Accurate measurement of particle number concentration in liquid at NMIJ: with applications for biomedical and cellular analyses

National Metrology Institute of Japan (NMIJ)
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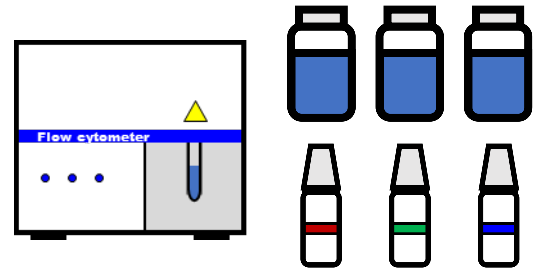
NMIJ's current status: PNC in liquid

NMIJ

M-OPC method: 600 nm – 10 µm

Under development: <600 nm

Calibration service
Technical consulting

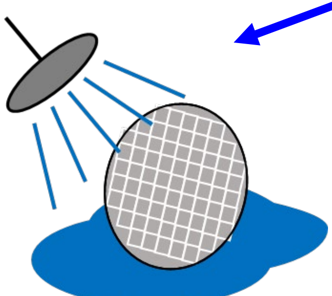


Manufacturers

Particle / Cell counters

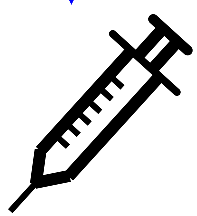
Standard PNC suspensions

Improvement of
Quality Control technique



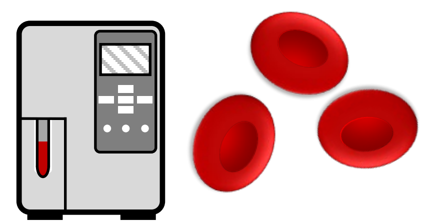
Semiconductor industries

600 nm – 2 µm



Pharmaceutical industries

≤10 µm



Clinical diagnoses

2 µm – 10 µm

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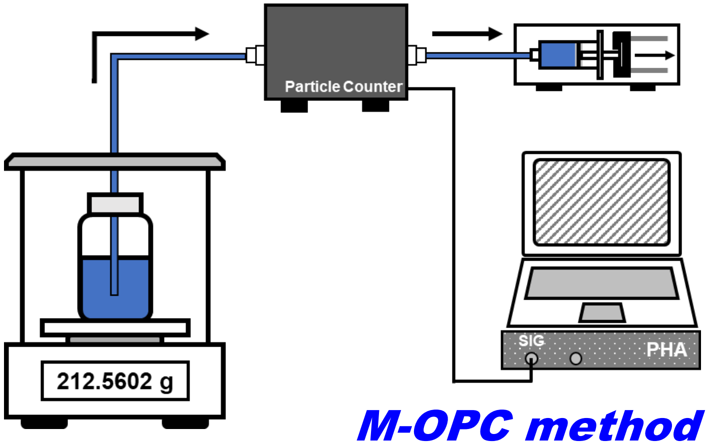
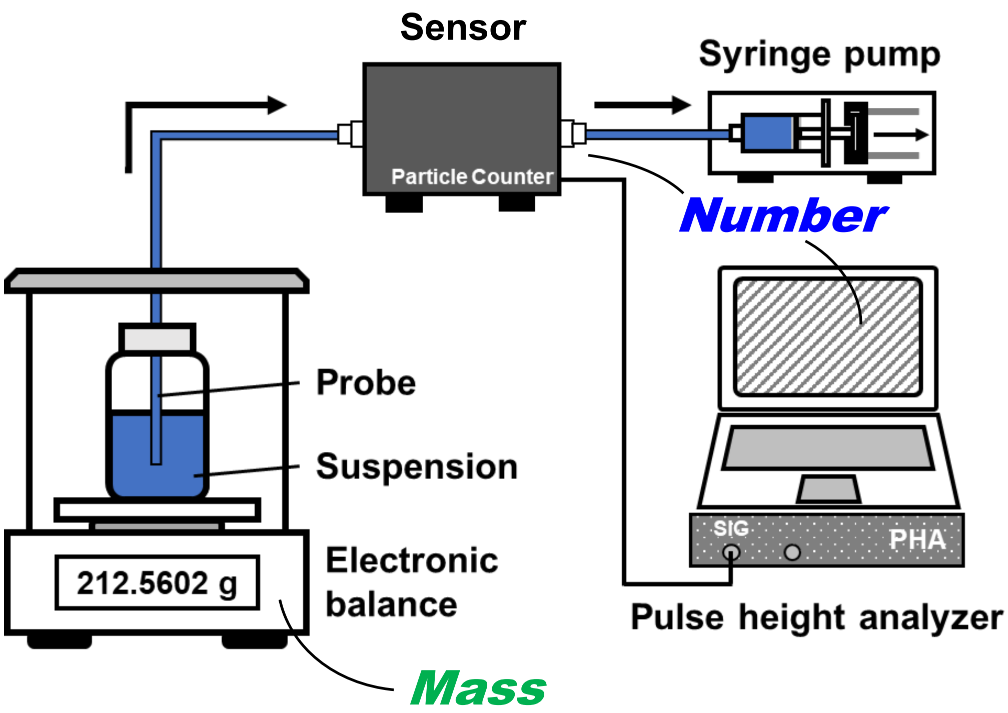
  - Validation of the reference method of blood cell counting
- Ongoing projects
  - Development of standard particles for biomedical analyses
  - PNC Intercomparison study (CCQM-P222)

