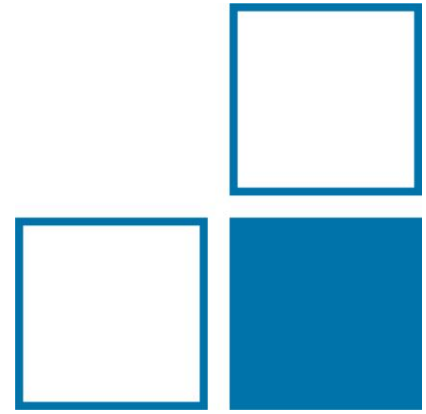
Physikalisch-Technische Bundesanstalt
Braunschweig and Berlin
National Metrology Institute

Overview of reference measurement system approach for blood cell counting

Andreas Kummrow, 8.32



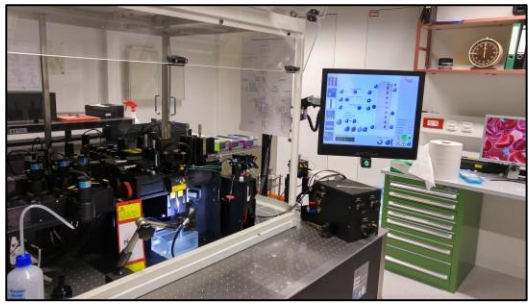
Physikalisch-Technische Bundesanstalt

Department of Biomedical Optics, WG 8.32, Berlin

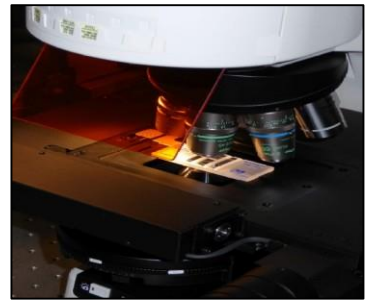
EMPIR EURAMET BioStand SEPTIMET AntiMicroResist Infect-met BioSITrace

Capabilities & Expertise:

Flow cytometry



Microscopy

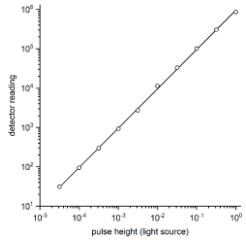


Digital PCR and qPCR

ISO standardization

- **ISO TC276/WG3**: At present two horizontal standards

flow cytometer
counting chamber
CFU, coincidence…



ISO20391-1: Biotechnology - Cell Counting – Part 1. General guidance on cell counting methods.

- published 01/2018

ISO20391-2: Biotechnology - Cell Counting – Part 2. Experimental Design and Statistical Analysis to Quantify Counting Method Performance

- published 08/2019

in preparation

ISO23033: Biotechnology - Analytical methods – General guidelines for the characterization and testing of cellular therapeutic products

…

DIN NA 063-03-06-AA Haematology

Guidelines of the German Medical Association on Quality Assurance in Medical Laboratory Examinations - Rili-BAEK

NV: nominal value

RMV: reference measurement values

Reference procedures:

		German Med Assoc	JCTLM
• DIN 58932-3:2017-01	**erythrocytes**	RMV	submitted
• DIN 58932-4:2003-07	leukocytes	RMV	
• DIN 58932-5:2007-10	**platelets**	NV [1]	listed
• DIN 58932-6: in preparation	CD4+ cells	--- [2]	

Note 1: reference method works for fresh blood, platelet concentrates but not for control blood used in Germany

Note 2: EQA for CD4+ T cells is currently only listed in Rili-BAEK B2 (Qualitative medical laboratories examinations)

