Development of New Reference Material for Biopharmaceutical Advancement

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CASSS – Consultant's Network
March 23, 2026

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

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These opinions, recommendations, findings, and conclusions do not necessarily reflect the views or policies of NIST or the United States Government.

Why are reference materials important?

Reference materials help with:

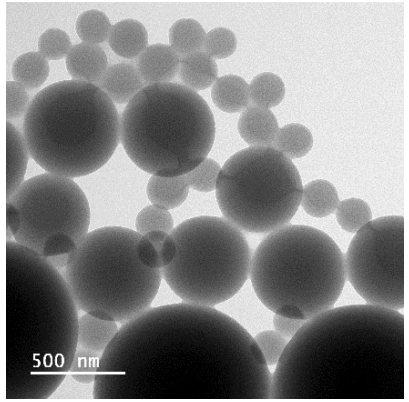
- **Verifying instrument performance -** Ensure instruments work correctly
- **Validating and calibrating measurement systems -** Ensure that analysis is accurate across industries
- **Developing and quality controlling products -** Ensure products meet quality control standards
- **Standardizing reporting -** Ensure published studies are comparable across different assays, instruments, and labs
- **Estimating measurement uncertainty –** Quantify instrument sensitivity
- **Training purposes**

Particle-Based Reference Materials In Development

RGTM 10231 silica-based

- Sub-micrometer (< 1 µm) particles with lower refractive index than typical polymer-based spheres
- Multi-modal sample covering wide size range

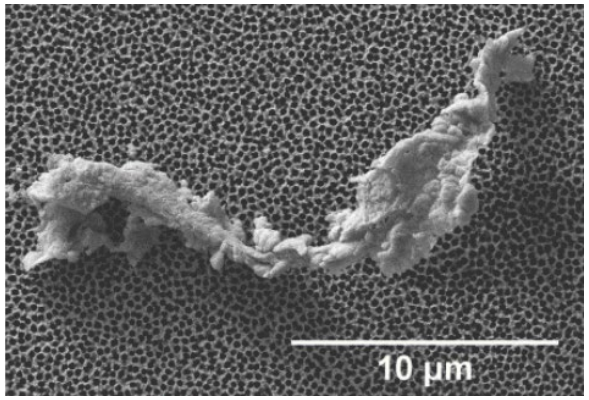
Benkstein et al. (2022) J. Pharm Sci. 111:699.



RM 8634 ETFE particles

- Subvisible proteinaceous particle surrogate
- Enables more accurate monitoring of particles in biotherapeutics

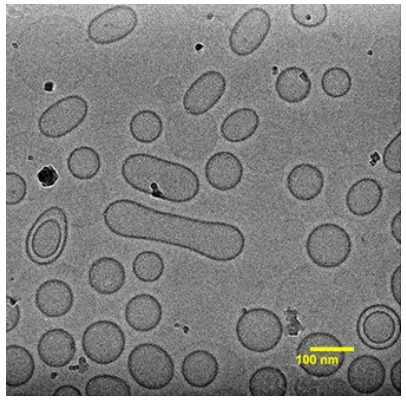
NIST Special Publication 260-193
Available 2019



RGTM 10237 liposome-based

- Test material relevant for characterization of lipid-based particles loaded with therapeutic vectors
- Development underway to examine material stability

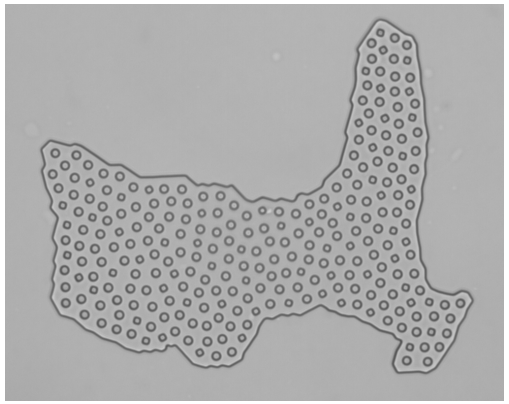
Lehman et al. (2023) Langmuir. 39:12313.