Accurate measurement method for the particle number concentration (PNC) in liquid developed at NMIJ



Mass-measurement-type Optical Particle Counting: *M-OPC method*



$$C = \frac{N}{M}$$

C : Number concentration* [particles g^{-1}]

N : Number of particles [particles]

M: Mass of suspension [g]

*Simplified formula

Kuruma et al. (2020), *Adv. Powder Technol.*, 31, 848-858.
Kuruma et al. (2021), *Metrologia*, 58, 045007.
with some updates…

- Particle number concentration, C, can be obtained by simultaneous measurement of number of particles, N, and mass of suspension, M.

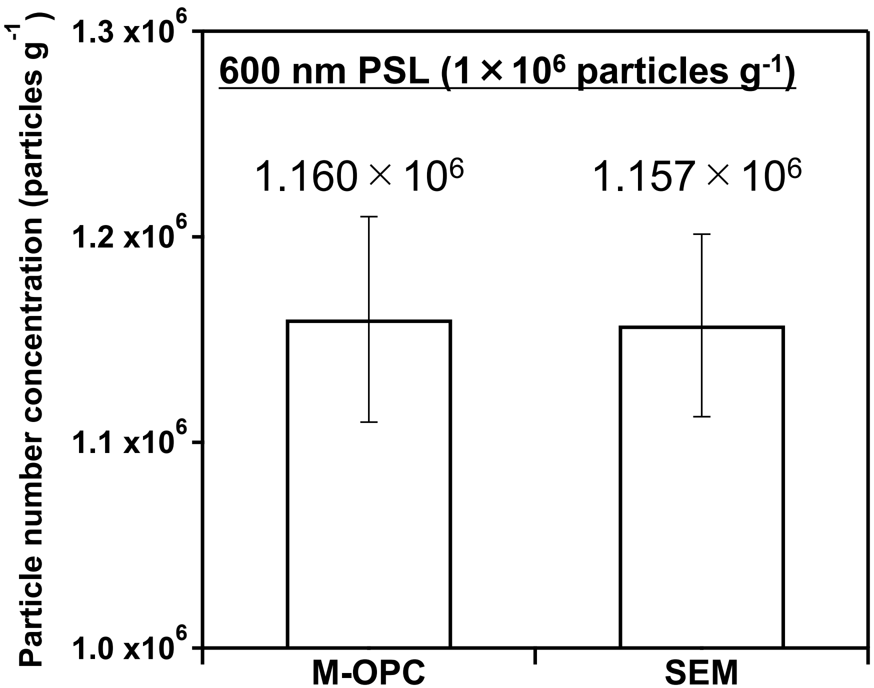
Comparison with the former primary standard

FCM: flow cytometer (optical)