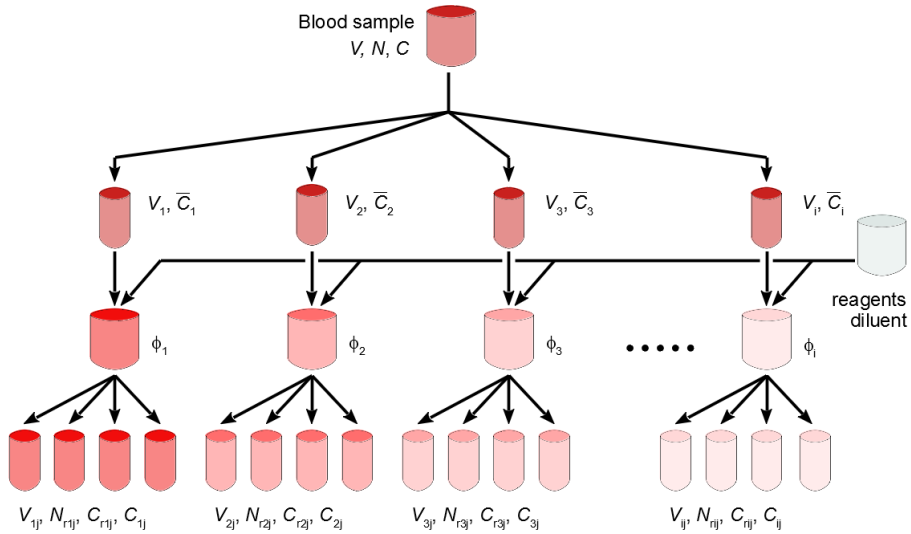
CCQM study P102 for CD4+ cell count is completed

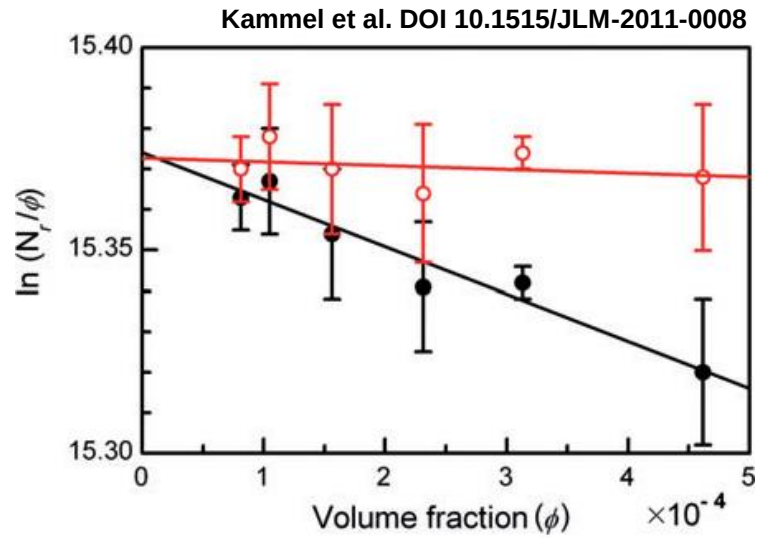
DOI: 10.1002/cyto.a.22614 , 10.1002/cyto.a.22634

DIN series for blood cell counting

Concept for concentration measurement in DIN procedures:

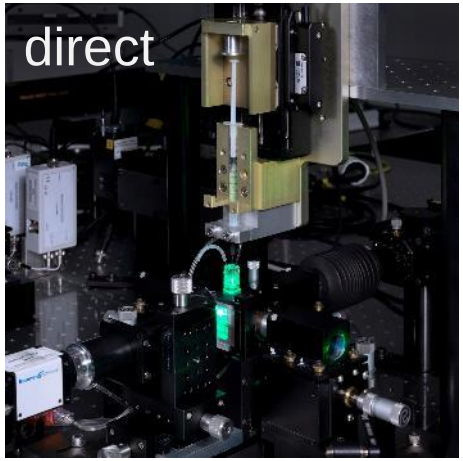
- Technical requirements for identification and counting of target cells
- SI traceable measurement of sample volume including dilution
- Dilution series with repeat measurements
- Consider coincidence loss (depends on instrument and sample)
- Consider influencing factors (e.g. adhesion loss)
- Recommendations for uncertainty analysis and quality control



