Small Particle Flow Cytometry:

Size matters and other considerations

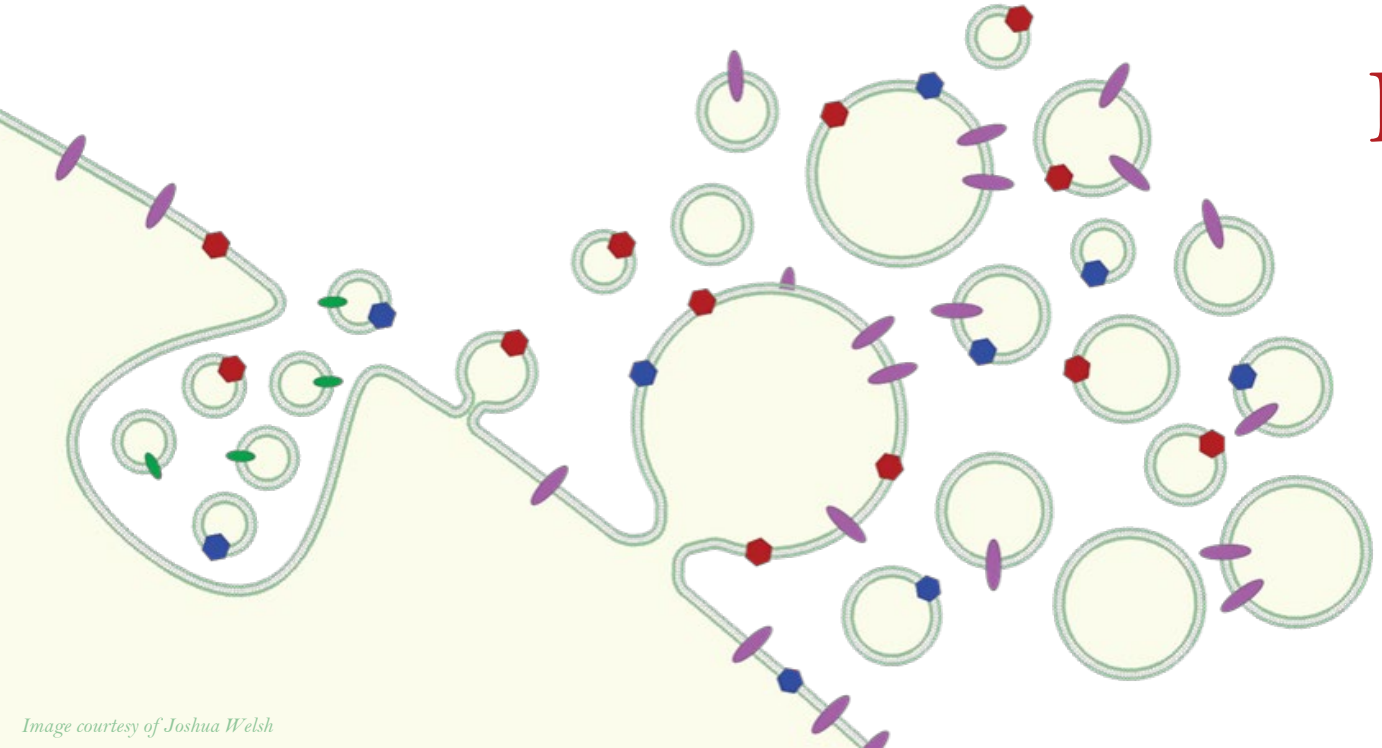

Image courtesy of Joshua Welsh

References & Links

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University of Ottawa

ISAC SRL Emerging Leader (2018-2023)
Canadian Cytometry & Microscopy Association (co-president)

Reporting Guide for Nanoscale FCM

J Extracell Vesicles. 2020; 9(1): 1713526.