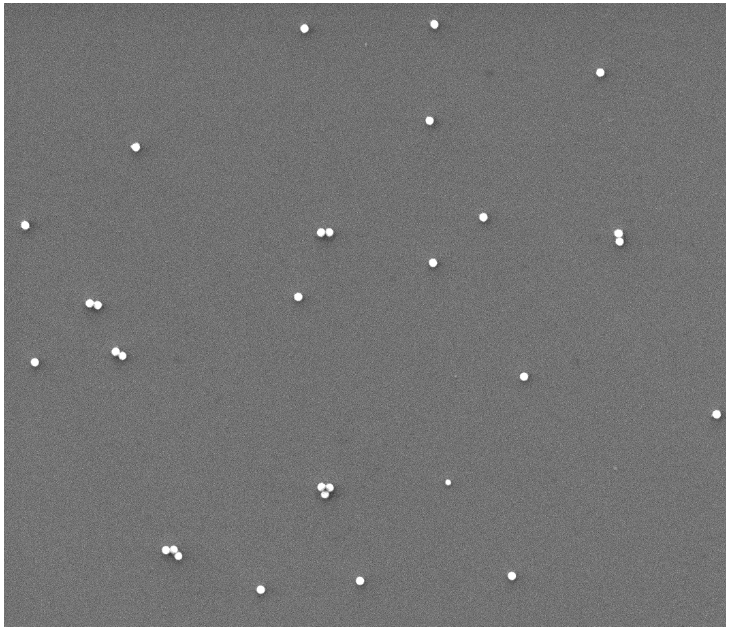
	M-OPC (Current primary standard at NMIJ)	**FCM** (Former primary standard at NMIJ)
Applicable size range	**600 nm** – 10 µm	2 µm – 10 µm
Throughput	High (≥10 mL/min)	Low (<0.2 mL/min)
Measurement uncertainty @ 2 µm PSL (k = 2)	1.5%	6.8%
Required operation time for the measurement	A day	Several days
Concentration range	500 – 2 000 000 particles g^{-1}	
References	Kuruma et al. (2020), *Adv. Powder Technol.*, 31, 848-858. Kuruma et al. (2021), *Metrologia*, 58, 045007.	Sakaguchi and Ehara (2011), Meas. Sci. Technol., 22, 024010.

- ✓ Reduction of the measurement time and uncertainty
- ✓ Extension of the size range from the micrometer to the sub-micrometer level

Validation of the M-OPC method by independent another method



Error bar： Expanded uncertainty, U (k = 2)
M-OPC： 4.3%, SEM： 3.8%



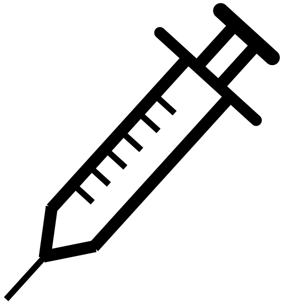
SEM image of 600 nm PSL

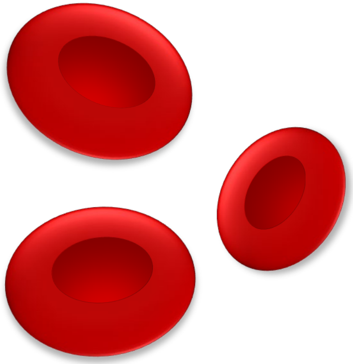
[Kuruma et al. (2021), *Metrologia*, 58, 045007.]

✓ The M-OPC method is validated by comparison with an independent* SEM method in size range between 600 nm to 10 µm.

*Both methods have independently established SI traceability.

Applications for biomedical and cellular analyses





Application 1: Insoluble particulate matter test for injections

[The Japanese Pharmacopoeia XVIII]

6.07 Insoluble Particulate Matter Test for Injections

1. Method 1. Light Obscuration Particle Count Test

1.1. Apparatus

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. ◆It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year.◆

1.1.4. Sensor

There is a possibility of changes of particle size resolution and counting rate of particle-detecting sensor in each sensor by assembling accuracy and parts accuracy even in the same type sensor. The threshold accuracy also needs to be confirmed. Testing should accordingly be performed for each of particle size resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PSL spheres having mean diameter of approximately 10 μm, of a concentration at 1000 particles/mL ±10%, not more than 5% of CV value).

Pharmacopoeia defines the test method for insoluble particulate matter in injections.

JP18, 6.07.
USP36-NF31, 788.
EP8.5, 2.09.10.

Test method:
Light obscuration particle count

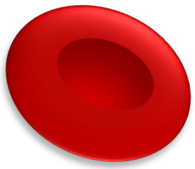
Counting accuracy test of LO sensor:
Reference standard suspensions*
with known PNC

*10 μm PSL suspension.

NMIJ provides calibration service for the manufacturers of commercial standard PNC suspensions.

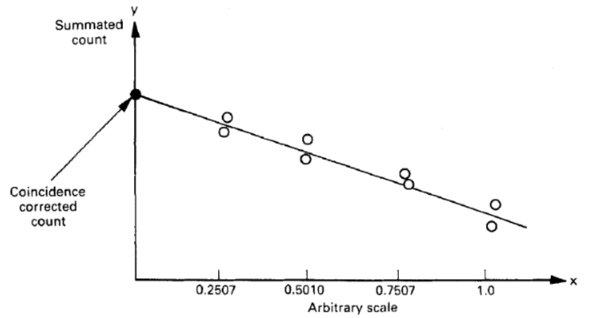
✓ M-OPC method contributes to produce the reliable standard PNC suspensions.