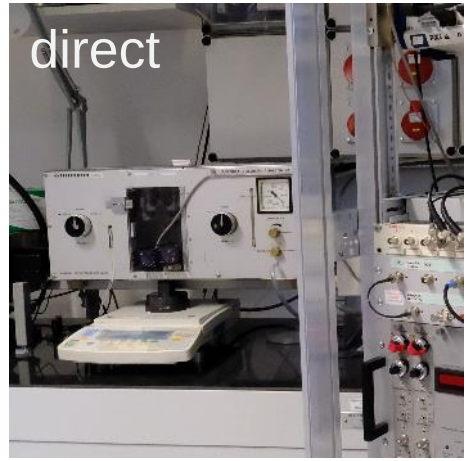


SI traceable volume measurement

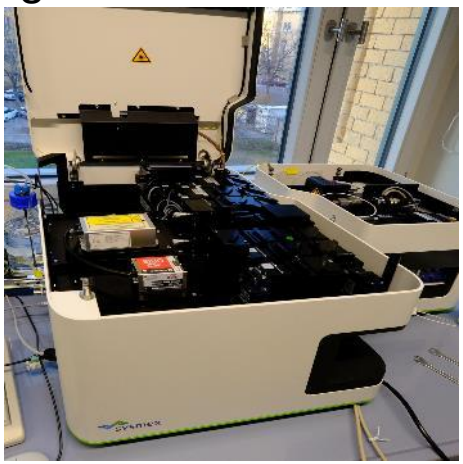
calibrated syringe



weighing



gas buffered



calibrate start-stop

Counting CD34 cells