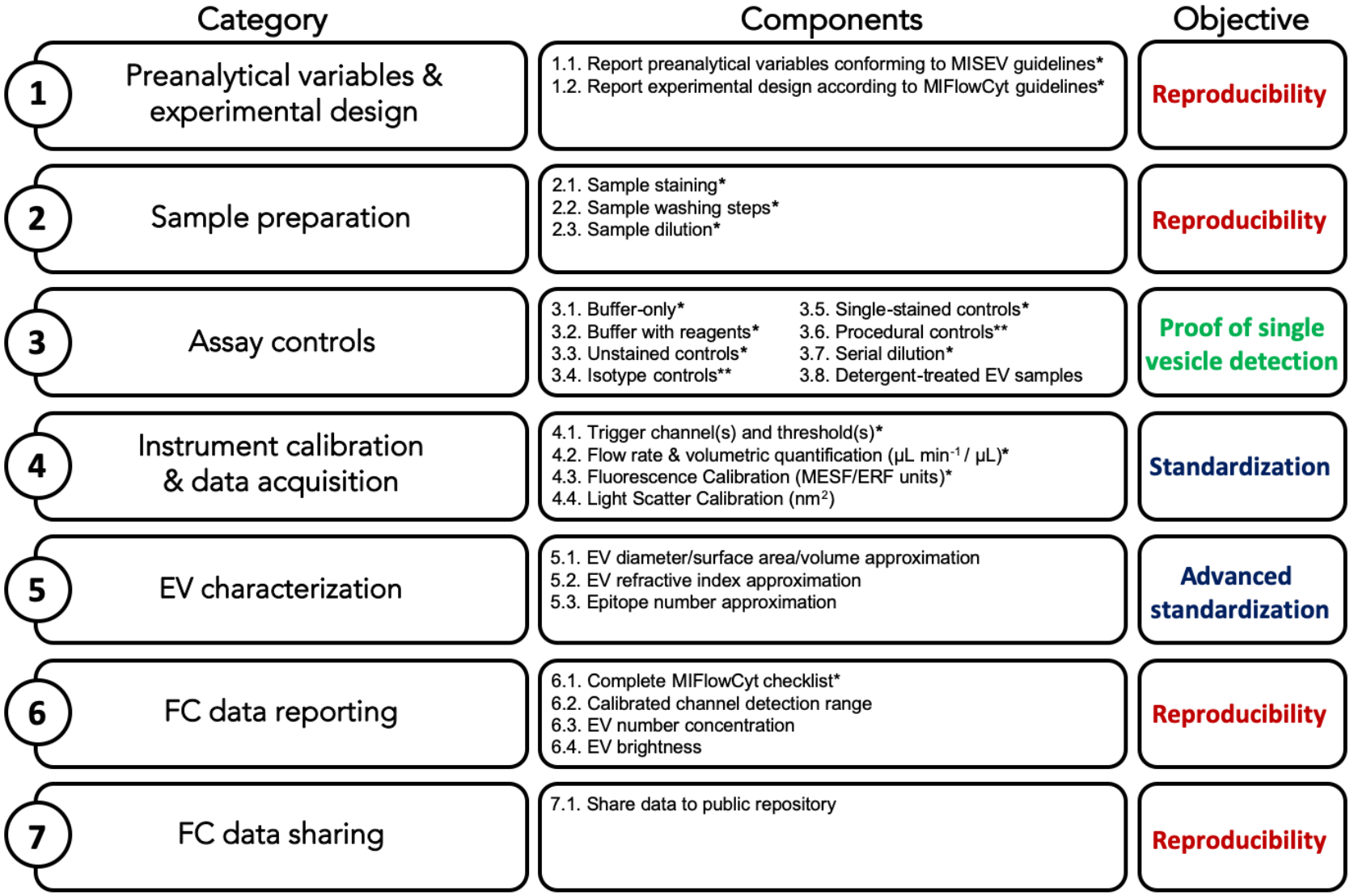
Minimum Information about a Flow Cytometry experiment on EVs *and other small particles* (MIFlowCyt-EV)

Contributing Societies:

