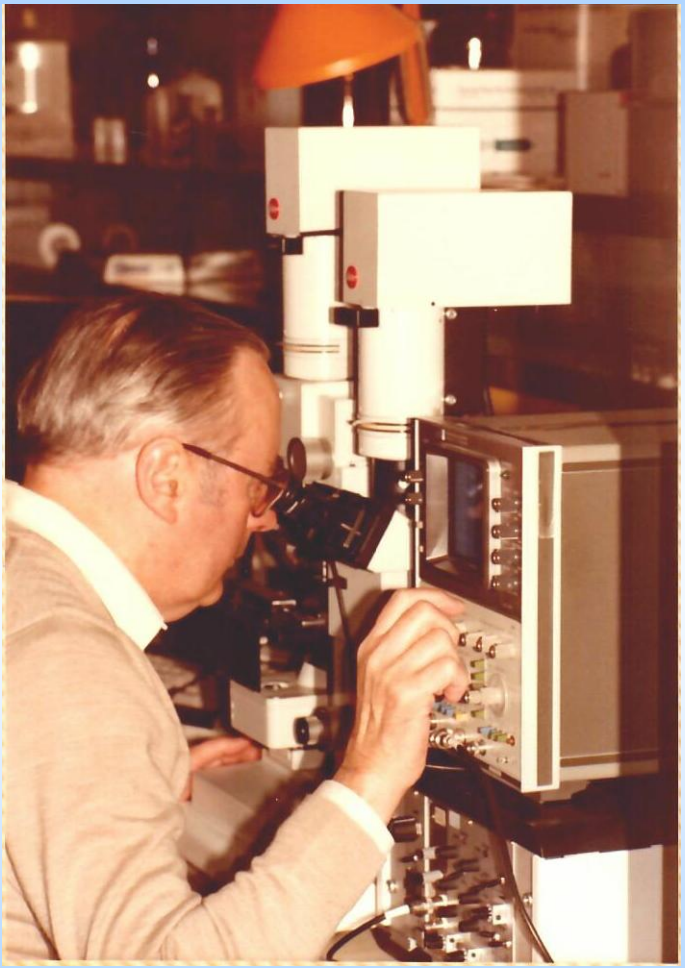
Vollständige Meßanlage zur Partikelvolumen-Analyse

Reinhard Thom
Westendkrankenhaus
Berlin sowie
A.Hampe, G.Sauerbrey
Physikal.Techn.Bundesanstalt
Braunschweig

- acquired by Coulter Electronics (Hialeah, Fl)
- market removal

1 channel hydrodynamically focused Coulter cell volume instrument

Leitz (Wetzlar) MPV-Compact Flow Cytometer (1982)

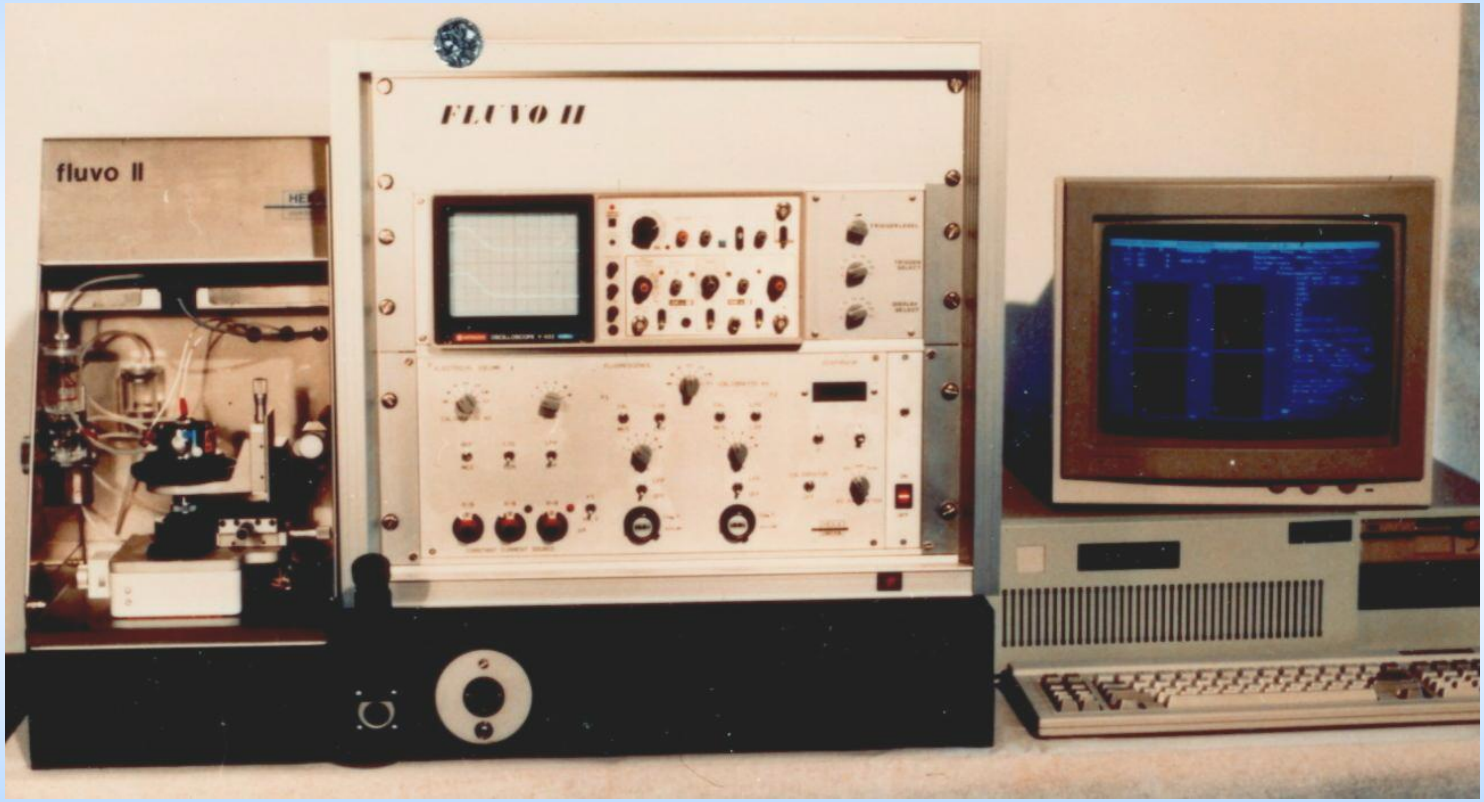


Harold Steen
Oslo (Norway)

This flow cytometer was particulary conceived for small particle analysis, like bacteria, cell nuclei, or mitochondria

High pressure mercury arc lamp, 2 light scatter + 2 fluorescence channels

HEKA (Lambrecht/Pfalz) FLUVO-Metricell (1983)



Volker Kachel
Max-Planck Inst.
für Biochemie
Martinsried

Hydrodynamically focused Coulter cell volume with high pressure mercury arc lamp and 2 fluorescence channels

Kratel Instruments (Böblingen) Flow Cytometer (1984)

Wolfgang Eisert Wolfgang Beisker

Gesellschaft für Srahlenforschung (GSF, Hannover)

- The instrument was particularly conceived for precise particle length measurements and small particle analysis (clean water in clean room environments)

Argon laser excitation, 2 fluorescences

Cytometry Organizations

-Flow and image cytometers, software, stains and applications were developed in the 1970s by around 150-200 **scientists** worldwide mostly from US, Germany and Norway.

