Physikalisch-Technische Bundesanstalt
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Traceable Quantification of Antibodies using Flow Cytometry

Motivation

- **Flow Cytometry (FC)** is a **standard** for blood measurements
- A **better quantification of cellular markers** would provide a **better diagnostic basis** for several diseases (e.g. HIV, CLL or sepsis)
- Intra and inter lab **comparability of measurements** is in need of improvement

❖ Before starting my PhD at PTB in November 2020, I was doing my Master´s degree in physics working on Raman and MALDI-TOF-MS applications in dermatology

Concept

1. Select **target** to be investigated and prepare
2. Measure **Mean Fluorescence Intensity (MFI)** of target cells with FC
3. Obtain **Antibody Binding Capacity (ABC)** via subsequent Time Of Flight Mass Spectrometry (TOF-MS)
4. **Relate** measured MFI and ABC

Aim 1
Improved diagnostics of various diseases via **enhanced standard blood testing**

Setup & Basics

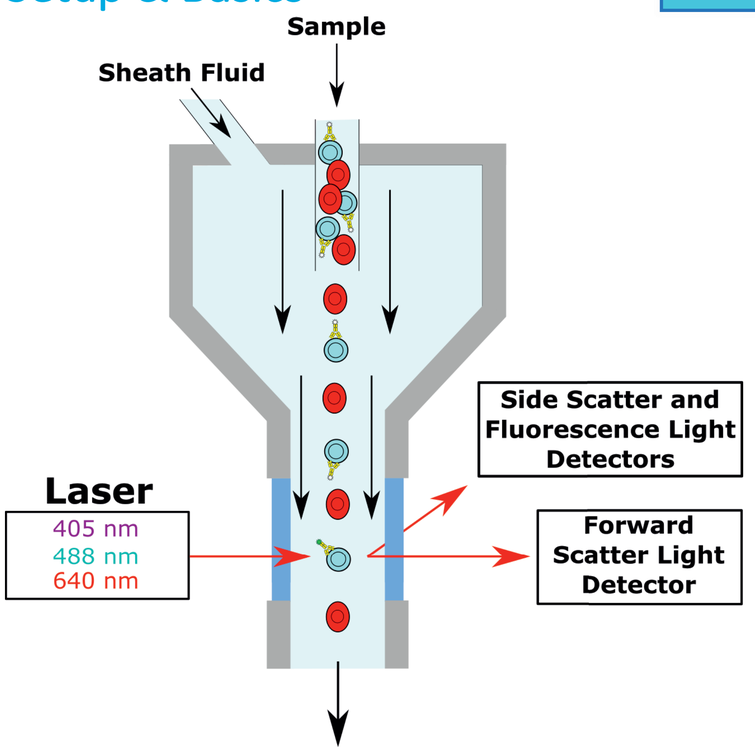

Figure 1: FC scheme. The accelerated sample cells are interacting with an incident laser beam one by one. Scattered and fluorescent light are detected for each interaction event.

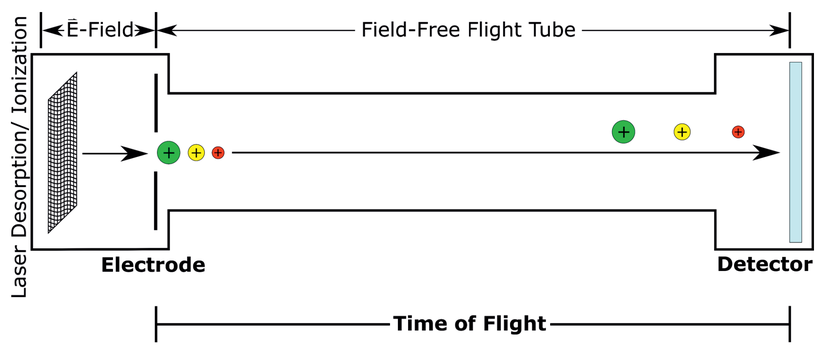

Figure 2: Time of flight scheme. Particles are accelerated towards the detector and their mass is calculated by time of flight.

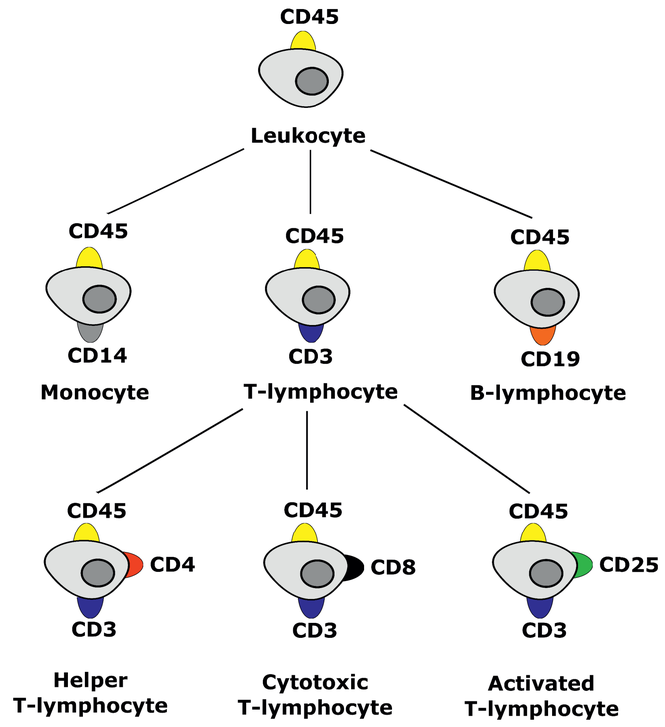

Figure 3: Cellular expression scheme. Cell type or cell activation is perceivable by CD surface markers via specific antibodies. Thereby, labeled antibodies can be used for cell type or status identification.

Aim 2
Develop **reference method** for traceability of Antibody Binding Capacity to the SI

Blood Testing Today

- Only some quantitative tests available, which provide **relative quantities** to an internal standard
- **No traceable reference materials**
- **No External Quality Assurance (EQA)** available for ABC measurements
- Lack of absolute quantification **hinders comparability** between devices or labs

Blood Testing in the Future

- **Reference method** provides **absolute quantities** for ABC **traceable to the SI**
- **Reference method** is used to **calibrate reference materials**
- **EQA** via reference materials and/or round robin tests for ABC
- **Traceable absolute quantification provides comparability** of ABC between devices and labs enhancing measurement precision and quality of diagnostics

Challenges

Double labeling
Specificity
Sensitivity
Correlation of measurands
Technical Feasibility
Repeatability

Summary

- **Today** there **is no absolute quantification or EQA** for ABC measurements
- Connecting fluorescence measurements in FC to MS may enable the **traceability of ABC** to the SI
- Our method would thereby provide a **basis for EQA** schemes
- **A traceable quantification** of ABC in the future would be important to enable **good quality clinical diagnostics** and should be a matter of course

References

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