International societies for extracellular vesicles, advancement of cytometry and thrombosis and haemostasis (**ISEV-ISAC-ISTH**)

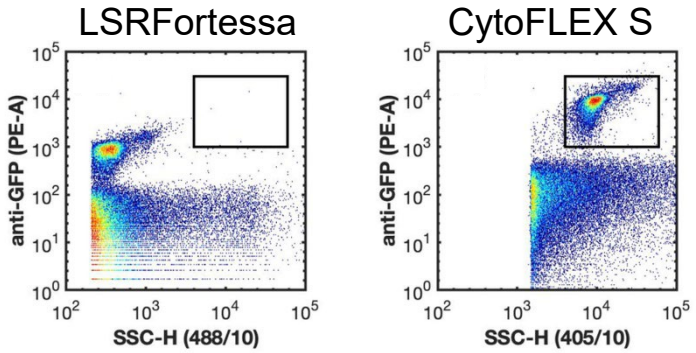
Goal:

To improve the quality of EV and small particle flow cytometry data



J Extracell Vesicles. 2020; 9(1): 1713526.

Calibration: *What & Why?*

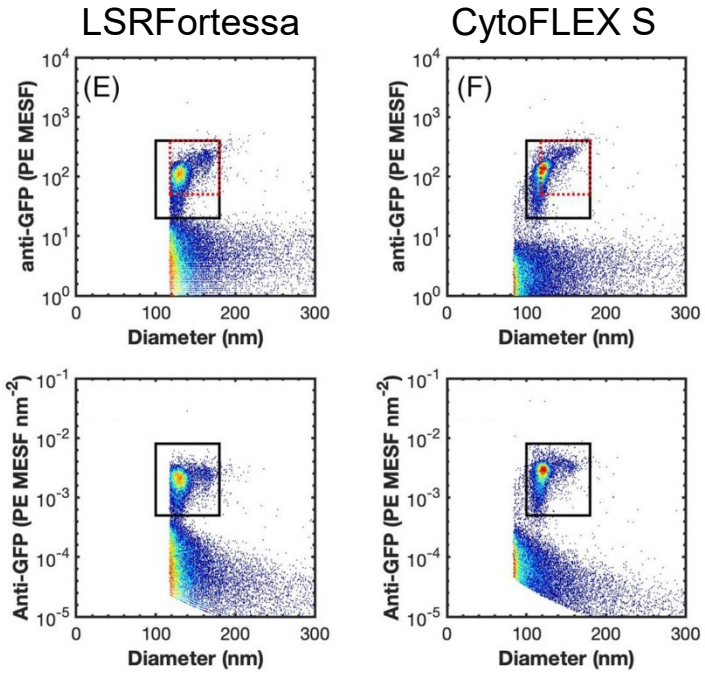


FL (a.u.) & SSC (a.u.)

Different instruments

- settings & configuration
- 488 vs 405 nm
- Sample – MLVsfGFP + anti-GFP-PE

calibrate



Antigen Density (MESF/nm²)

Calibration allows for data to be reported in **Standard Units** instead of Arbitrary Units of fluorescence & scatter

Futureproof Your Data!

Welsh JA, Jones JC, Tang V, **Fluorescence and light scatter calibration allow comparisons of small particle data in standard units across different flow cytometry platforms and detector settings.** Cytometry Part A, doi: 10.1002/cyto.a.24029

Methods for Calibration - Software

- **Scatter Calibration**
- **Fluorescence Calibration**
- **Detector Optimization**

Download: https://nano.ccr.cancer.gov/fcmpass/ Free for academic use
Protocol: https://currentprotocols.onlinelibrary.wiley.com/doi/10.1002/cpcy.79

Materials required: MESF or ERF beads for FL calibration, NIST-traceable polystyrene and silica beads (non-fluorescent)*