-The **Phywe ICP-11** flow cytometer introduction stimulated preferentially *clinical* tumor cell DNA aneuploidy measurements, resulting in a boost of publications (60 mostly German versus 44 searchable US publications between 1969-76).

-**Duplicate** result presentations at European and US cytometry meetings prompted 1978 the **foundation** of the Society for Analytical Cytology (SAC) at the American Engineering Foundation meeting in *Schloss Elmau* near *Mittenwald* (Germany), conceived by *Sandford Cole* and organized by *Klaus Goerttler* Deutsches Krebsforschungs-zentrum Heidelberg (DKFZ)

Foundation of the Society for Analytical Cytology

European effort

motivator: W.Göhde

Phywe ICP-11/22 mercury arc cytometer

1972 Heidelberg (M.Andreeff)
1973 Nijmegen (C.Haanen)
1975 Münster (W.Göhde)
1977 Vienna (D.Lutz)
1979 Voss (O.Laerum)
1980 Rome (F.Mauro)

American Engineering Foundation

conference organizer: Sandford S.Cole

BioPhysics/Ortho/Coulter/BD laser cytometers

1972 Saxton River, VE
1973 Asilomar, CA
1975 Asilomar, CA
1976 Pensacola, FL
1978 Schloß Elmau/Mittenwald

foundation of the Society for Analytical Cytology (SAC)
& journal Cytometry
in Elmau 1978, first meeting 1981
in Wentworth by the Sea (NH)

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SAC Founding Committee at Schloss Elmau, Germany 1978

back: Myron Melamed, Scott Cram, Sandford Cole, Mort Mendelsohn, Hans Aus, Klaus Goerttler, Jim Tucker, Paul Mullaney, Volker Kachel, unidentified, Brian Mayall, Mac Fulwyler

front: Leon Wheeless, Ted Young, Marvin vanDilla, Dennis Rutovitz, Tom Jovin

Centralized SAC or International Cytometry Network ?

- Fast flow cytometer development, high US company investments and instrument sales **increased** SAC US membership within a few years to around 600 with SAC and later ISAC understanding itself as an international scientific **marketing** organization for US cytometers with affiliated national societies.

- **European** cytometry scientists strengthened their position by founding
  - ESACP (1986) and the ACP journal (today **Cellular Oncology**, IF 4,19)
  - national cytometry societies like DGfZ (1989)
  - by setting up the worldwide first *cytometry society* Internet servers (ESACP, DGfZ 1994) and
  - by organizing the first European flow cytometry course for scientists (**Martinsried** 1985-1993) with initially *only* European cytometers

- Today, the national cytometry societies are associated with ISAC as an international **cytometry network**

1.-6.Martinsried Flow Cytometry Courses (1985-1993) for >200 scientists

PROGRAM

1.MARTINSRIED FLOW CYTOMETRY COURSE

4/15-4/19/1985

photographs by *Rudi Kratel*:
https://www.classimed.de/mk85phot.pdf

Mildred-Scheel-Laboratory for Cancer Cell Research
Max-Planck-Institute for Biochemistry
8033-Martinsried near Munich

ESACP

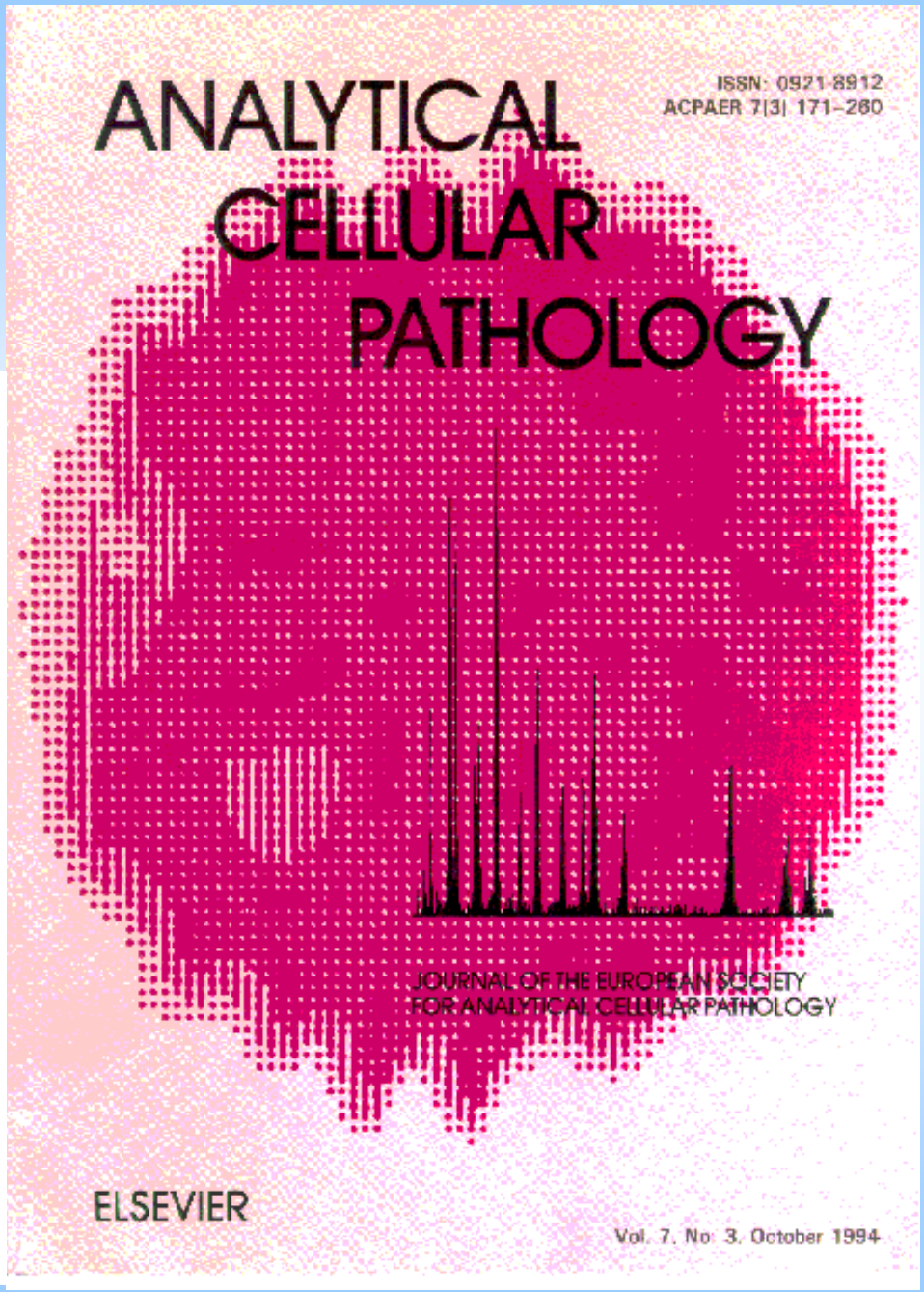
1986: foundation of

European Society for Analytical Cellular Pathology (ESACP) by:
G.Auer, P.Benedetti, G.Brugal, G.Burger, Y.Collan, C.J.Cornelisse, O.A.N.Husain, A.Reith, F.Rilke, D.Rutovitz, E.Sprenger, B.Stenkvist, G.Valet, P.Viallet, L.Vindelov, G.P.Vooijs.