↓

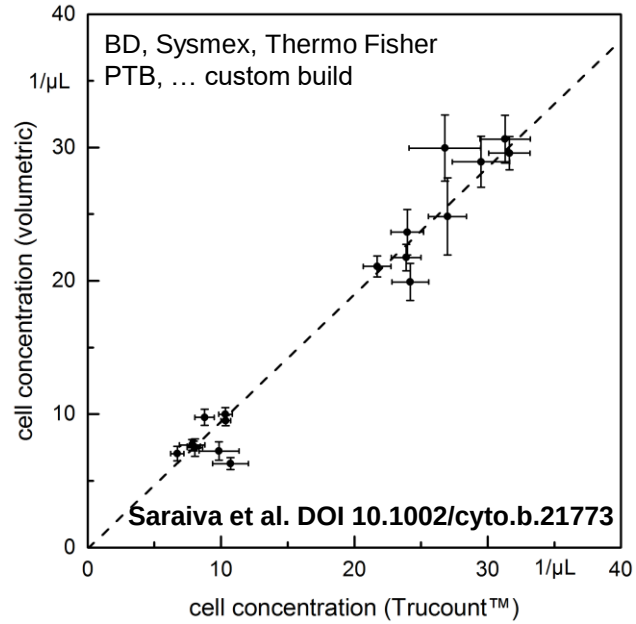
Dual-Platform

↓ ~1999

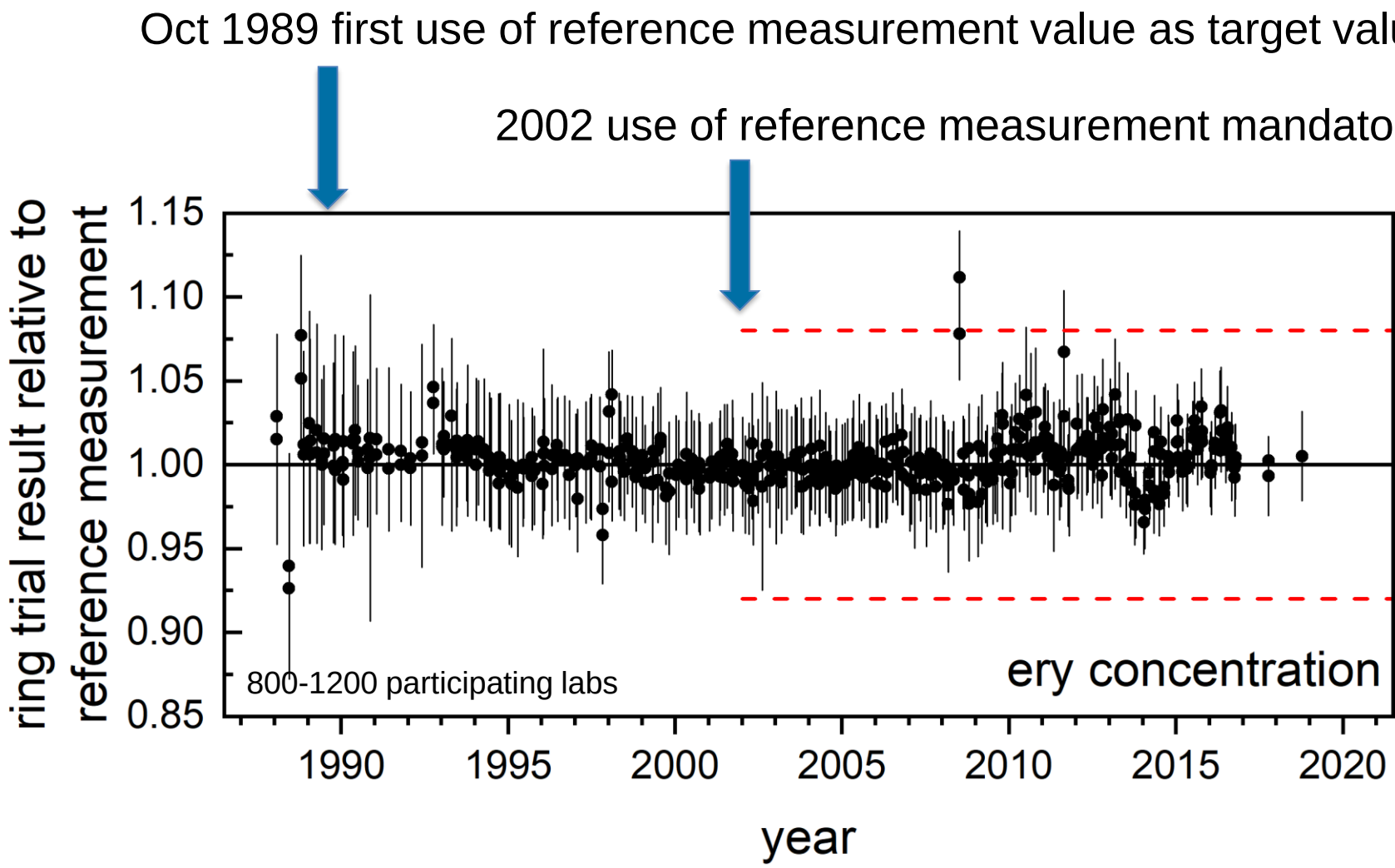
Single-Platform

↓ future option

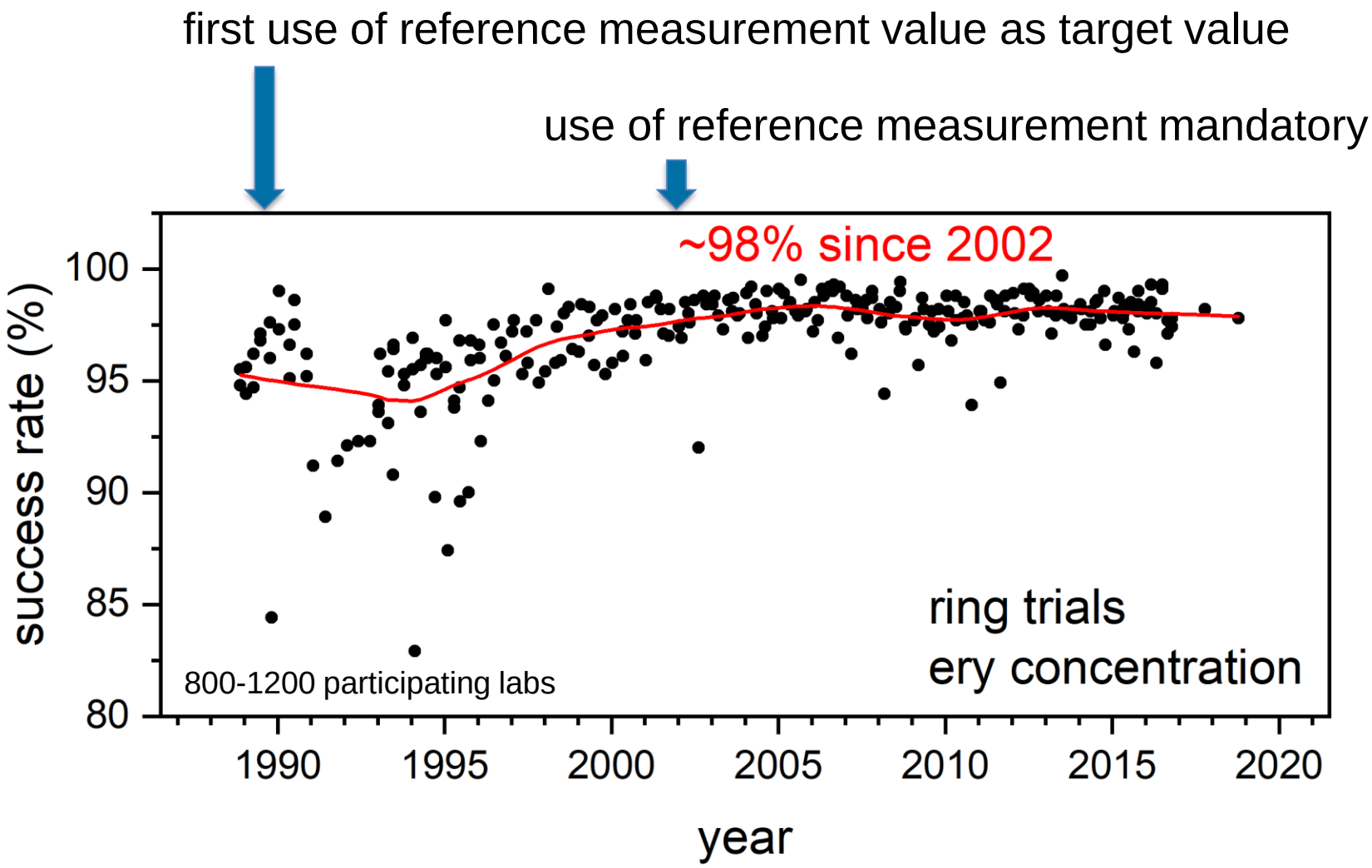
Volumetric CCQM P165



