

Reference measurement procedures for cell concentration

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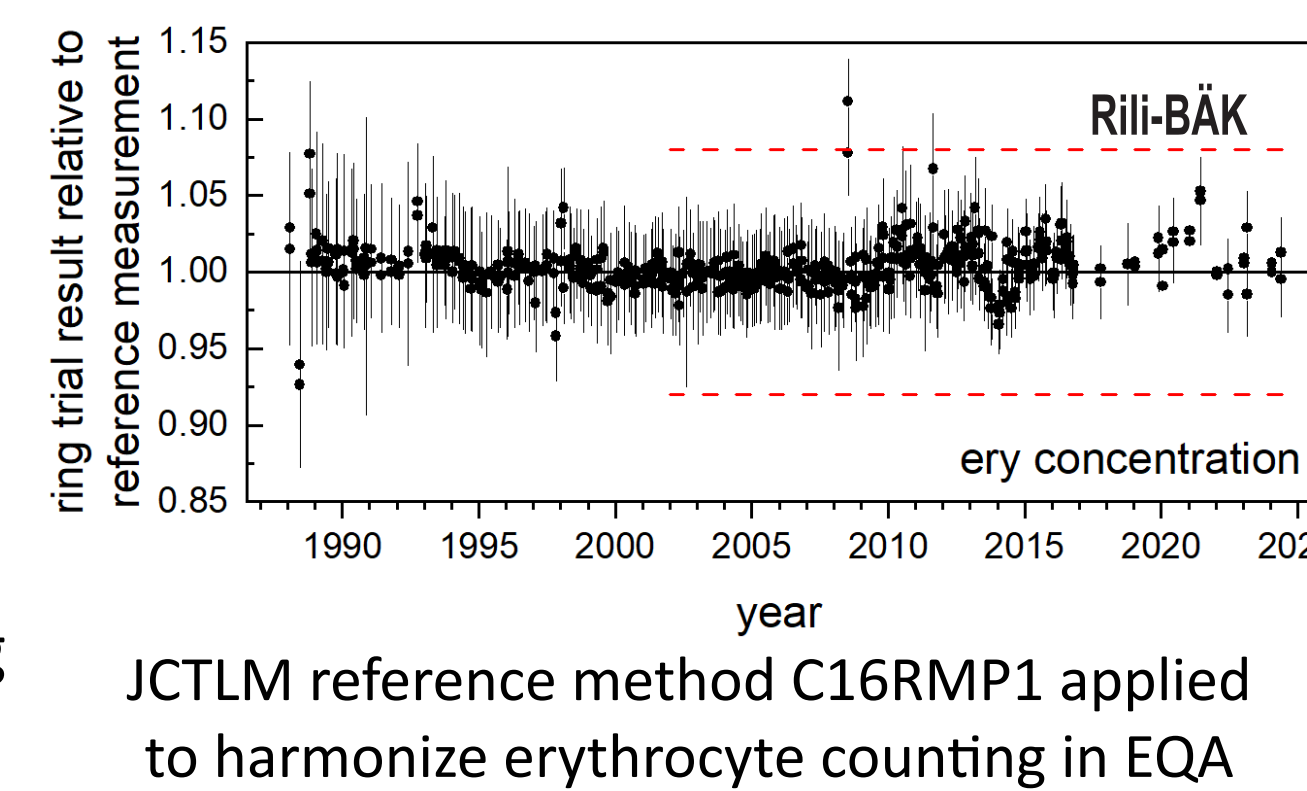


Introduction

Cell concentrations are key parameters for medical diagnostics and in bioprocessing. The most frequently used methods for measuring cell concentration are flow cytometry and microscopy. Flow cytometry allows higher accuracy for analysis of cell suspensions, since uncertainties arising from counting statistics can be reduced rather easily in most applications. A challenge in pushing the limits for uncertainty is the uncertainty of the sample volume. This poster describes three approaches to meet this challenge.