with the ACP journal
G.Burger, G.Valet, P.Vooijs, G.Brugal

continued since 2003 as:

International Society for Cellular Oncology (ISCO) with the Cellular Oncology journal (IF 4.191 in 2019)



DGfZ Foundation 1989

foundation committee
K.Goerttler
C.Cornelisse
G.Feichter
W.Göhde
H.Hoehn
F.Otto
A.Radbruch
G.Valet

membership cohesion
DGfZ treasurer
1990/2010
P.Schwarzmann

DGfZ presidents
90/92 K.Goerttler
92/94 G.Valet
94/96 A.Radbruch
96/98 J.Hemmer
98/00 M.Nüsse
00/02 R.Knüchel
02/04 M.Stöhr
04/06 A.Tarnok
06/08 G.Brockhoff
08/10 S.Müller
10/12 E.Endl

meetings
Heidelberg 1988-2004
K.Goerttler
M.Stöhr
K.Hutter
H.zur Hausen

Leipzig
A.Tarnok 2005/06
S.Müller 2009/10

Regensburg 2007
G.Brockhoff

Bremen 2008
G.Rothe

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Future

- Basic research: more parameters, miniaturization, mass cytometry, multidimensional data analysis ...
- Medicine: exploitation of the systemic potential of cytometry (system cytometry*) like predictive medicine by cytomics
- Concept: diseases are caused by molecular changes in cells and tissues (*cells know it first*). Molecular cell data patterns permit individualized disease course predictions

*) http://www.cyto.purdue.edu/cdroms/cyto3/8/valet/keyvirt1.htm (1997)

Basic Research

Dimension reduced multiparameter cluster display in vaccine development

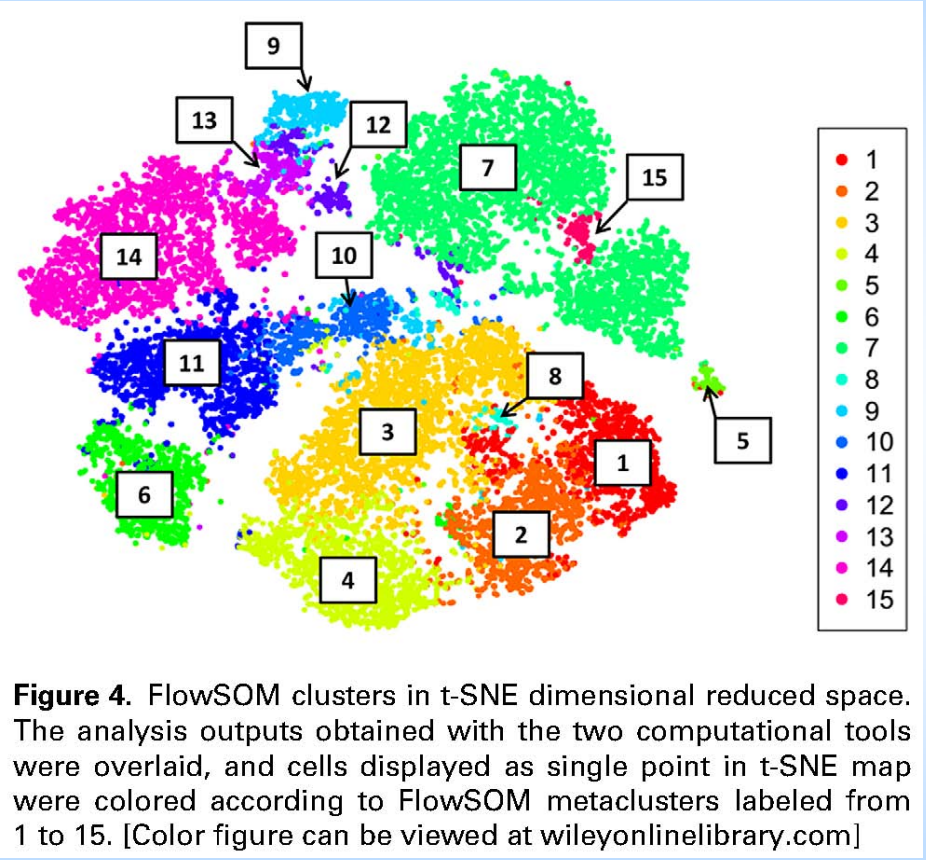


Figure 4. FlowSOM clusters in t-SNE dimensional reduced space. The analysis outputs obtained with the two computational tools were overlaid, and cells displayed as single point in t-SNE map were colored according to FlowSOM metaclusters labeled from 1 to 15. [Color figure can be viewed at wileyonlinelibrary.com]

Lucchesi S, Nolfi E, Pettini E, Pastore G, Fiorino F, Pozzi G, Medaglini D, Ciabattini A. Computational Analysis of Multiparametric Flow Cytometric Data to Dissect B Cell Subsets in Vaccine Studies. Cytometry (2020) 97A: 259–267

Medicine

- Goal: *Individualized* predictions for time course, outcome and disease occurence in patients
- Advantage cytometry: no compartment mixing and easier small entity detection than in genomics or proteomics
- Problem: Actual clinical therapy planning is group (like *Kaplan-Meier* statistics) and *not individual* patient oriented
- Improvement: Use of algorithmic data pattern predictions for *individual* patients instead of multidimensional statistics for patient groups.

https://www.classimed.de/classif1.html

Medicine

- **Status**: „***Over the past 20 years, there has been an exponential increase in the number of biomarkers. At the last count, there were 768,259 papers indexed in PubMed.gov directly related to biomarkers. Although any of these papers report clinically useful molecular biomarkers, embarassingly few are currently in clinical use.***“

(H.B.Burke, Biomarkers in Cancer 2016 8:89-99)

