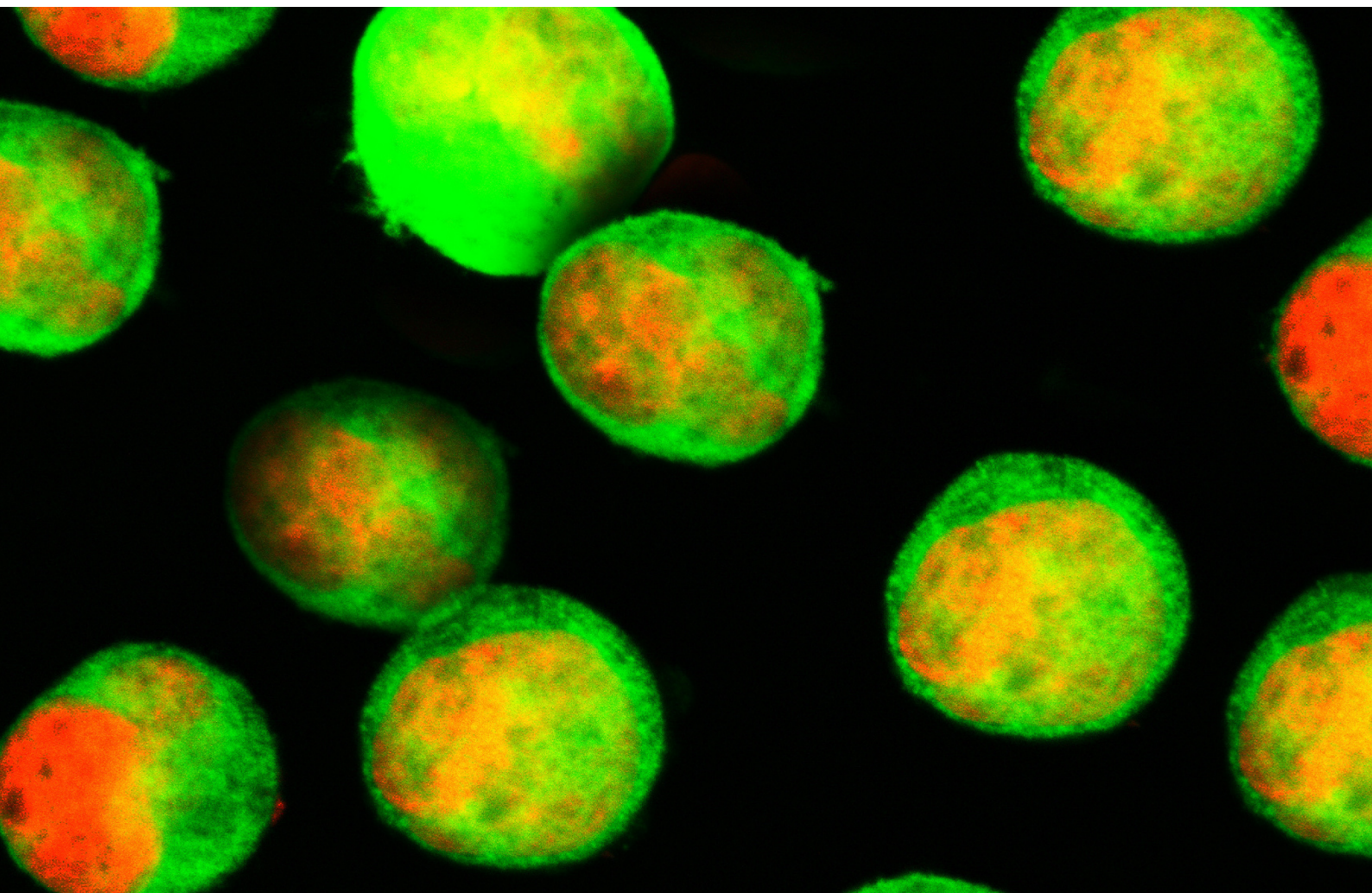




UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION

Bureau
International des
Poids et
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Metrology to Harmonize Cell and Particle Measurements and Advance Modern Medicine and Biotechnology

A NIST-led consortium of industry and academic partners has developed calibration beads with SI-traceable fluorescence intensities for accurate, quantitative flow cytometry, to underpin clinical diagnostics, the manufacturing of advanced therapies, and environmental and food safety monitoring

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The Challenge

Advances in cell and gene-based therapeutics, as well as other regenerative medicine products, have increased the need for high-quality, robust, and validated measurements for cell characterization. Flow cytometry is a sophisticated laser-based technology used to detect and measure the chemical, physical, and biological characteristics of highly heterogeneous cells and other biological entities, serving as a cornerstone of modern medicine and biotechnology. Its importance lies in its ability to analyze thousands of particles per second in high-speed fluid streams, in a highly multiplexed manner, providing unprecedented insights into complex biological systems.