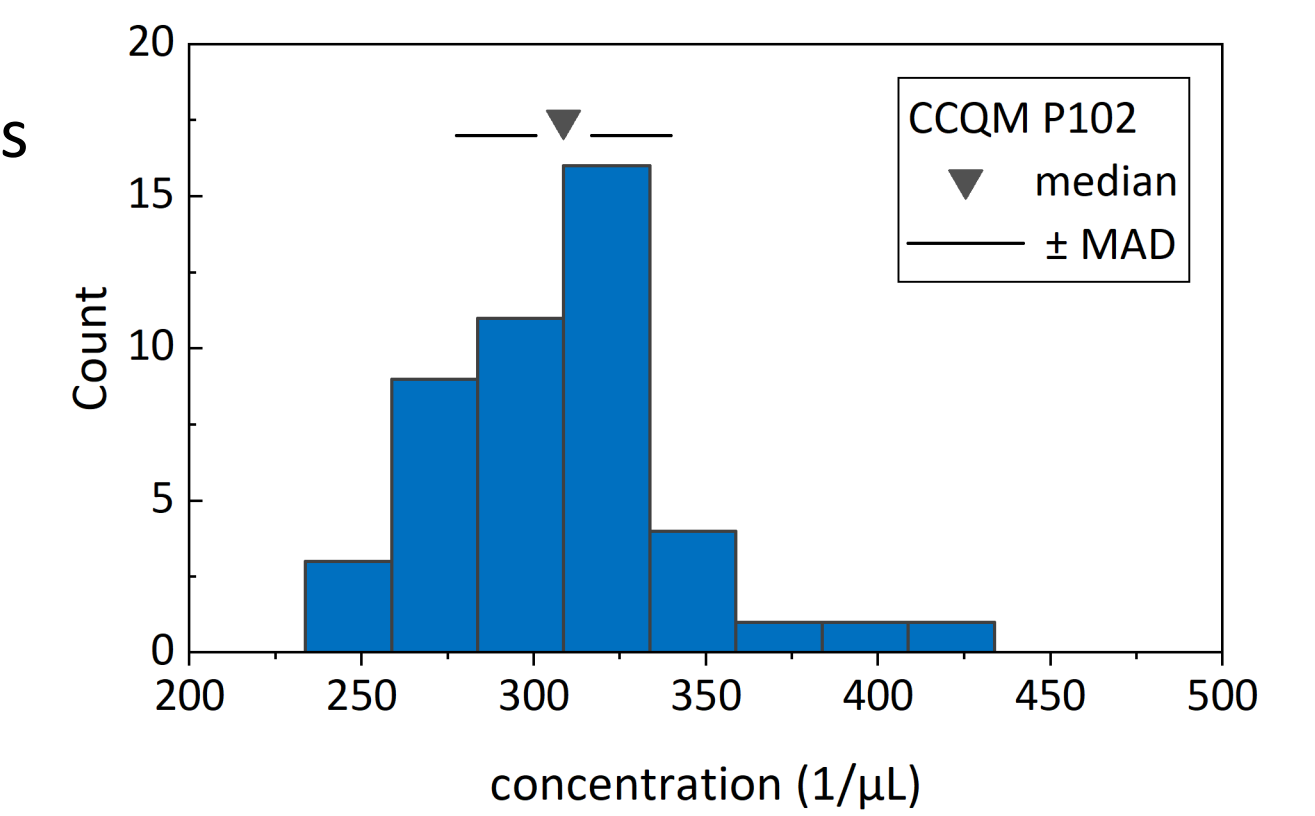
Applications for cell counting

Complete blood count is the most frequently requested medical test. A scatter of routine laboratory results of around 3% (RSD) is typical for erythrocytes in EQA ring trials (s. figure). Reliability of average results requires harmonization and standardization. ICSH has published several standards for blood cell counting based on flow cytometry primarily aiming at harmonization [7]. JCTLM listed reference methods on blood cell counting are all based on flow cytometry [8]. General requirements for cell counting are described in ISO 20391-1. Cell counting by microscopic imaging (e.g. CCQM-P123) is considered there, but is often limited to special applications, e.g. in case of challenging identification of the target population [9].