Statistics for Group oriented Gene Associations

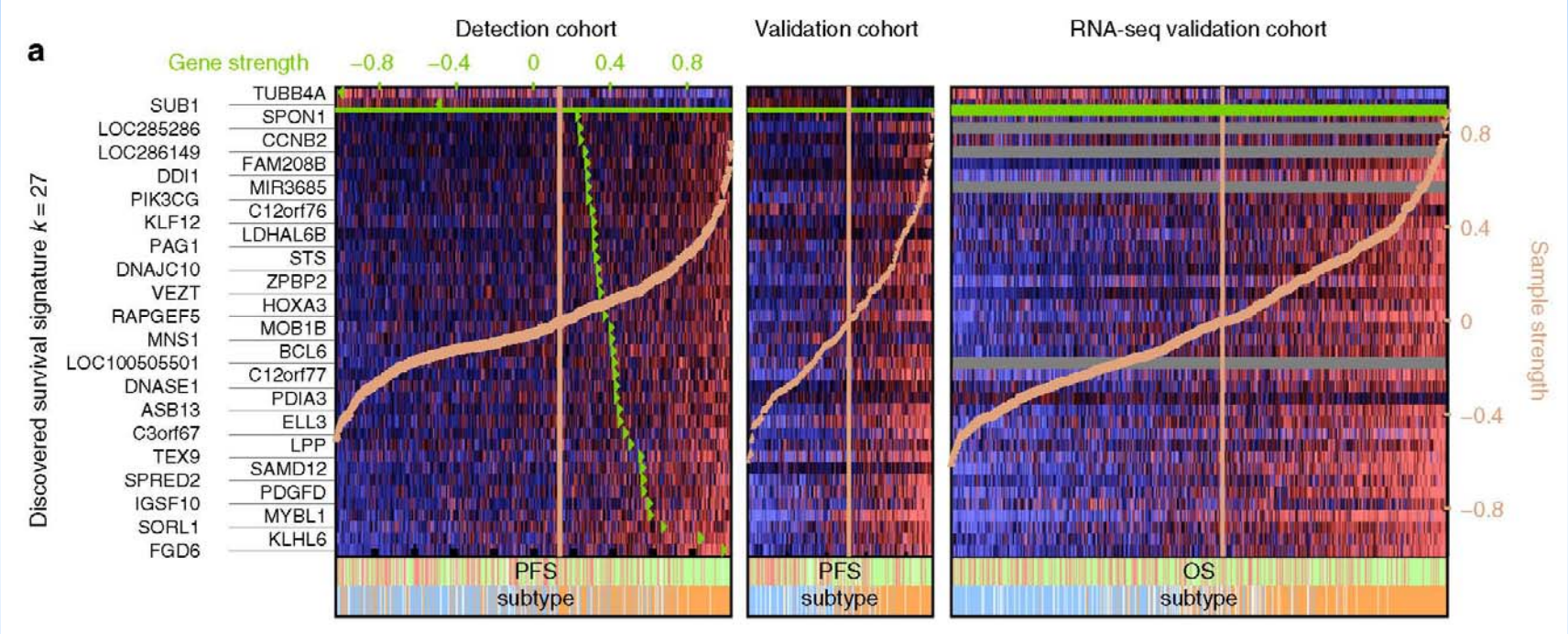
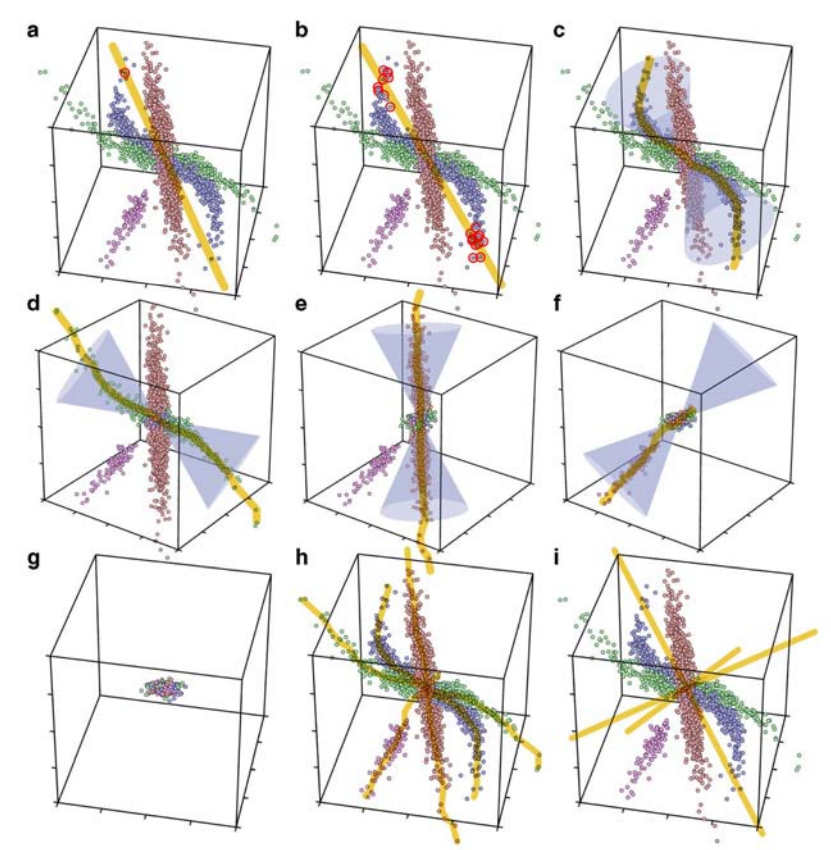


Fig. 5 Discovered survival signatures in diffuse large B cell lymphoma (DLBCL, RNA microarrays).

Grau M, Lenz G, Lenz P. **Dissection of gene expression datasets into clinically relevant interaction signatures via highdimensional correlation maximization. Nature Comm (2019) 10:5417**