Despite its enormous potential, broader adoption of flow cytometry as a definitive or reference method has been hindered by a lack of comparability across instrument vendors and models, due to differences in instrument design, including the lasers and detectors used in the detection. In the absence of physical artefact standards, cell and particle measurements have been highly dependent on the specific instrument model and operating conditions, greatly impeding metrological progress toward cell measurement comparability. When results are highly dependent on instrument models and operating conditions, the ability to collate results from different sources to identify trends is substantially weakened.

The Solution

The National Institute of Standards and Technology (NIST), as the US NMI, has collaborated with global flow cytometer manufacturers, reagent providers, and users to standardize flow cytometers in the NIST Flow Cytometry Standards Consortium.¹ NIST formed the consortium in December 2020, and the Consortium launched a large-scale study to collect the data needed to demonstrate, for the first time, a measurement approach² that enables the use of commercially available calibration microspheres (beads) based on assigned fluorescence intensity values. These calibration beads are SI-traceable through NIST-certified reference material SRM 1934 Fluorescent Dyes for Quantitative Flow Cytometry (Visible Spectral Range) with bead fluorescence intensity values, expressed in Equivalent Reference Fluorophore (ERF; see **Figure 1**) units. NIST assigns ERF values to commercial calibration beads via this new measurement service. This ultimately enables the calibration of the fluorescence scale for any

¹ NIST Flow Cytometry Standards Consortium. <https://www.nist.gov/programs-projects/nist-flow-cytometry-standards-consortium>

² De Rose et al. Assignment of Fluorescence Intensity Values to Calibration Microspheres and Nanospheres for Flow Cytometry. NIST Special Publication 260-264, 2026. <https://doi.org/10.6028/NIST.SP.260-264>

flow cytometer channel detector in an instrument-independent manner. In addition, NIST demonstrated the use of an instrument-dependent calibration value, Absolute Reference Intensity (ARI), on the same calibration beads. Together, these new derived units (ERF and ARI) and corresponding reference methods for measuring cells provide a comprehensive metrological framework for characterizing and standardizing global flow cytometer performance.³

The NIST Flow Cytometry Standards Consortium operates through a series of working groups (five in total) that work in collaboration towards the common goal of improving flow cytometry measurement capabilities:

1. *ERF-Based Instrument Calibration and Standardization*. This working group (WG1) develops standards, including reference materials, reference data, and reference methods, for the calibration of flow cytometers and the standardization of flow cytometry data.
2. *Flow Cytometry Assay Standardization*. This working group (WG2) designs and conducts interlaboratory studies to develop standards, assay control materials, and best-practice protocols that achieve flow cytometry assay standardization and generate high-quality reference datasets.
3. *Data Repository and Centralized Data Analysis*. This working group (WG3) organizes, analyzes, and disseminates data generated by Consortium interlaboratory studies (ILS), including developing and applying standardized metadata schemas that capture relevant aspects of ILS design, and the collaborative development of a robust data repository to catalogue input data, post-processed data, accessory files, and analysis reports.
4. *Gene Delivery Systems*. This working group (WG4) identifies and minimizes sources of variability in assays of gene delivery systems, including adenoviral, adeno-associated viral, and lentiviral vectors, lipid nanoparticles, and extracellular vesicles. Consensus-based methods, reference materials, and reference data are expected to be made available to the community as a result of these efforts.
5. *Artificial Intelligence and Machine Learning (AI/ML) Approaches*. This is the newest of the working groups (WG5) and will use high-quality reference datasets generated by Consortium interlaboratory studies to support the development and validation of emerging AI/ML models for flow cytometry

³ NIST Flow Cytometry Standards Consortium. <https://www.nist.gov/programs-projects/nist-flow-cytometry-standards-consortium>