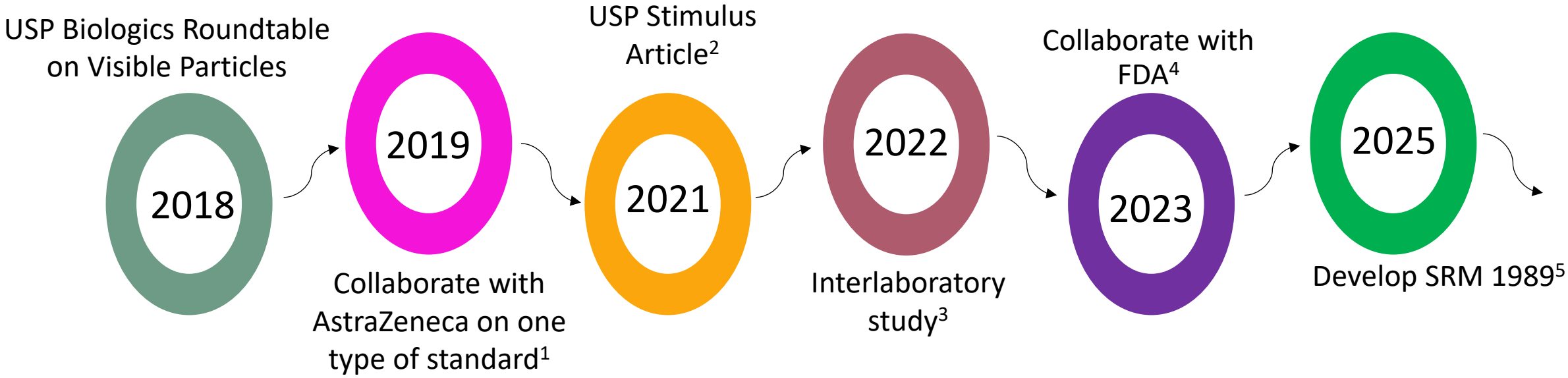
SRM 1989 visible particles

- Visible particle standard reference materials certified for size (3 varieties)
- Uniform, non-spherical

NIST Special Publication 260-255
Available 2025



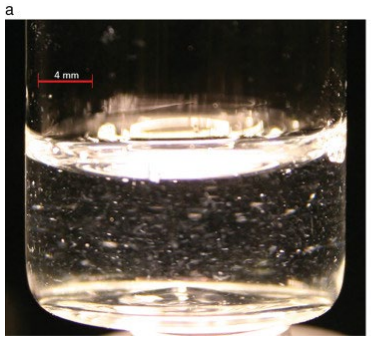
Development of The Visible Particle Standard



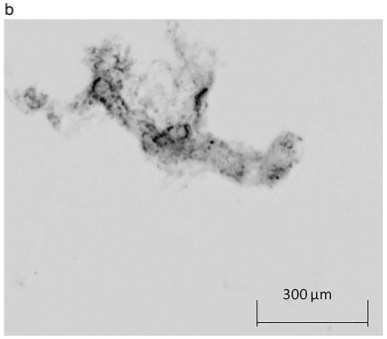
1. **Telikepalli S**, Gonzalez K, Dragulin-Otto S, Ripple D, Carrier M, Khan M. Development of protein-like reference material for semi-quantitatively monitoring visible proteinaceous particles in biotherapeutics. PDA J Pharm Sci Technol. 2019 Sep–Oct;73(5):418–432.
2. Narhi LO, Bou-Assaf GM, Gonzalez K, Mazaheri M, Messick SK, **Telikepalli SN**. Filling the pharmacopeial gaps of visual inspection: toward standardization and consistency of visible particle testing. USP Pharm Forum. 2021 May;47(3).
3. **Telikepalli SN**, Carrier MJ, Ripple DC, Barnett G, Bhirde A, Bolton D, Bou-Assaf GM, Ferrari E, Leigh S, Levitskaya-Seaman S, Menzen T, Nikels F, Riley A, Saggu M, Sahni N, Vernooij E, Wuchner K. An interlaboratory study to identify potential visible protein-like particle standards. AAPS PharmSciTech. 2022 Dec;24(1):18.
4. De Luna IF, **Telikepalli SN**, Carrier M, Ripple D, Srinivasan C, O'Connor T, Lute S, Bhirde A. Root cause analysis investigation of visible particulates in therapeutic protein drug product using morphologically directed Raman spectroscopy. Sci Rep. 2025 Nov;15:42026.
5. **Telikepalli SN**, Ripple DC, Carrier M, Steffens KL, Newton D, Montgomery C, Ritchie NWM, Asmar AJ, Halter M (2025) Certification of Standard Reference Materials® 1989: Monodisperse Irregularly Shaped Epoxy-Based Particles (Nominal 100 µm, 150 µm, 220 µm). (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-255. https://doi.org/10.6028/NIST.SP.260-255.