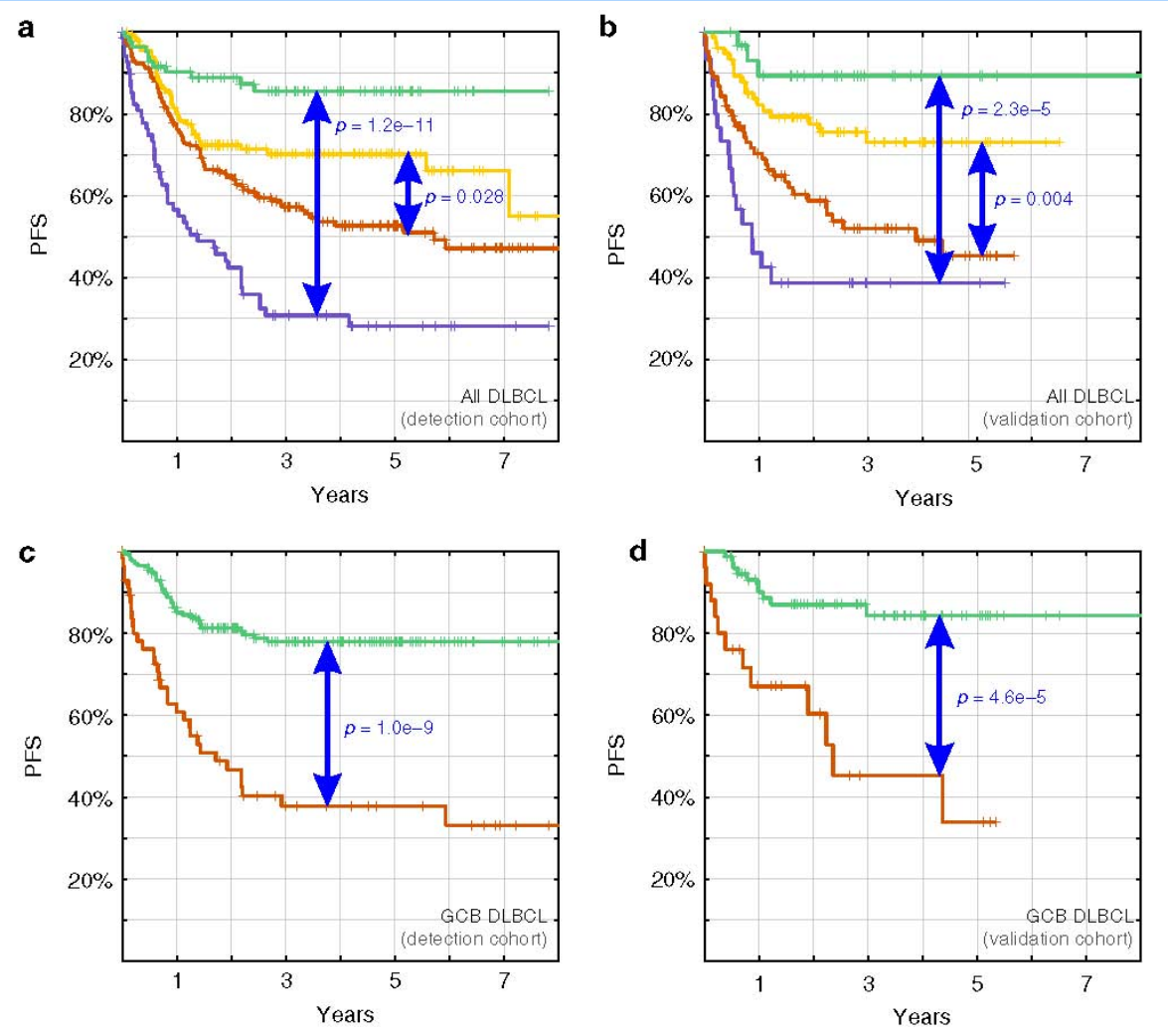
Statistics for Group oriented Gene Associations



Diffuse Large B-Cell Lymphoma (DLBCL)

Fig.1 Concepts of SDCM (Signal Detection by Correlation Maximation) illustrated by a 3-dimensional example.

Statistics for Group oriented Gene Associations



Diffuse Large B-Cell Lymphomas (DLBCL)

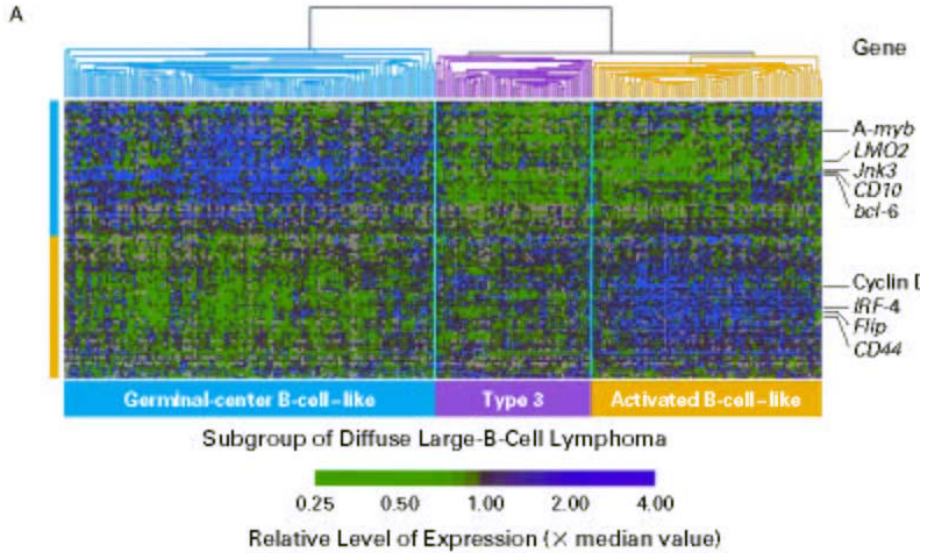
Problems with Group Statistics

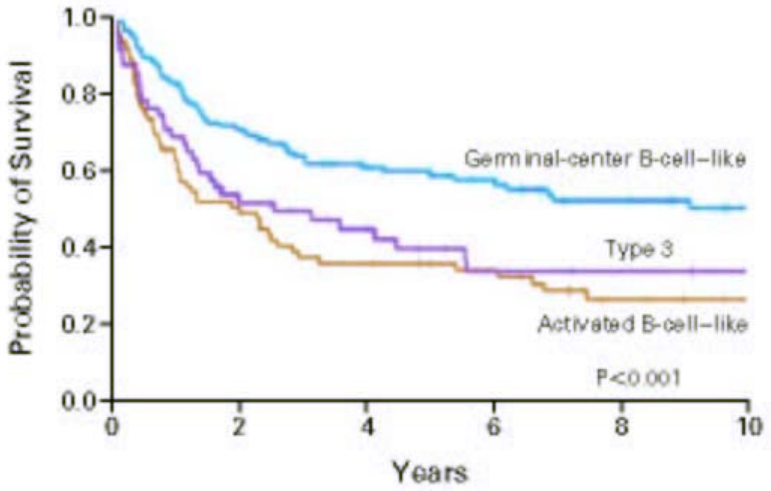
- only a fraction of patients profits from therapy, the others may suffer from therapeutic side effects
- non reactive patients cannot be identified in advance with no possibility for early alternative therapies
- patients are clearly more interested in *individual* disease course and outcome than in group statistics
- gene patterns of *individual* patients seem more informative for therapy and also for new hypotheses development on disease generating mechanisms
- Is it possible to achieve this ? Yes, it is !

Hierarchical Classification for Patient Groups

Diffuse Large B-Cell Lymphoma (DLBCL)

Rosenwald A et al NEJM 346:1937-47 (2002)