**FCM$_{PASS}$ recommended beads are listed in protocol reference*

EXOMETRY

Rosetta Calibration

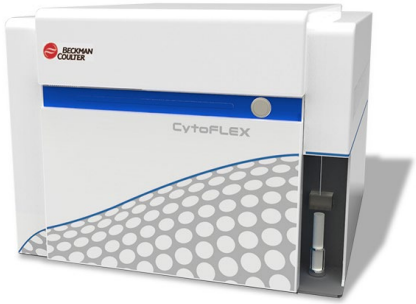
- **Scatter Calibration**

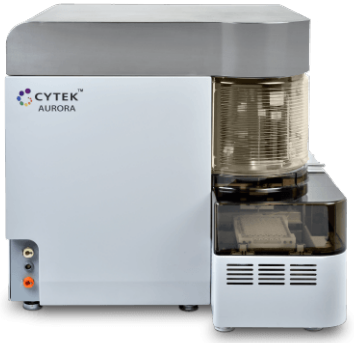
Available on FlowJo as a plug-in
Website for purchase: https://www.exometry.com/products/rosetta-calibration

Materials required: Rosetta Calibration Beads

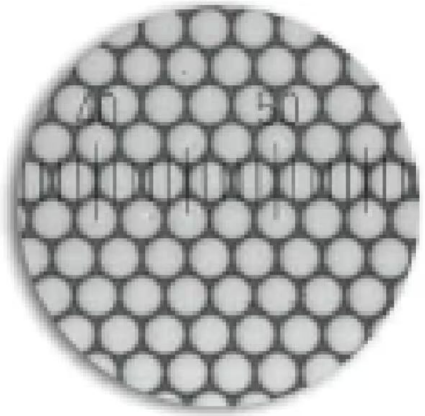
Materials Needed:

Flow Cytometer





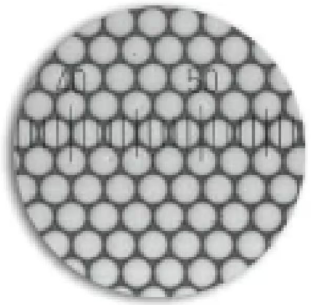
Reference Materials



Software

FCM PASS

Reference Materials:



Multi-peak Rainbow Particles

- QbSure Beads (Cytek B7-10005)
- 8-Peak Rainbow (Spherotech RCP-30-5A)

Light Scatter Calibration Beads

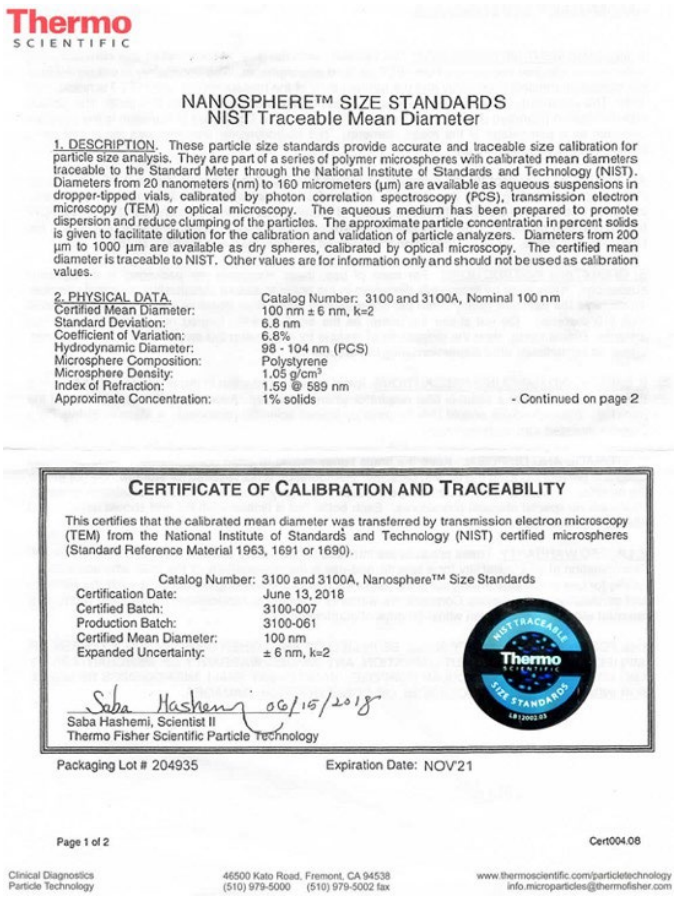
- NIST-treaceable size standards (Thermo Fisher 3000 series)