Visible Particles

Visible particles is **one of the main reasons** for drug recall; 31 % of recalls from 2017 to 2024 were due to particulate contamination (FDA Recalls, Market Withdrawals, and Safety Alerts Database)[1]



Vial containing VP



An image of a representative particle.

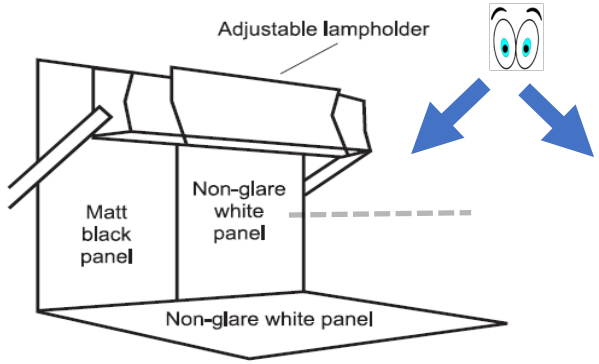
Non-proteinaceous: not acceptable

Proteinaceous: needs to be monitored & controlled

Ensure quality & manufacturing consistency

May affect safety of the drug

Manual



Semi-Automated

Instrument

Automated

Instruments qualified with reference particulates & training sets

Industry trying to move towards automated methods & we are interested in supporting that

1. https://www.fda.gov/drugs/drug-safety-and-availability/drug-recalls and https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/recalls-biologics