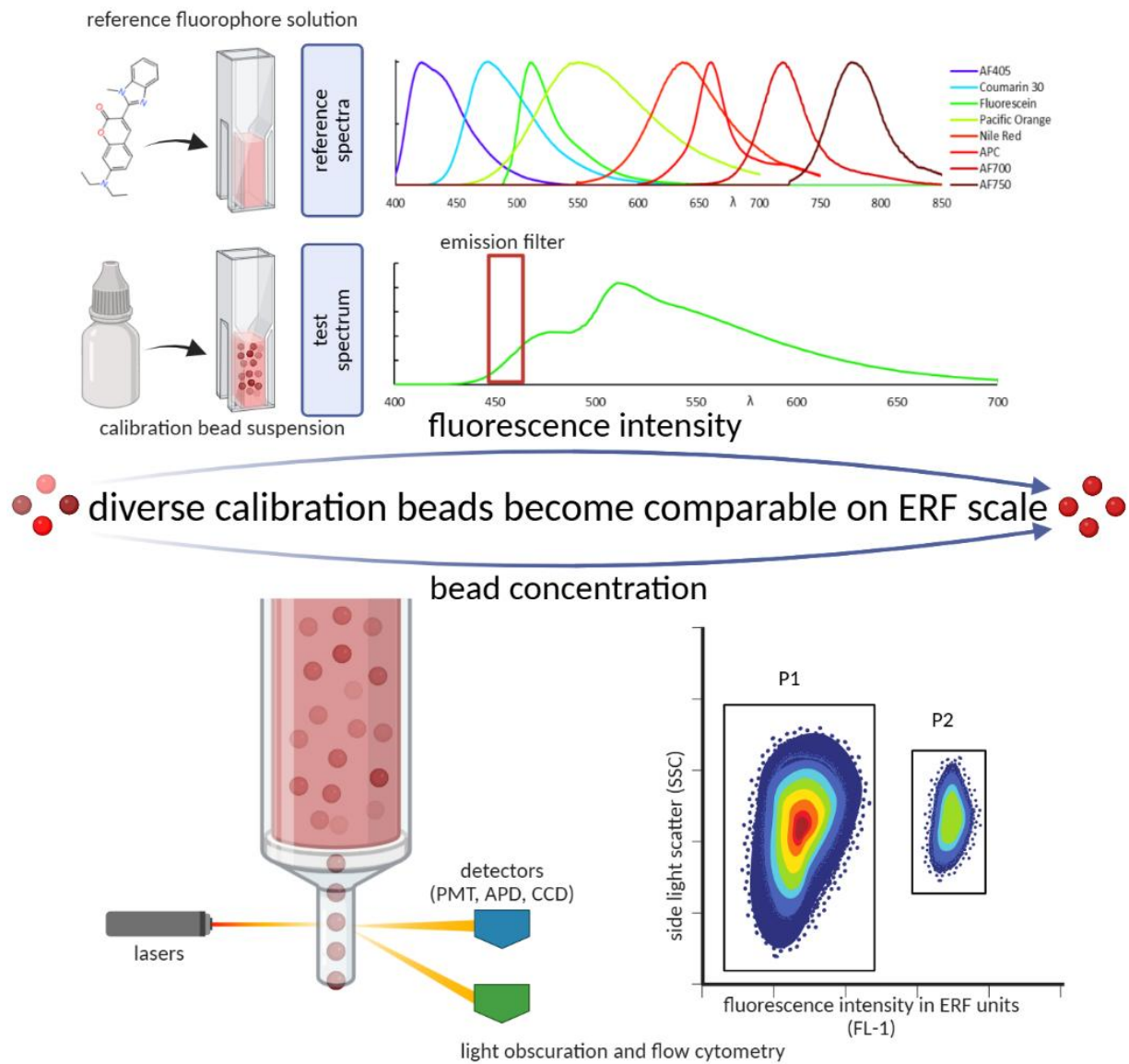


Figure 1. Schematic representation of the Equivalent Reference Fluorophore (ERF) value assignment through comparing fluorescence emissions of reference fluorophore solutions and bead suspensions and quantifying bead concentrations.

The Consortium meets regularly with members to continue advancing the goals of the private-public partnership. Since 2017, the Consortium has held several workshops (see **Figure 2**) to bring stakeholders together and collaborate to identify new measurement challenges.

A Quantifiable Impact

As a result of this work, metrological traceability for fluorescence measurement procedures is being adopted across the global cytometry industry. Through the Consortium's working groups and activities, NIST provides ERF assignment of commercially-available cytometer calibration beads as a measurement service to many global diagnostics and health care companies, as well as to the cytometry professional societies, thereby enabling instrument standardization. More importantly, this work provides the foundational metrology needed to transform flow cytometers from relative to absolute quantification of cells and particles. The potential impact of this work on global health and commerce is profound. In clinical settings, the ability to assign equivalent reference fluorophore values to cells enables standardized analysis of biomarkers, which is essential for diagnosing diseases, selecting therapies, monitoring immune responses in patients after treatment, and ensuring the safety of advanced therapies, such as lifesaving cell and gene therapies.



Figure 2. Dr Lili Wang, NIST Flow Cytometry Standards Consortium Principal Investigator, discussing the Consortium's work at the 2025 International Society for the Advancement of Cytometry's CYTO25 Meeting.