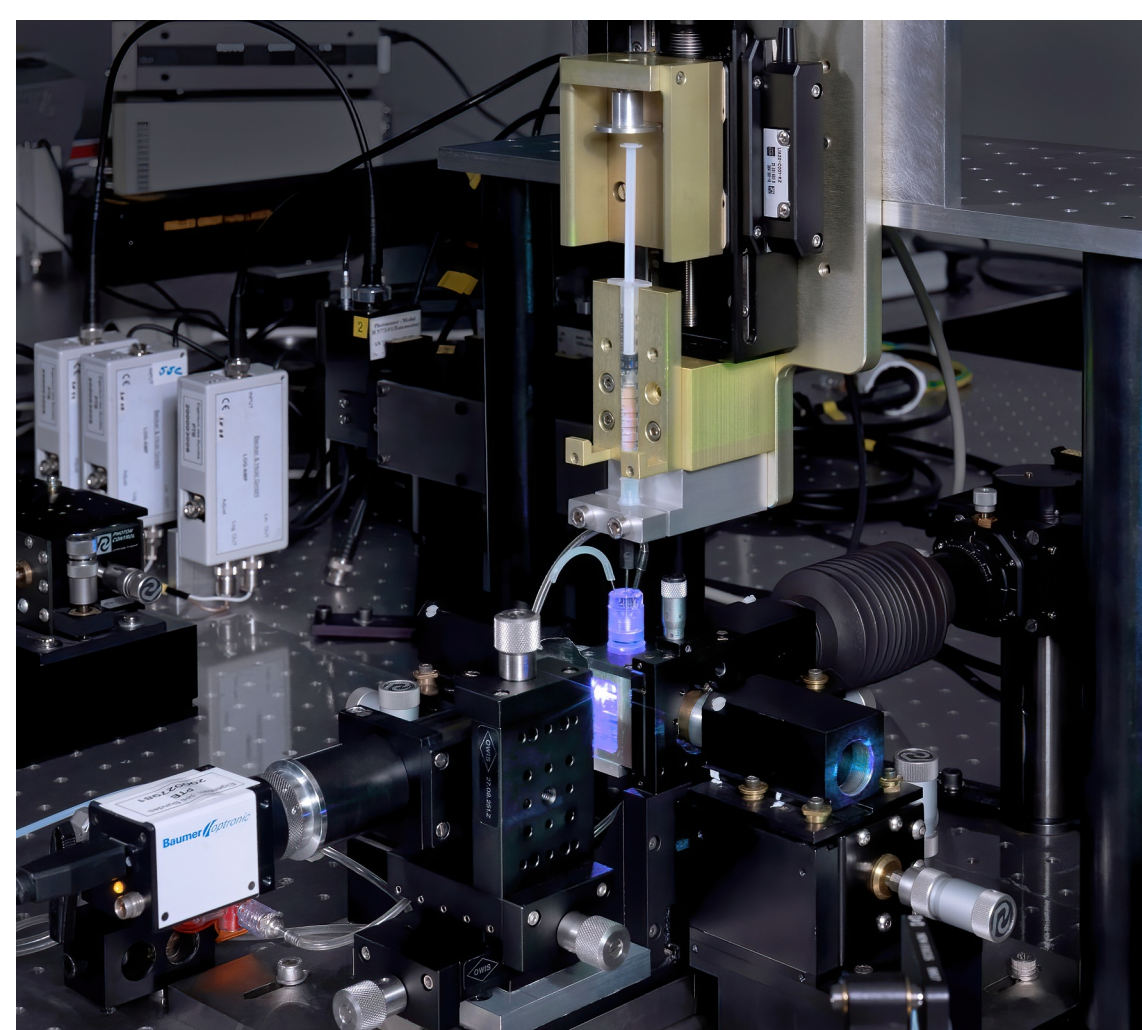
CCQM studies

The precision of cell concentration measurements has been investigated in several CCQM studies. CCQM-P102 [10] showed a comparatively large scatter of CD4 cell concentration measured by different participating laboratories (relative standard deviation of about 10% estimated with robust statistics [11]). Similar results were reported for the more recent CCQM-P165 study on CD34 cells [12]. Fresh cell suspension samples are usually measured within a few hours. CCQM studies require long term stability of the test material. This makes correct cell identification a particular challenge. This may explain why the observed accuracy is worse than expected from existing JCTLM reference methods.