Challenges with Visible *Proteinaceous* Particles

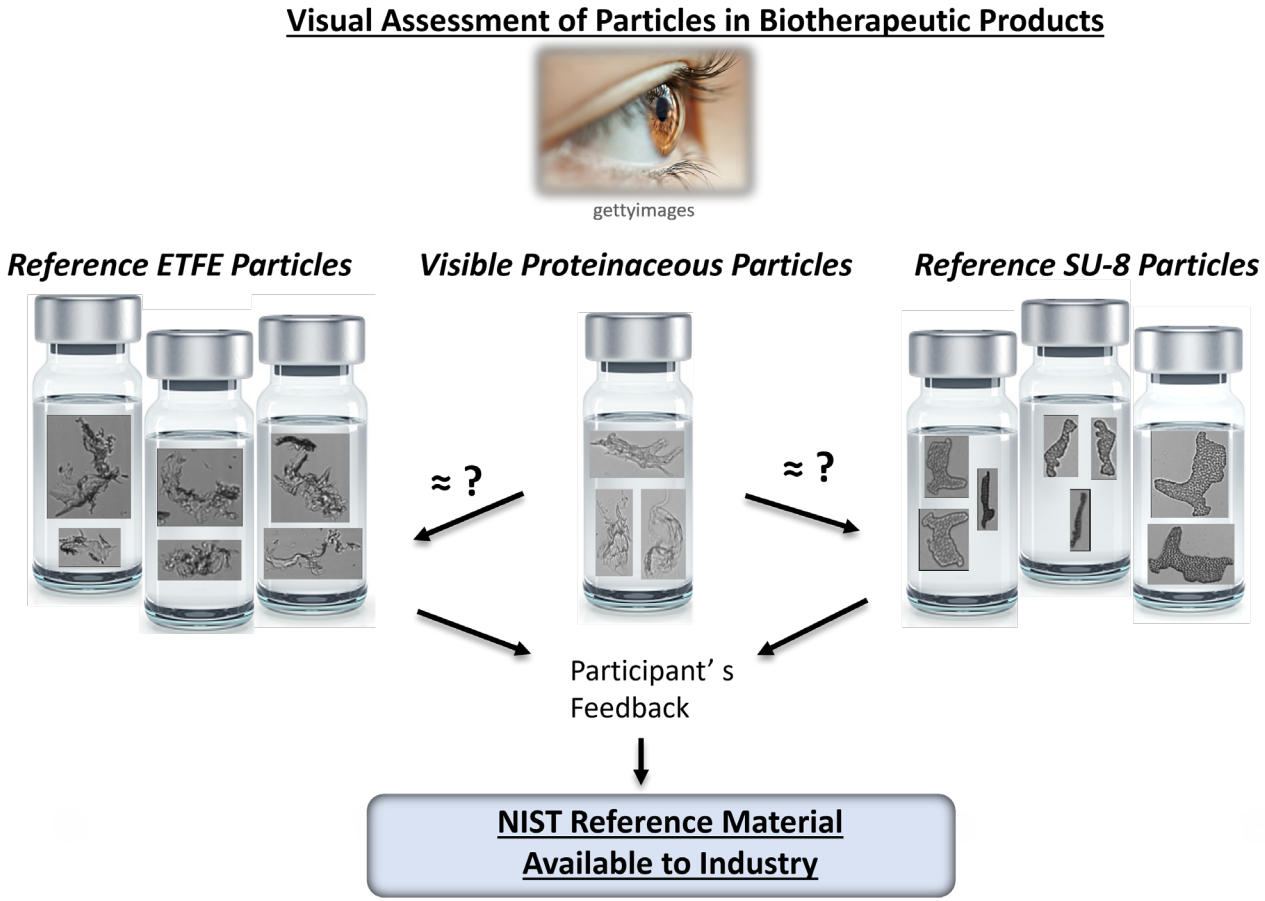
Technical & Methodological Challenges

- **Subjective & non-quantitative**: No clear size cut-off for particle being visible to human eye
- **Visibility is ≠ 100% detection**: probabilistic (ex: 150 µm bead POD 70%, fibrillar particle might need to be a lot bigger)
- **Many parameters effect visual inspection** (operator, illumination, observation time, type & speed of rotation, fill volume & primary packaging, location of particulate—floating or stuck, **type of particle**—transparency, size, density, shape, etc.)

Regulatory & Standardization Gaps

- **Regulatory guidelines may be vague**
- **No commercial protein-like particle reference standards** for monitoring proteinaceous VP & for training
- **Company specific/ product specific limits & criteria**
- **No standard definition** of "visible" for irregular, translucent proteinaceous particles

Visible Particle Interlab Study



List of participants in the study

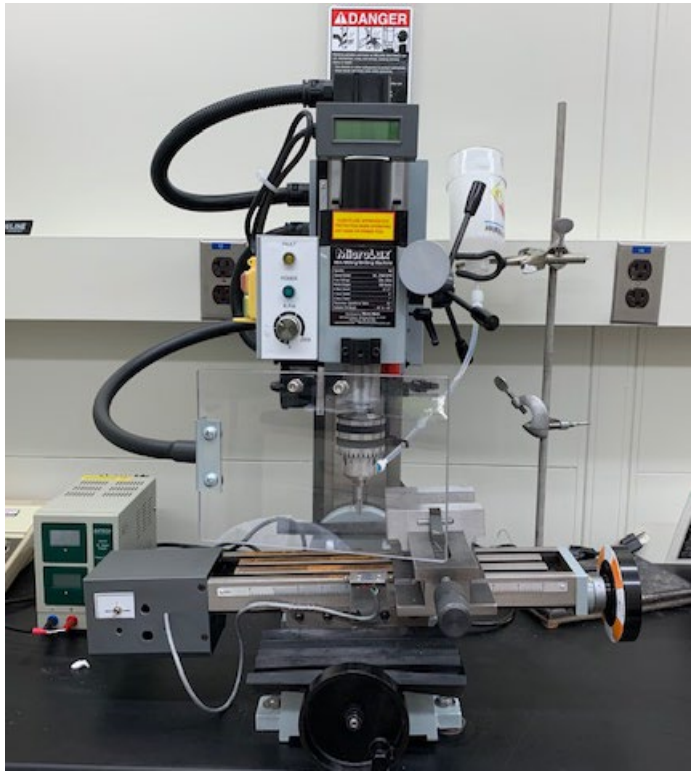
Amgen, Inc.
AstraZeneca
Biogen
Boehringer Ingelheim Pharma GmbH
Coriolis Pharma
Eli Lilly and Company
Fibrogen
Food and Drug Administration
Genentech, Inc., Roche Group
GlaxoSmithKline
Janssen R&D, DPDS BioTDS Analytical Dev.
Macrogenics
Porton Biopharma Ltd
Sanofi, BioAnalytics

Qualitative study to get input from industry
Goal was to use feedback to develop a RM