EQA for erythrocyte concentration in DE

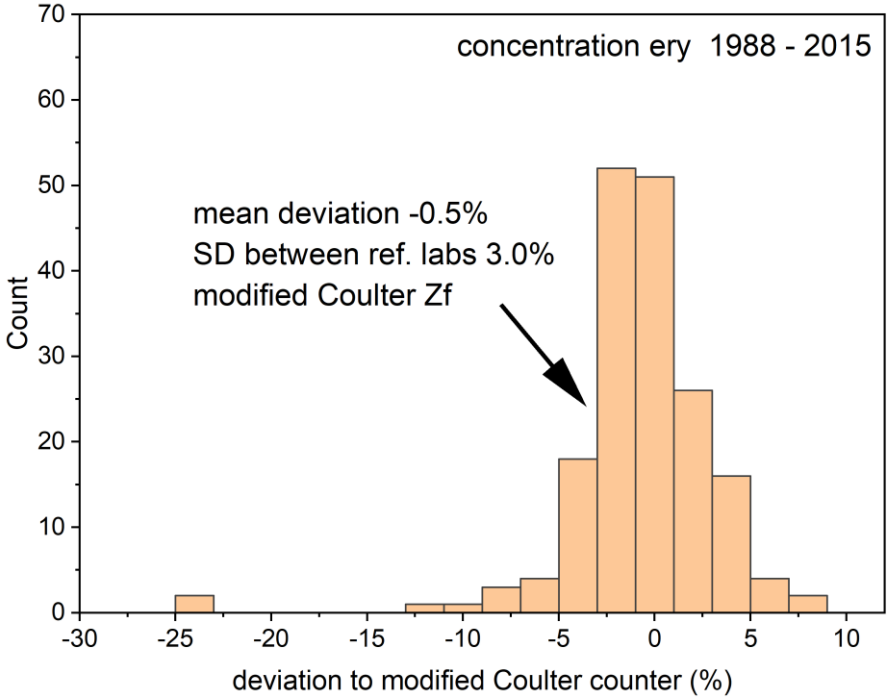


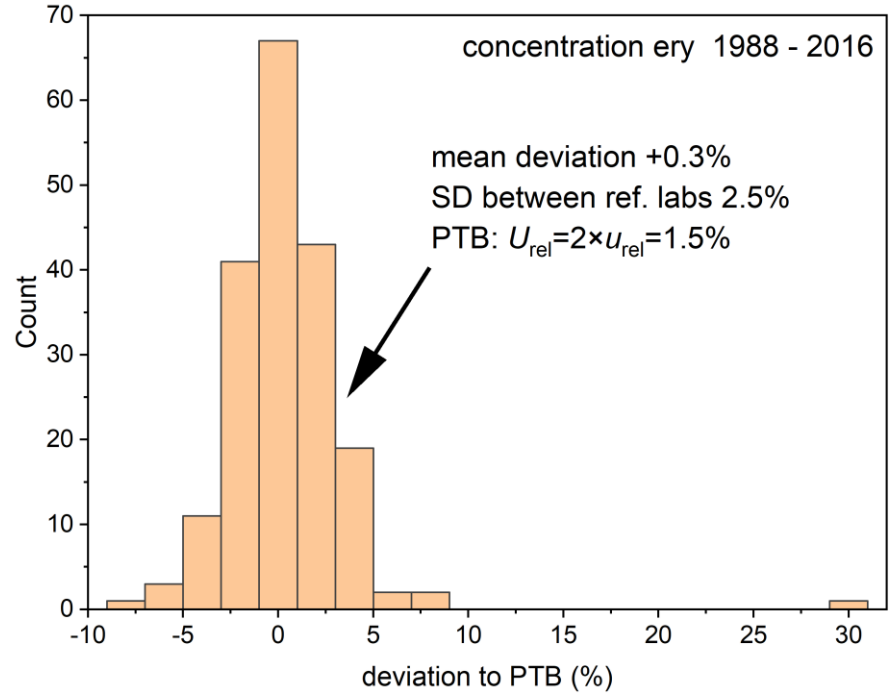
EQA for erythrocyte concentration in DE



Performance of reference method for Ery

- Reference method ready 1988, now DIN 58932-3:2017-01 in EN
- Deviation between reference instruments on average **<0.5%**
- Scatter between ref. labs dominated by sample tube-to-tube variation (3%)