Implementation of traceable volume measurement

Development of traceable volume measurement at PTB started with impedance counters for complete blood count. In those devices the volume can be measured by gravimetric control [4]. Optical flow cytometry offers a large number of parameters to correctly identify cells, which was needed e.g. in the CCQM studies P102 and P165. For optical flow cytometers volume measurement was implemented using calibrated syringes [1]. The cell suspension sample can be injected directly into the flow cell, or a gas buffer is used.



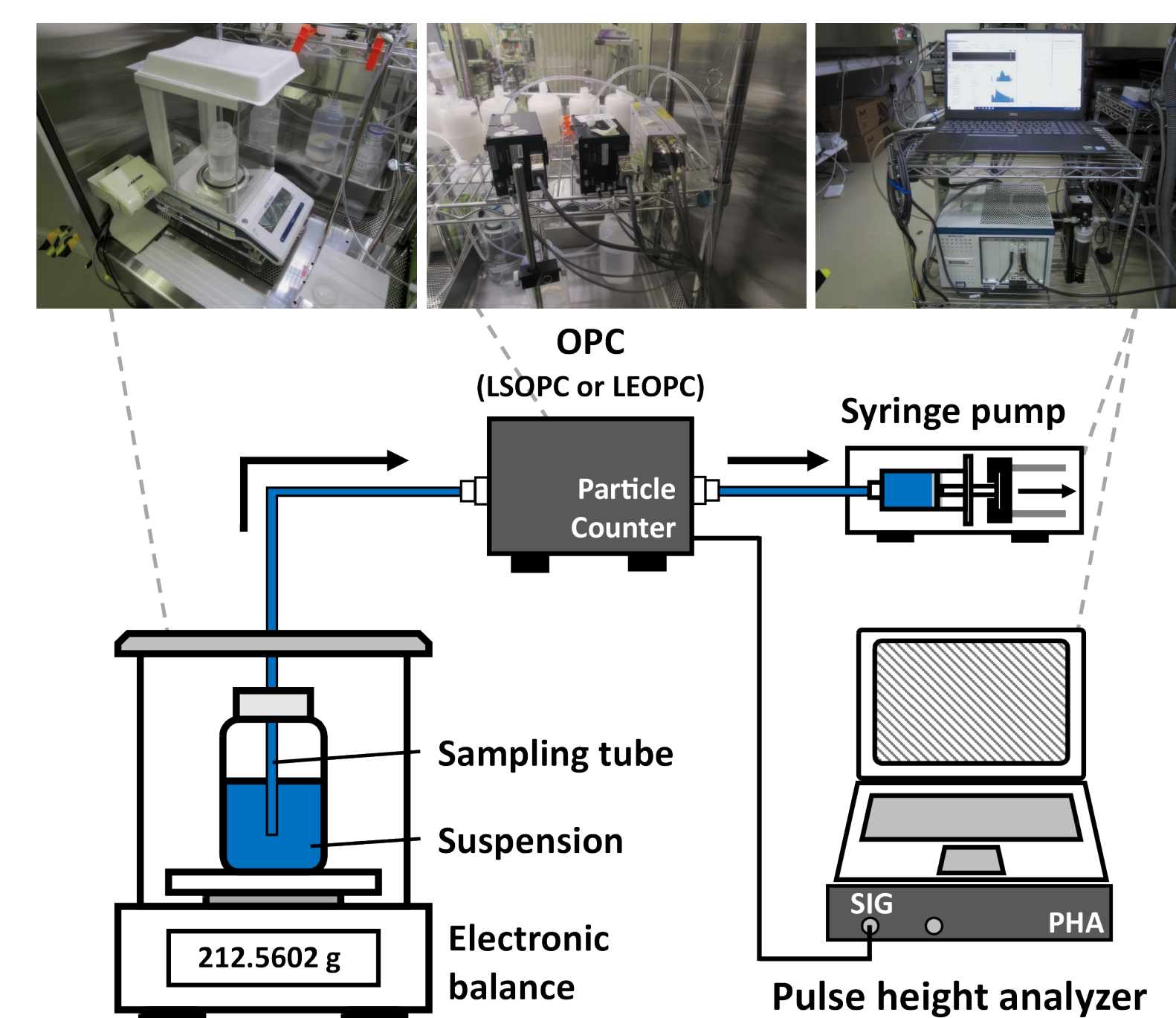
Direct injection device: typically $u_{rv} = 0.17\%$