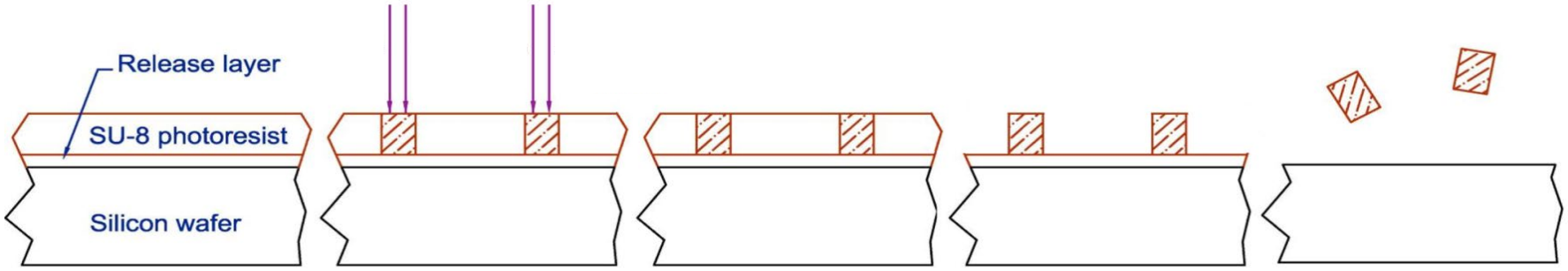
Telikepalli et al. AAPS PharmsciTech. (2022), 24:18

Production of the Two Particle Standards

ETFE Particles



Photolithographic Particles (SU-8)



1. Coat wafer with the release layer (Omnicoat) followed by SU-8 2005
2. Expose wafer to UV light with noncontact exposure tool
3. Post exposure bake at 95°C
4. Remove unexposed SU-8 in developer solution
5. Dissolve Omnicoat layer and release particles into solvent

Test kit for participant evaluation.

Considerations for Interlab Study

Overall Perspective on 2 Potential Visible Particle Standards: ETFE and Photolithographic SU-8

- Company Ratings
- Lab Ratings (Form, Analytical, QC, Manufacturing)
- Usability & Handling
- Challenges

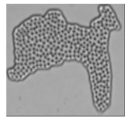
Particle Size

Monodisperse SU-8: 150 µm, 250 µm, 300 µm

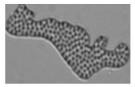
Polydisperse ETFE: Enriched in (75 to 150) µm, (106 to 150) µm, (150 to 250) µm

Particle Morphology

SU-8



AR ~ 0.6



AR ~ 0.3

ETFE

AR ~ 0.2 to 0. 7

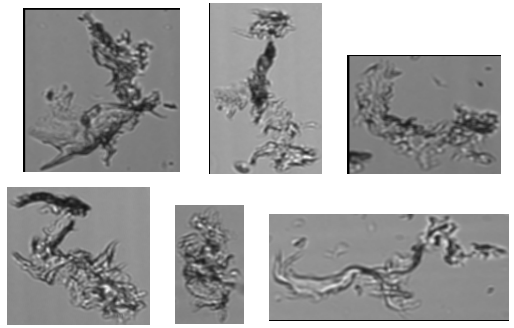
Background Solution

Clear/ slight opalescence/ slight viscous/ slight opalescence & viscous

Two Standards - Two Applications for Visual Inspection

Designed 2 particle candidates: 1) mechanically produced ETFE (larger) 2) photolithographically produced SU-8

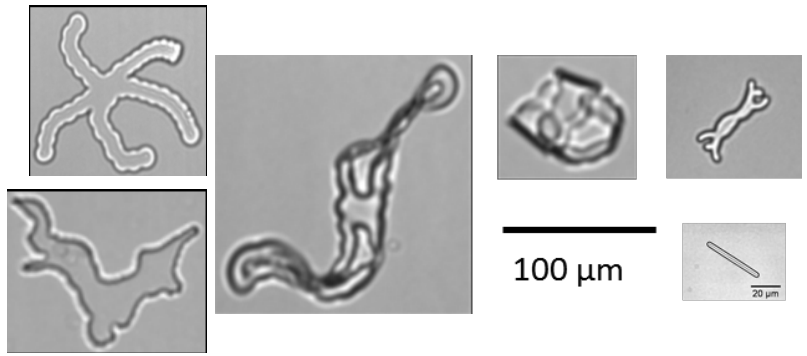
1) ETFE

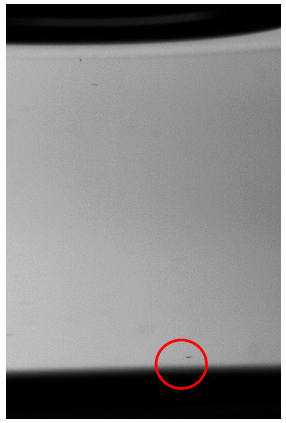


Looked "more proteinaceous" Polydisperse haze of proteinaceous particles in a vial

- Semi-Quant Use[1]
- Formulation Dev
- Stability testing
- Qualification & Training

2) Photolitographically produced (SU-8)





Monodisperse, distinct particles (150 or 250) µm

- Sensitivity of inspection
- POD studies
- Define "visible"
- Qualification & Training

[1]Telikepalli S, et al. PDA J Pharm Sci Technol. 2019;73(5):418-32.