Application 2: Validation of the reference method for blood cell counting



Reference method for the enumeration of erythrocytes and leucocytes

INTERNATIONAL COUNCIL FOR STANDARDIZATION IN HAEMATOLOGY; PREPARED BY THE EXPERT PANEL ON CYTOMETRY →**ICSH**



[ICSH (1994), *Clin. Lab. Haemat.*, 16, 131-138]

- Red Blood Cells (RBCs) counting by blood cell counters based on electrical sensing zone principle
- Coincidence-loss correction by dilution series measurement (a potential major uncertainty source)

- Random components of measurement uncertainty have been evaluated, but some of systematic components, such as the counting efficiency of blood cell counters, haven't been sufficiently evaluated so far.

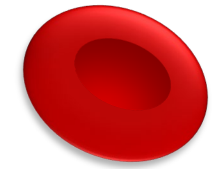
▼

- For accurate RBC count, establishment of SI traceability, including uncertainty evaluation, and eventually validation is needed for the ICSH method.

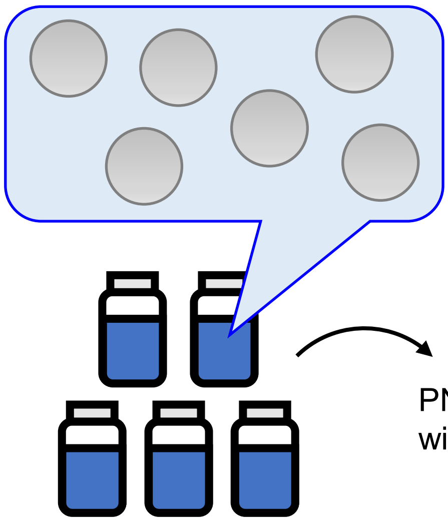
▼

✓ Comparison with something reliable is needed for accurate evaluation and validation.

Application 2: Validation of the reference method for blood cell counting



- Use of stable artificial particle suspension with known PNC as a reference standard for validating the ICSH method



PSL particles as
surrogate of RBC
(5 µm ≅ 65 fL)

PNC evaluation
with the M-OPC method

Preparation of 5 µm PSL suspensions
with known PNC

PNC measured by a blood cell counter and reference value determined by the M-OPC method agreed within the uncertainty.

[Kuruma et al., *in preparation*]

✓ Counting efficiency of the blood cell counter was evaluated.
✓ The ICSH method including coincidence-loss correction was well validated.

[This study was collaboratively conducted with Sysmex Corporation, Kobe, Japan]

Ongoing projects

Ongoing project 1: Development of standard particles for biomedical analyses

Quality Control (QC) challenge for biopharmaceutical products

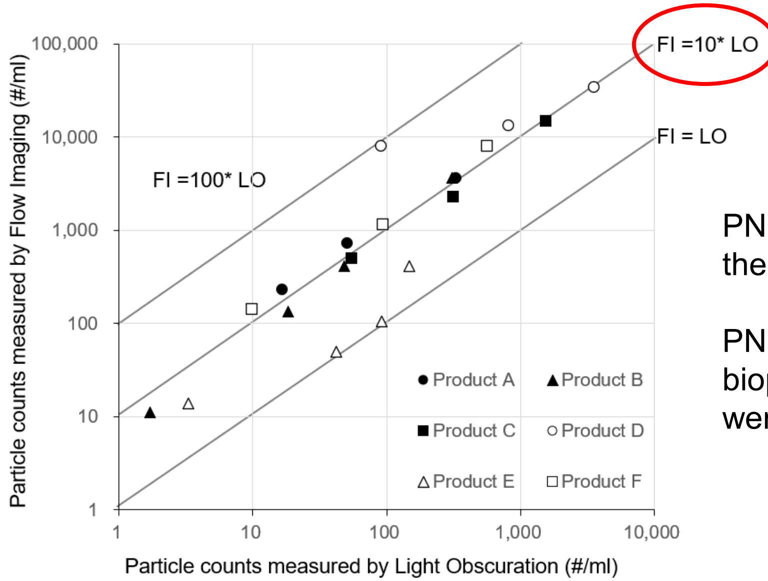
Flow Imaging (FI)

https://www.yokogawa.co.jp/solutions/products-platforms/life-science/flowcam-flow-imaging-microscopy/flowcam-8000/

Light Obscuration (LO)

https://www.rion.co.jp/product/particle/liquid_borne/pharmacy/kl-05.html

FI



LO

PNC measurements of various therapeutic protein injections by LO and FI

▼

PNCs of sub-visible particles* (SVPs) in biopharmaceuticals obtained by LO and FI weren't consistent.