Gas buffered device. Despite use of pressure and temperature sensors the uncertainty for the volume is higher: typically $u_{rv} = 0.25\%$

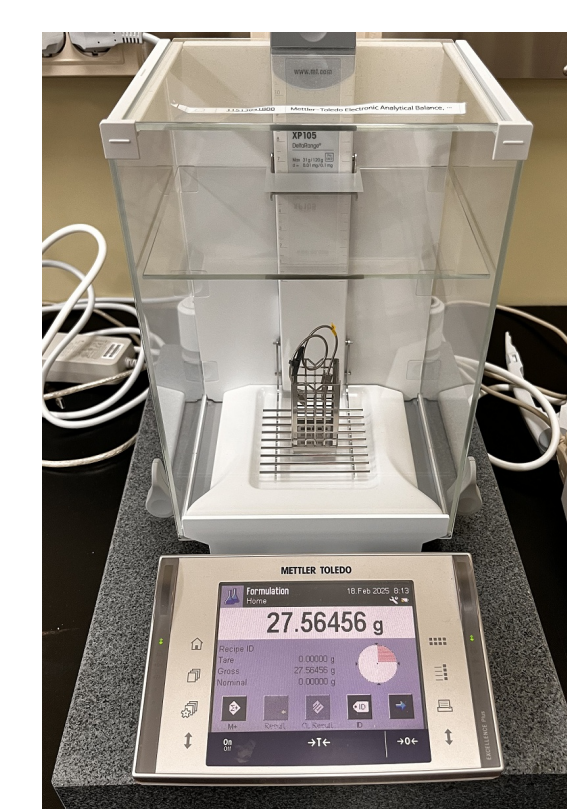
Traceable mass (or volume) measurement system has been developed at NMIJ for the calibration of polystyrene particle standard suspensions. This system counts particles using a light-scattering or light-extinction optical particle counter (LSOPC or LEOPC) [5,6]. Currently, NMIJ provides calibration service for number concentration in the size range of 600 nm to 20 μm . In addition, this system is being used to evaluate a 5 μm monodisperse polystyrene particle suspension as a study material in the ongoing CCQM study P222.

For optical particle counters, mass (or volume) measurement can be implemented by continuously measuring the mass of the particle suspension as it is sampled by the syringe pump [2]. If necessary, the mass unit (M) can be converted to the volume unit (V) by measuring the density (ρ) using a calibrated density meter: $V = M/\rho$.