hierarchical classification according to Kaplan Meier group statistics

- germinal center B-cell lymphomas
- type 3
- activated B-cell like

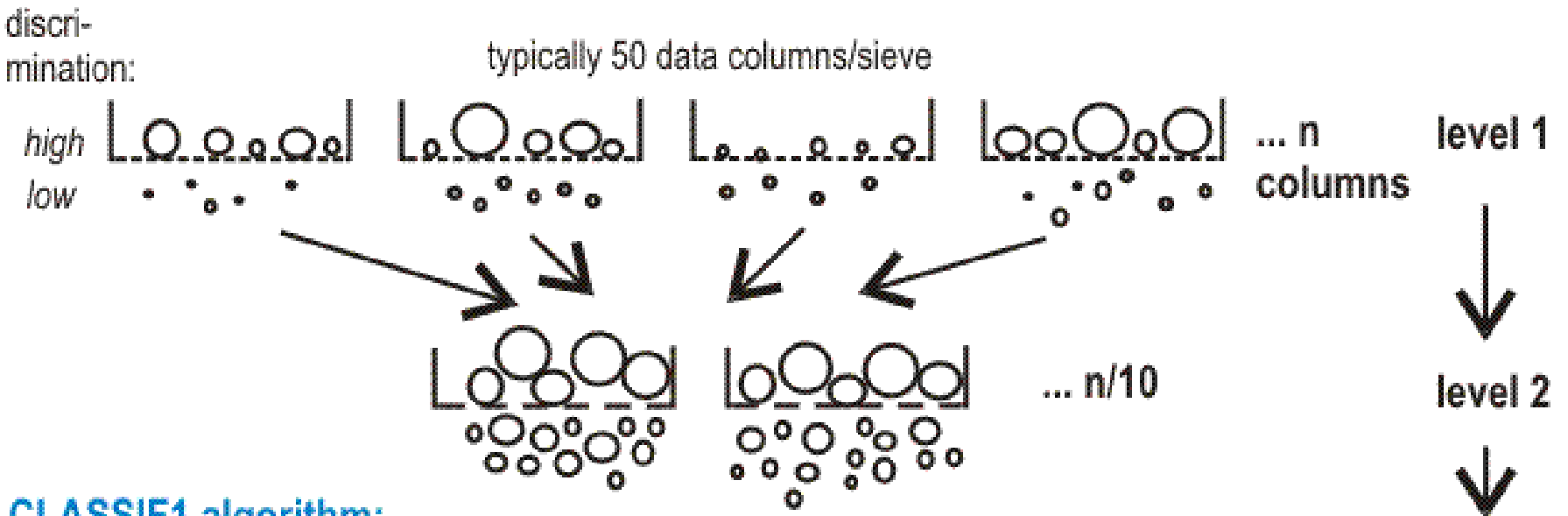
“Lymphochip“ RNA expression search using 7399 DNA sequencs microarray

Algorithmic Classification of Individual Patients

by data pattern analysis:

- unsupervised
- non hierarchical
- no statistics
- no neuronal network
- no mathematical assumptions
- robust against missing values and outliers
- standardizable

Information Enrichment by Data Sieving



CLASSIF1 algorithm:

- sequential data partitioning into 50 parameter (data columns) wide data sieves
- select 5 most discriminatory parameters in each sieve by percentile analysis
- combine selected parameters, repartition, sieve & repeat procedure until 50 columns or less remain
- classify remaining data columns for most discriminatory triple matrix data pattern
- algorithm characteristics: surface data mining (no models), unsupervised exhaustive knowledge extraction to access unknown knowledge spaces

GVAL

Data Pattern Classification of Individual Patients

DLBCL Outcome Prediction

data from: NEJM 346:1937-47(2002)

mask pos nr	parameter & UniGene access number	S	NS
5	glutathione synthetase |Hs.82327|	- 0	+
8	MAD mothers against decapentaplegic homolog 4 (Drosophila) |Hs.75862|	- 0	+
10	bone morphogenetic protein 7 (osteogen.prot.1) |Hs.170195|	- 0	+
17	caspase 6, apoptose-related cystein protease |Hs.3280|	- 0	+
20	intercellular adhesion molecule 2 |Hs.347326|	- 0	+
21	chemokine (C-X3-C) receptor 1 |Hs.78913|	- 0	+
23	lymphocyte antigen CD117 |Hs.88411|	- 0	+
1	MAD mothers against decapentaplegic homolog 5 (Drosophila) |Hs.37501|	0 +	-
7	nuclear receptor subfam.3, group C, memeber 3 |Hs.75772|	0 +	-
11	HLA-DPalpha1 |Hs.914|*H60848	0 +	-
12	HLA-DPalpha1 |Hs.914|*H62848	0 +	-
13	solute carrier fam.2(facil.glucose transporter) memb.3 |Hs.7594|	0 +	-
15	IFNg inducible protein 30 |Hs.14623|	0 +	-
16	fructose-1,6-biphosphatase 1 |Hs.574|	0 +	-
18	CD9 antigen (p24) |Hs.1244|	- 0	+
19	adenosine kinase |Hs.94382|	- 0	+
2	|||LC_28024	- 0	+
3	DKFZP434F2021 protein |Hs.78277|	- 0	+
4	ESTs |Hs.22635|	- 0	+
6	hypothetical protein MGC4189 |Hs.334808|	- 0	+
9	h.sapiens mRNA, cDNA DKFZp586L 141 |Hs.140945|	- 0	+
22	|||LC_20218	0 +	-
14	hypothetical protein FLJ10116 |Hs.79741|	0 +	-
24	ESTs weakly similar to ALU1_human ALU subfamily J (h.sapiens) |Hs.159556|	0 +	-
val/hoe			

- 24 gene data pattern selected from 7399 spot DNA microarray by data sieving
- Individualized data patterns coincide only partially (HLA-DPalpha1) with group patterns

Valet GK, Hoeffkes HG
Data pattern analysis for the individualised prethe-rapeutic identification of high-risk diffuse large B-cell lymphoma (DLBCL) patients by cytomics Cytometry (2004) 59A: 232–236

Data Pattern Classification of Individual Patients

Pretherapeutic Risk Assessment in Diffuse Large B-Cell Lymphoma Patients

classify: 7399 array parameters

learning set

clinical outcome	pat. (n)	CLASSIF1 prediction (%) surv	non surv
survival	71	98.6	1.4
non surv.	86	39.5	60.5
neg/pos predval		67.3	98.1

unknown test set

clinical outcome	pat. (n)	CLASSIF1 prediction (%) surv	non surv
survival	29	82.8	17.2
non surv.	47	61.7	38.3
neg/pos predval		45.3	78.3

25-75% percentile thresholds, S1R12P25.BI4

classifiable patients: learn: 157/160 (98.1%), test: 76/80 (95.0%)

Discrimination more informative than Correlation

Individualized Pretherapeutic Risk Assessment in Diffuse Large B-Cell Lymphoma Patients

Data pattern classification of 54630 chip spot parameters (a,b) versus 105 correlation gene signatures (c,d)

a) chip spot parameter classification				**c)** signature classific.	
category	patients	surv(%)	nsurv(%)	surv(%)	nsurv(%)
survivor	151	spec **98.7**	fpos 1.3	**80.1**	19.9
non surv	84	fneg 46.4	sens **53.6**	54.8	**45.2**
predval(%)		neg **79.3**	pos **95.7**	**72.5**	**55.9**
b) test set validation				**d)** test set validation	
survivor	152	**92.7**	7.3	**73.0**	27.0
non surv	83	73.5	**25.5**	60.0	**40.0**
predval(%)		**69.8**	**66.7**	**69.0**	**44.6** gval21

abbreviations: spec=specificity, sens= sensitivity, fp/fn=false positive/ negative, neg/pos= negative/positive predictive values

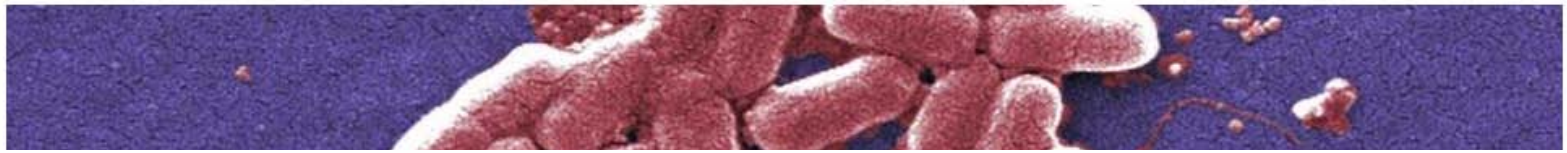
Sepsis

KE Rudd, SC Johnson, KM Agesa et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study.
Lancet (2020) 395:200-211.