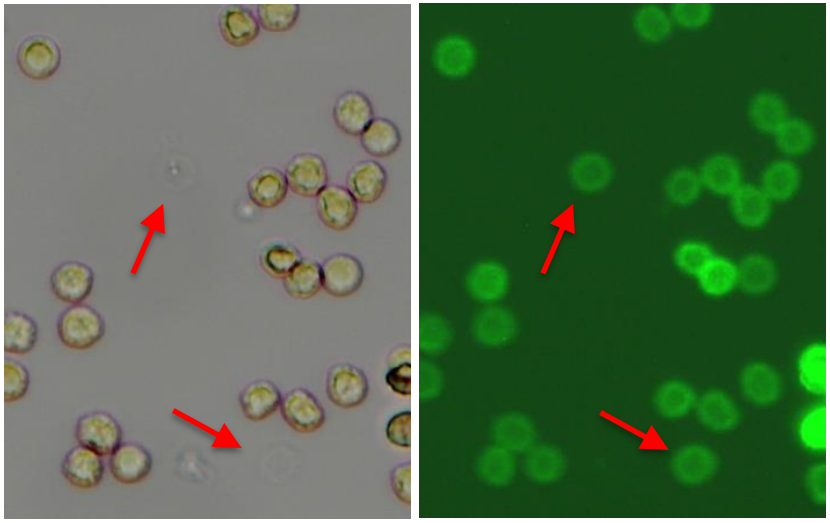




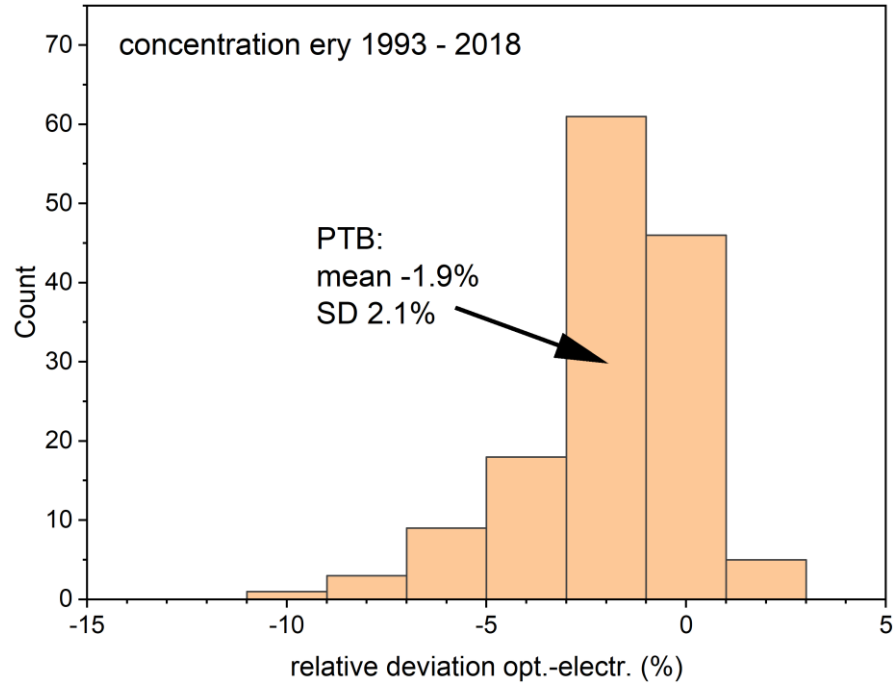
Challenge: optical reference instruments

- PTB reference instrument for Ery conc. is impedance counter
- Reference instruments based on optical detection are more flexible
- For counting Ery: erythrocyte ghost issue for control material

control blood +21d



CD235a-FITC



ICSH and DIN method for platelets

- ICSH* aims **measurement precision**: 'reference method' i.e. 'clearly and exactly defined technique'
- DIN** aims **measurement accuracy**: 'inaccuracy as small as possible'

similar:

	ICSH	DIN
platelet identification	CD41, CD61 (and CD41b, CD42, CD42a)	CD41, CD61 (and CD42a, b, c, d)
sample	EDTA blood	EDTA blood
container	suitable (PS, PP)	suitable (PP)
dilution series	serial (pipetting)	separate, gravimetric controlled
diluent	PBS with BSA	PBS with BSA