Fluorescence Calibration Beads

- FITC MESF (Bang Labs Quantum-5 MESF)
- PE MESF (BD Quantibrite PE)

Biological Reference Materials

- Not needed for calibration
- Used to validate assays – staining, isolations, etc.
- Recombinant EVs (not a bead! GFP+ EV reference material, Sigma SAE0193)



Thermo SCIENTIFIC

NANOSPHERE™ SIZE STANDARDS
NIST Traceable Mean Diameter

1. DESCRIPTION. These particle size standards provide accurate and traceable size calibration for particle size analysis. They are part of a series of polymer microspheres with calibrated mean diameters traceable to the Standard Meter through the National Institute of Standards and Technology (NIST). Diameters from 20 nanometers (nm) to 160 micrometers (µm) are available as aqueous suspensions in dropper-tipped vials, calibrated by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM) or optical microscopy. The aqueous medium has been prepared to promote dispersion and reduce clumping of the particles. The approximate particle concentration in percent solids is given to facilitate dilution for the calibration and validation of particle analyzers. Diameters from 200 µm to 1000 µm are available as dry spheres, calibrated by optical microscopy. The certified mean diameter is traceable to NIST. Other values are for information only and should not be used as calibration values.

2. PHYSICAL DATA.

Catalog Number:	3100 and 3100A, Nominal 100 nm
Certified Mean Diameter:	100 nm ± 6 nm, k=2
Standard Deviation:	6.8 nm
Coefficient of Variation:	6.8%
Hydrodynamic Diameter:	98 - 104 nm (PCS)
Microsphere Composition:	Polystyrene
Microsphere Density:	1.05 g/cm³
Index of Refraction:	1.59 @ 589 nm
Approximate Concentration:	1% solids

- Continued on page 2

CERTIFICATE OF CALIBRATION AND TRACEABILITY

This certifies that the calibrated mean diameter was transferred by transmission electron microscopy (TEM) from the National Institute of Standards and Technology (NIST) certified microspheres (Standard Reference Material 1963, 1691 or 1690).

Catalog Number:	3100 and 3100A, Nanosphere™ Size Standards
Certification Date:	June 13, 2018
Certified Batch:	3100-007
Production Batch:	3100-061
Certified Mean Diameter:	100 nm
Expanded Uncertainty:	± 6 nm, k=2

Saba Hashemi 06/15/2018
Saba Hashemi, Scientist II
Thermo Fisher Scientific Particle Technology

Packaging Lot # 204935 Expiration Date: NOV'21

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Useful Links:

Practice Datasets:

- rEV Serial Dilution: genboree.org/nano-ui/dataset/1754681130
- FL DSI: genboree.org/nano-ui/dataset/1754681134
- SSC DSI: genboree.org/nano-ui/dataset/1754681135
- NIST Bead Calibration: genboree.org/nano-ui/dataset/1754681131
- Cross-Calibration (FL): genboree.org/nano-ui/dataset/1754681132

Literature for Reference

- A compendium of single extracellular vesicle flow cytometry – Journal of Extracellular Vesicles https://doi.org/10.1002/jev2.12299

- Quantitative flow cytometry (qFCM) enables comprehensive optimization and cross-platform extracellular vesicle studies. Cook et al. 2023 CR-METHODS-D-23-00115R2

  This manuscript is under final review in Cell Reports Methods with associated protocols in submission to STAR Protocols. It summarizes a workflow for EV flow cytometry analysis from optimization of instrument detector settings to data calibration for FL and SSC parameters.