*Protein aggregates, silicone oil droplets etc.

[Shibata et al. (2022), *J. Pharm. Sci.*, 111, 648-654.]

- There is a gap in the refractive index between SVPs and calibration particles (PSL).

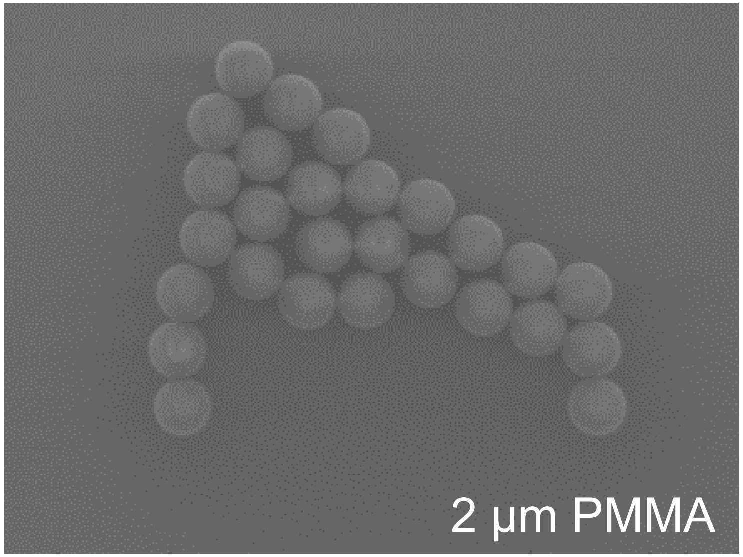
▼

- LO which is calibrated with PSL significantly underestimates the size of measured SVPs, which results in significant underestimation of the PNC of SVPs.

▼

✓ Use of standard particles with **a refractive index close to that of SVPs** is a key to resolve the inconsistency.

Ongoing project 1: Development of standard particles for biomedical analyses



Two size level of monodisperse PMMA particles (200 nm, 2 µm)
as a new forum standard for QC of biomedical analyses

- ✓ PNC (2 µm) and mean diameter determination: ***SI traceable***
- ✓ Spherical and highly monodisperse: **CV value is <3%**
- ✓ Low refractive index: ***n* = 1.5** (cf., PSL: *n* = 1.6)

■ We are planning an inter-comparison study with domestic partners in 2023.

University, Biopharmaceutical company, Testing laboratory etc.

[This research was supported by AMED under Grant Number JP20ae0201002]

Ongoing project 2: PNC Intercomparison study (CCQM-P222)

Project overview

- CCQM-P222 inter-comparison study is intended for the first step to establish validated methods to understand the NMIs' capability of absolute particle number concentration (PNC) measurement for cellular analysis, especially blood cell counting.

    1. To confirm the NMI's measurement capability of PNC.
    2. To investigate difference between the measurement principles/instruments.
    3. To elucidate the applicability of the artificial particles to the evaluation of measurement capability of cell analyzers.
    4. Establishment of a solid protocol for future key comparisons and a basis for CMC registrations for measurement of number concentration of cells/particles with artificial particles.

- In this pilot study, we use non-fluorescent 5 μm polystyrene latex (PSL) particles as surrogate of Red Blood Cells (RBCs).

Pilot labs.

National Metrology Institute of Japan (NMIJ)

Physikalisch-Technische Bundesanstalt Braunschweig und Berlin

Physikalisch-Technische Bundesanstalt (PTB)

Ongoing project 2: PNC Intercomparison study (CCQM-P222)

Study material specification

- Material
  →Aqueous suspension of PSL microspheres
- Nominal mean diameter
  →5 μm (65 fL)
- Coefficient of variation in diameter
  →1 %
- Dispersion medium
  →Water
- Nominal particle number concentration:
  →0.5×10^{6} – 1.5×10^{6} particles g^{-1}
- Volume per bottle
  →50 mL
- Packaging bottle
  →Polypropylene bottle with screw cap
  (sealed in an aluminum pouch to prevent evaporation)

✓ Homogeneity
✓ Long-term stability

✓ **15 laboratories will participate in P222 study.**