Stern Januar 2020

GEFAHR IM BLUT

Sepsis: Jeder fünfte Todesfall weltweit geht auf das Konto des "unbekannten Killers"

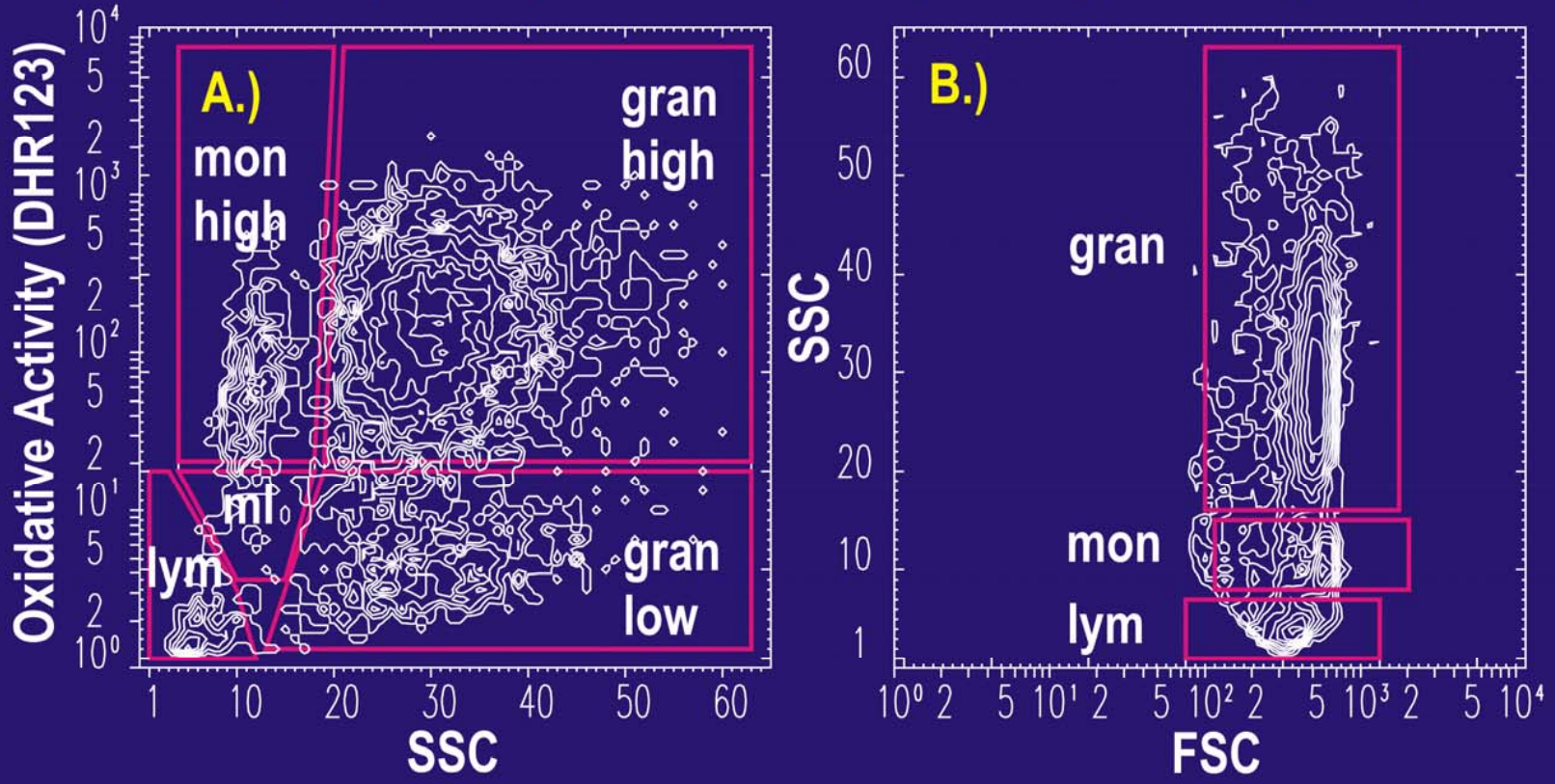


20.01.2020, 20:06 Uhr

Eine Sepsis ist früh erkannt gut zu behandeln, doch verläuft oft tödlich, weil Symptome falsch gedeutet werden. Eine Studie zeigt nun, dass die Krankheit weit mehr Menschen betrifft als bislang angenommen.

Oxidative burst (DHR123) in viable buffy coat blood leukocytes (30min,heparin)

Septic patient, PMA stimulation (100nM), DHR123 (1uM)



Survival prediction (3-15d) for intensive care patients on admission (0d)

#	selected parameters (DHR123)	S0	D0
1	SSC lym (F)	0+	-
2	DHR/FSC high DHR mon (F)	0+	-
3	DHR/FSC all mon (F)	0+	-
4	SSC/FSC all grn (F)	0+	-
5	SSC lym (F+T)	0+	-
6	DHR/FSC lym (F+T)	-0	+
7	% mon of all vital cells (F+T)	-0	+
8	FSC low DHR grn (F+T)	-0	+
9	DHR/FSC low DHR grn (F+T)	-0	+

30-70% percentiles, 9 out of 90 parameters (LSLEARN.BI4)
F = FMLP, F+T = FMLP+TNFalpha

gval

Sepsis Risk: Data Pattern Analysis for Individual Patients

Nr.	classification category	category abbreviation	class coinc	ref.classifi-cation masks
1	survivor	S0	1.00	000000000 ++++++----
2	non surv.	D0	1.00	----++++

Nr.	clinical outcome patients: LSLEARN.BI4	CLASSIF1 prediction	class coinc factor	patient classification. masks (.=no value)
2	KE1343 S0	S0	0.67	00+0-0+0+
3	KE1344 S0	S0	0.67	-000--+00
4	KE1349 S0	S0	0.67	+-0-+--+-
5	KE1367 S0	S0	0.78	+00+0-+-+
7	KE1378 S0	S0	0.89	0++00000+
8	KE1379 S0	S0	0.67	00--000+0
10	KE1386 S0	S0	0.78	+0-0+00+-
12	KE1296 S0	S0	0.67	0++-+++-0
13	KE1298 S0	S0	0.89	0+00000-+
14	KE1299 S0	S0	0.78	0+0+---+-
16	KE1277 S0	S0	0.78	--0+00-00
17	KE1292 S0	S0	0.67	-0++-+000
18	KE1398 S0	S0	0.56	+--0++-+0
		mask column coincidence (%)		777767666 777797929
1	KE1334 D0	D0	0.67	---0-+0+0
6	KE1376 D0	D0	0.89	-----+0++
9	KE1380 D0	D0	0.56	+0--0++0+
11	KE1382 D0	D0	0.78	-----0++0
15	KE1301 D0	D0	0.56	-++0-++0+
		mask column coincidence (%)		868688666 000000000

gval

Survival prediction (3-15d) for intensive care patients on admission (0d)