*| DOI 10.1309/91PR-E4G6-XBAF-N8DY, **| DOI 10.31030/9866520

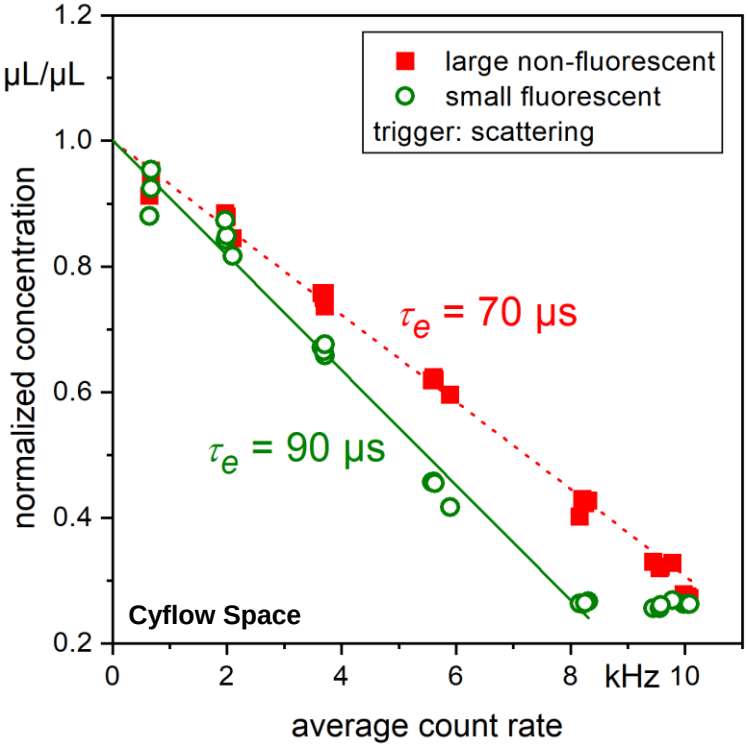
Example: Platelet counting

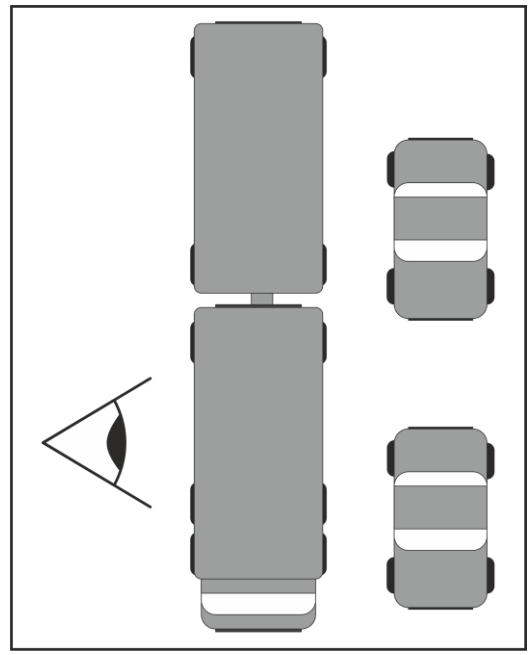
- Differences between ICSH and DIN method:

	ICSH	DIN
optical flow cytometer	FSC, FL; trigger FSC	FSC, FL; trigger FL
volume measurement	dual platform, based on RBC	volumetric instrument
coincidence correction	not allowed	Poisson correction allowed
volume	Precision 1% + sample variation	Accuracy 0.3%
PLT count error	3.2%	2.5% (c >20 nL^{-1})
recommended QA	none	annual SI traceable calibration for volume, mass and density measurement
PLT concentrates	not included	included

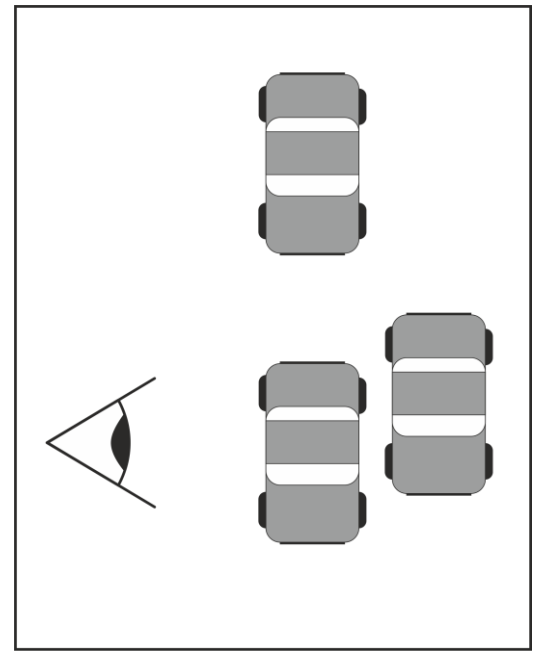
Coincidence loss in complex situations

- Complex situations may occur if :
  - more than one detection channel needed (e.g. FLS, FL) or
  - several sub-populations involved (e.g. Ery, Plt)
- Loss depends on signal height, signal processing, count ratio





no Poisson correction



Poisson correction

Example: Platelet counting

- Differences between ICSH and DIN method:

	ICSH	DIN
optical flow cytometer	FSC, FL; trigger FSC	FSC, FL; trigger FL
volume measurement	dual platform, based on RBC	volumetric instrument
coincidence correction	not allowed	Poisson correction allowed
volume	Precision 1% + sample variation	Accuracy 0.3%
PLT count error	3.2%	2.5% (c >20 nL^{-1})
recommended QA	none	annual SI traceable calibration for volume, mass and density measurement
PLT concentrates	not included	included

Summary

- Rili-BAEK distinguishes
  NV: nominal value
  RMV: reference measurement values
- For blood cell counting: procedures to determine RMV published as DIN-Standards
- ICSH standards have mostly similar requirements
- Notable differences related to volumetric measurement,
  used in German EQA for 30 years
- Volumetric flow cytometers are now available from several vendors.
  This should allow to establish SI traceable concentration measurements on a broader scale
- Note: method for best accuracy not necessarily gives best precision

Physikalisch-Technische Bundesanstalt
Nationales Metrologieinstitut

This work was supported by the project 16SIP01 which has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme

Physikalisch-Technische Bundesanstalt
Braunschweig and Berlin
Abbestr. 2-12
10587 Berlin

Andreas Kummrow
Telefon:+49 (0)30 3481-7643
E-Mail: andreas. kummrow@ptb.de

https://www.ptb.de/