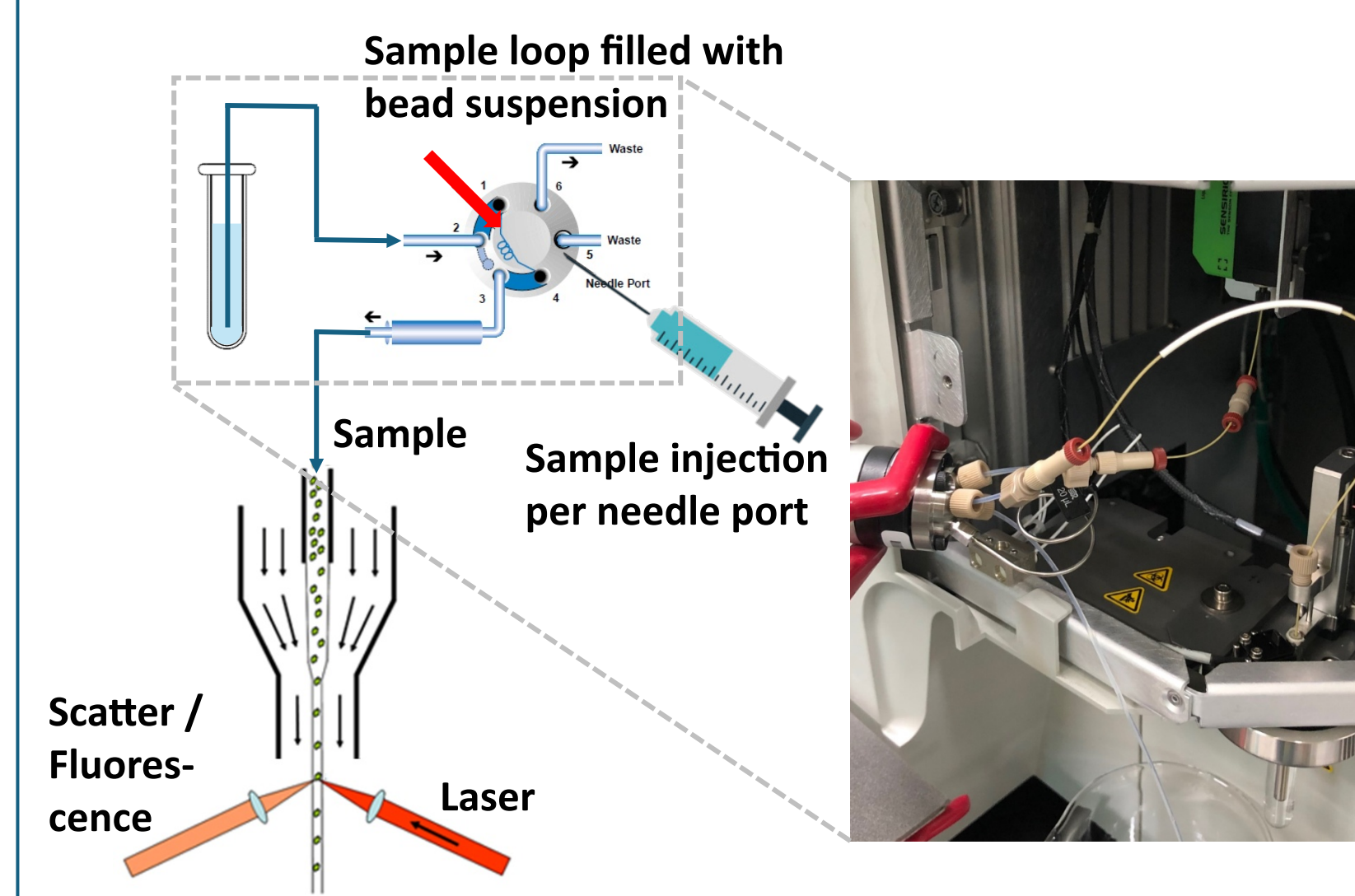


Primary standard for the number concentration of liquid-borne particles developed at NMIJ. The uncertainty for the mass is typically $u_{rm} = 0.22\%$ to 0.46% , depending on the flow rate.

At KRISS, a traceable number concentration measurement system for flow cytometry has been developed [3]. It uses a defined-volume sample loop, where particles are loaded before flowing through the detection area for exhaustive counting once measurement begins. Traceability is ensured through gravimetric measurement of the sample loop's volume. This system has been used in the ongoing CCQM P222 study and for the development of cell and bead number concentration standards.