- oxidative burst in blood leukocytes by flow cytometry
- **stimulation**:
  FMLP (100nM),
  TNF-α (10ng/ml, 2x10 U/mg prot)
- data pattern heat map for informative parameters:
  (-)=diminished, (0)=unchanged, (+)=increased
  LSLEARN.BI4

*Valet GK , Roth G, Kellermann W . R*isk assessment for intensive care patients by automated classifi-cation of flow cytometric data. In: Phagocyte Function, Eds. JP Robinson, GF Babcock, Wiley-Liss Inc, New York 1998, p 289-306.

Sepsis: Data Pattern Analysis for Individual Patients

Prediction of Sepsis Survival on Admission

A. Oxidative Activity (DHR123)

clinical outcome	pat. (n)	CLASSIF1 prediction (%) *	
		surv	non surv
surv	12	100.0	0.0
non surv	5	0.0	100.0
neg/pos predval		100.0	100.0

B. Serine Proteinases (R110)

surv	13	100.0	0.0
non surv	5	20.0	80.0
neg/pos predval		92.9	100.0

* 25-75%, 20-80% percentiles
LSLEARN,KDLEARN.BI4

Challenge

- Cytometrists should address the systemic potential of single cell analysis for individualized disease course predictions

- A human cytome project*) aiming at *predictive medicine by cytomics*, a molecularly standardized *disease classification system* and the establishment of a *periodical system of cells* should be envisaged at the European level

*) https://www.classimed.de/val170.pdf

Goal: Individualized Predictions instead of Group Statistics

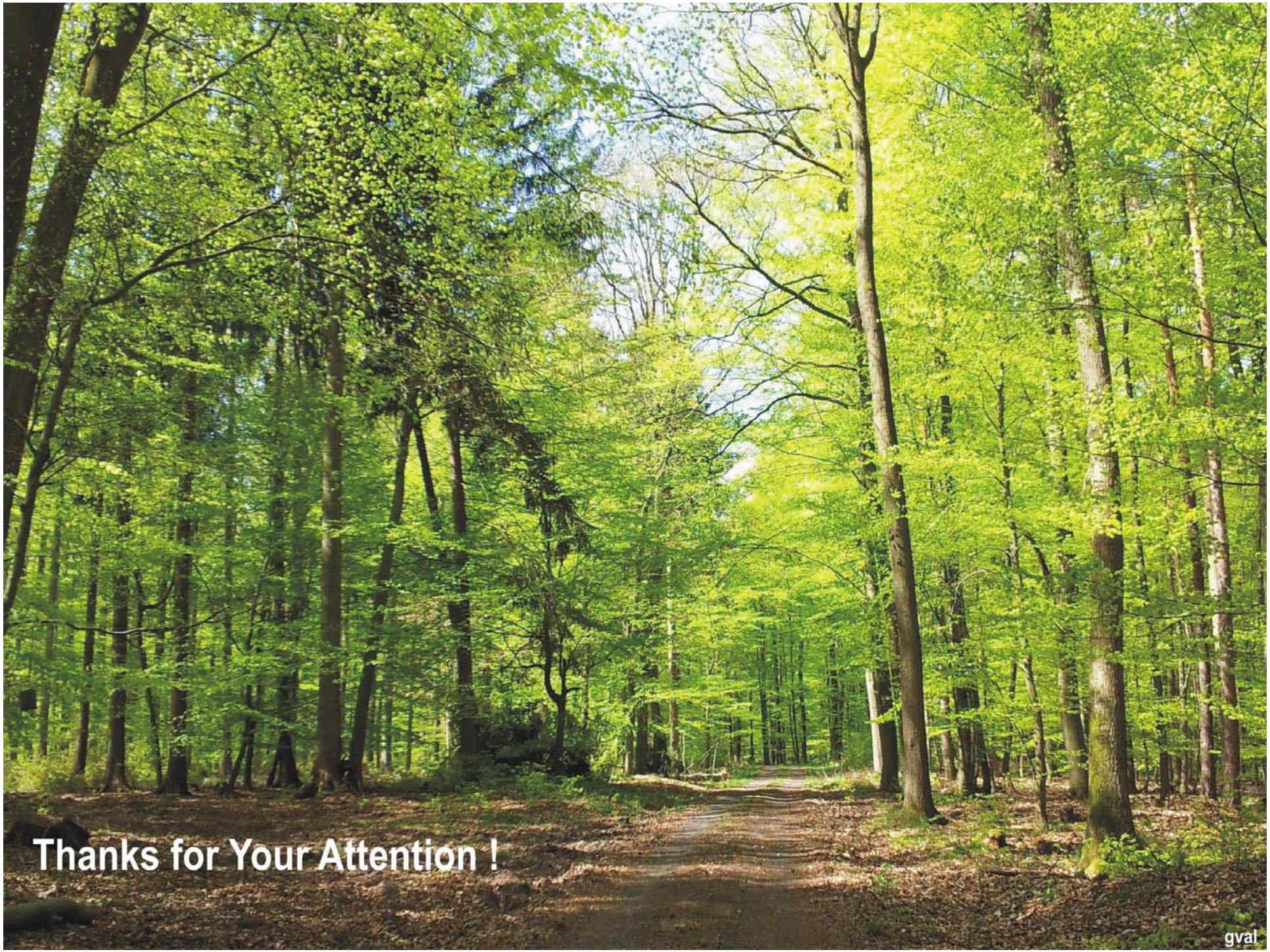
- Core unit cytometrist + clinician
- Retrospective prospective (metaanalysis) data pattern classifications of all available patient information (clinical, clinical chemistry, flow cytometry) to elaborate interlaboratory standardized classifiers
- Cytometry data standardization:
  - Intralaboratory by calibration particles
  - Interlaboratory by measurements of a certain number of fresh blood donor leukocyte samples, followed by normalization of cytometry patient results onto the donor leukocyte means in each laboratory
- Indistinguishable blood donor databases by data pattern classification indicate interlaboratory data comparability
- Initial freedom of parameter choice for all participating laboratories
- Gradual selection of the most discriminatory parameters assures self focusing interlaboratory classifier improvement

https://www.classimed.de/classif1.html#chap6

30 years DGfZ

- My best wishes to the society for efficient future activities and stimulating new ideas for the successful implementation of patient oriented applications together with my

- sincere gratitude to:
  - all collaborators and collaborating scientists
  - my wife Hanna for longterm outstanding work
  - my son Michael for essential software developments
  - the Mildred-Scheel foundation for generous funding
  - the Max-Planck Society as working background for many years


Thanks for Your Attention !
gval