Over 90 % of globally-available flow cytometry instrument platforms (47 instrument models from 7 instrument manufacturers) participated in the 2024-2025 NIST Flow Cytometry Consortium-led interlaboratory study.⁴ Seven major global cytometry instrument manufacturers are members of the NIST Consortium and underpin the health industry across clinical diagnostics, drug development, immunological research, and cell and gene therapy biomanufacturing. Preliminary results from this study across sites and cytometer platforms indicate successful calibration of fluorescence intensities, which varied by seven orders of magnitude (10^7) prior to the metrological intervention.⁵ This has clearly advanced the capabilities of the global health industry through more quantitative and comparable measurements.

NIST has provided this measurement service to the majority of global cytometry instrument manufacturers that utilize reference materials. Furthermore, these manufacturers are collaborating within the NIST Flow Cytometry Consortium to further advance measurement accuracy and comparability across different cytometer platforms. These measurement services are also necessary for the broader adoption of newer flow cytometry instrumentation that offers higher multiplexing and increased resolution or sensitivity.

The study also suggests an unexpected preliminary finding that relatively inexpensive, shelf-stable calibration beads are often sufficient to achieve SI-traceable calibration of flow cytometry measurements. In cytometry, there has been a long-held expectation that beads functionalized with the actual fluorophores used in the assay will function best for fluorescence intensity calibration. Since fluorophores can include exotic dyes and recombinant fluorescent proteins, fluorophore-functionalized beads can be expensive and typically must be used within days of preparing a working suspension. However, for six of the ten fluorescence emission channels included in the study, the results show near- equivalent calibration performance whether a simple “hard-dyed” bead or a fluorophore-functionalized bead is used as the ERF-assigned calibrant. Some hard-dyed beads are 100x less expensive compared to fluorophore-functionalized beads, and many are shelf-stable for years. The Consortium will continue to investigate this result as it has the potential to make cell cytometry standardization more accessible and cost-effective, in addition to making the results more comparable and SI-traceable.

Industry stakeholders also view this non-destructive particle analysis technology as not only accelerating results for measuring cells or cell-based components, but also contributing to new measurement capability for larger-scale anthropogenic components, such as microplastics:

⁴ Wang et al. 2026, Standardizing A Human TBMNK Cell Assay Across Instrument Platforms: A New Framework for Producing High-Quality, AI-Ready Reference Dataset, *Frontiers in Immunology*, <https://doi.org/10.3389/fimmu.2026.1946801>.

⁵ Wang et al. 2026, Standardizing A Human TBMNK Cell Assay Across Instrument Platforms: A New Framework for Producing High-Quality, AI-Ready Reference Dataset, *Frontiers in Immunology*, <https://doi.org/10.3389/fimmu.2026.1946801>

“Flow cytometry is a critical technology across a variety of industries, and we see it expanding to new spaces where particle analysis is also needed, such as aquatic presence of microplastics. The applications are extensive and give users access to answers rapidly versus genomic or proteomic methods.”

Matthew Goff, Head of Hardware for Flow Cytometry, Beckman Coulter Life Sciences

With a value exceeding 5 billion USD in 2025, the global flow cytometry market is projected to double by 2033. This growth is anticipated from classical applications in clinical diagnostics, advanced therapy development and manufacturing, and patient immune health monitoring; emerging uses in environmental water monitoring, food and beverage fermentation, and agricultural biotechnology; and increased adoption of flow cytometric methods in healthcare and manufacturing across Asia.⁶ Furthermore, as a method that records tens of measurands from each of thousands of individual cells or particles in a matter of minutes, flow cytometry is poised to be transformed by, and to transform, artificial intelligence and machine learning (AI/ML) tools for researchers, manufacturers, and clinicians.

Qualitative flow cytometry has underpinned cutting-edge clinical and biotechnological advances as an investigative tool for decades. Now, *quantitative* flow cytometry, grounded in the solid metrological foundation of calibrant ERF assignment, is a standardizable measurement tool. Standardizing flow cytometry in the biopharmaceutical and clinical diagnostics research and development industry sectors is essential to support assay development, decision-making, and cross-site collaboration by ensuring that data generated in different laboratories can be compared. It helps to minimize sample processing and analytical variability, making it easier to distinguish true biological signals from noise and background signals from analytical instrumentation or other technical processes. Standardization also supports clinical trials by enabling cost-effective, reproducible transfer of assays to contract research organization (CRO) partners, ensuring that trial data are suitable for downstream interpretation, regulatory submission, and strategic decisions. It is also critical for QC and release testing in cell therapy and immune-oncology manufacturing, where standardized assays help ensure consistent product characterization, quality, and compliance. NIST, through its partnerships and measurement services, is enabling standardized, quantitative flow cytometry to underpin similar advances for decades to come.

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⁶ Mahajan and Nanadikar. Flow Cytometry Market – Global Forecast to 2033. MarketsandMarkets, 2025. <https://www.marketsandmarkets.com/PressReleases/flow-cytometry.asp>