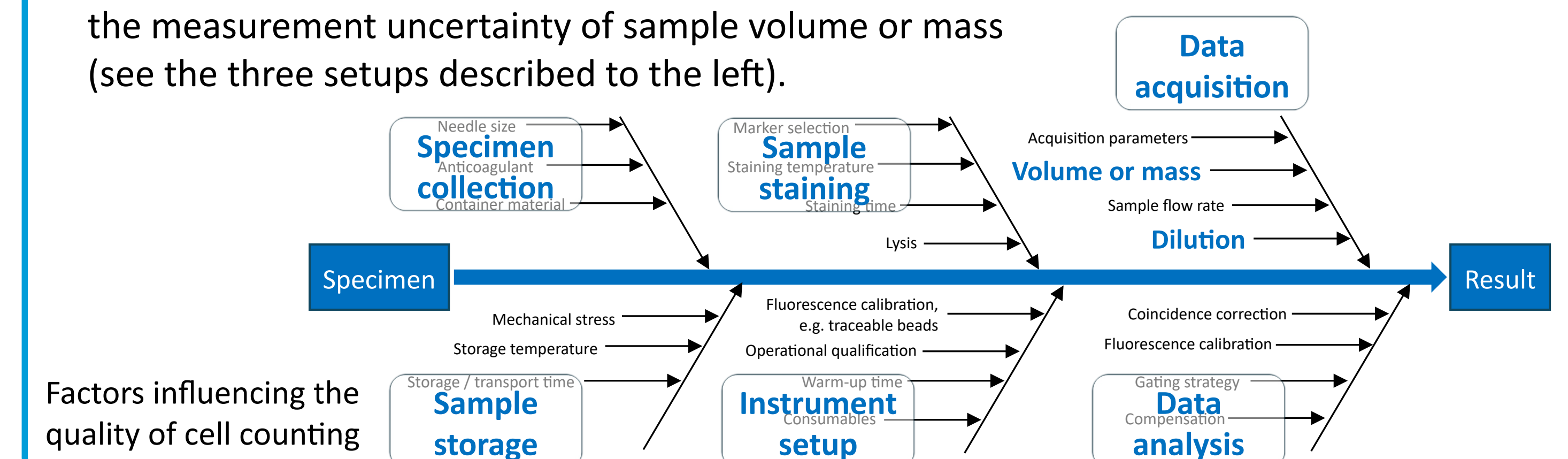
Gravimetric sample volume measurement: typically $u_{rv} = 1.1\%$



Measurement setup – schematic and photographic representation: The cell suspension is loaded into a sample loop via an injection valve, and the cells are then transported to the detection area for measurement.

Quality of cell counting

Many factors influence the quality of cell counting, particularly pre-analytical steps. The uncertainty contributed by measurements is in the data acquisition step. The uncertainty in gravimetrically controlled dilution is small. Ultimately, the limiting factor is the measurement uncertainty of sample volume or mass (see the three setups described to the left).



Discussion and Conclusions

JCTLM listed methods allow a relative uncertainty for volume measurement up to 0.3%. While such stringent requirement can be useful for specific cell types, an uncertainty of up to about 1% is sufficient in general, since other steps make higher contributions to combined uncertainty for cell concentrations (*cf.* previous CCQM studies). Even for erythrocyte count, the most accurate measurand, a traceable volume with uncertainty around 1% can be acceptable for standardization. The currently running P222 study, which is based on artificial material, explores to what extent worldwide standardization is possible. In the future, flow cytometers with traceable and direct sample volume measurements, as described here, could be used for quantitative counting, and offer the potential to be used to value assign bead-based calibration materials typically used in routine concentration measurements of cell subpopulations like CD4 or CD34 cells.

Literature

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- [5] ISO 21501-2: 2019 Light scattering liquid-borne particle counter
- [6] ISO 21501-3: 2019 Light extinction liquid-borne particle counter
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