Applications based on their physical properties.
Each type of particle serves a different but complementary role in visual inspection practices

Interlab Comparison: Two Paths Forward

Overall Findings: 1) No perfect standard
2) Can't meet user need with a single RM
3) Both types could be useful for training purposes

Photolithographic (SU-8) Particles

Pros

- Used as **size** standard (irregular particles)
- More uniform
- Easier to characterize

Cons

- Harder to produce
- SU-8 not perfect mimic

- 100 µm, 150 µm, & 220 µm
- Certify for size, concentration
- 1 morphology (may expand later)

ETFE Particles

Pros

- Used as **conc.** standard (irregular particles)
- Semi-quant use favored
- Looks more like protein particles

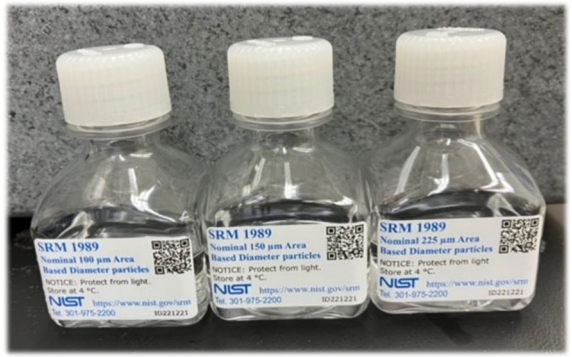
Cons

- Harder to characterize
- Sizing limits harder to define

- 150 µm to 300 µm
- Certify for particle size distribution
- Details TBD

General Schematic for SRM 1989 Production

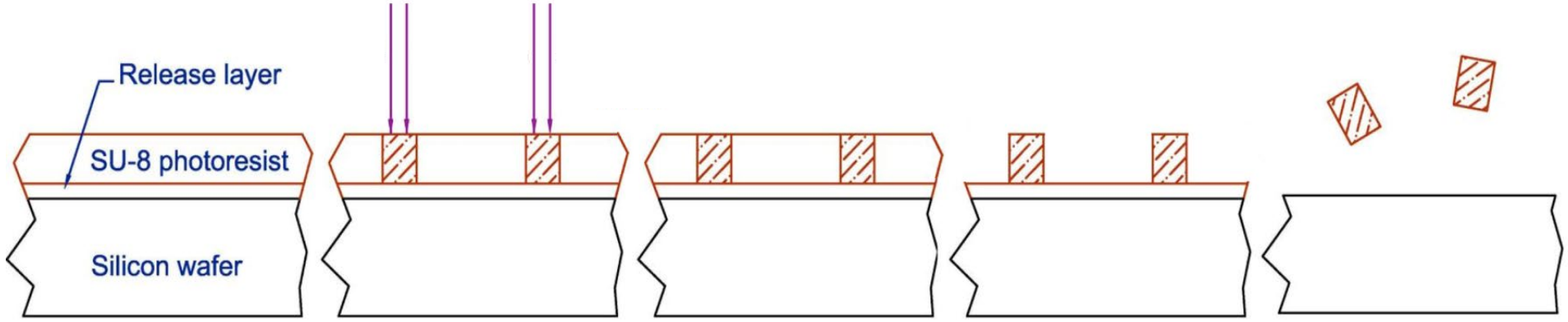
1. Optimize photolithographic procedure - high yield & quality (size & shape uniformity)
2. Release particles of each wafer into solution
3. Combination of multiple wafer contents
4. Remove contamination (unintended particles)
5. Concentration measurements of batch
6. Fill (small batch)
7. Characterization of batch, homogeneity, stability, and assessing biases of the methods



Telikepalli SN, Ripple DC, Carrier M, Steffens KL, Newton D, Montgomery C, Ritchie NWM, Asmar AJ, Halter M (2025) Certification of Standard Reference Materials® 1989: Monodisperse Irregularly Shaped Epoxy-Based Particles (Nominal 100 µm, 150 µm, 220 µm). (NIST, Gaithersburg, MD), NIST SP 260-255. https://doi.org/10.6028/NIST.SP.260-255

Fabrication of an Irregular Shaped Visible Particle

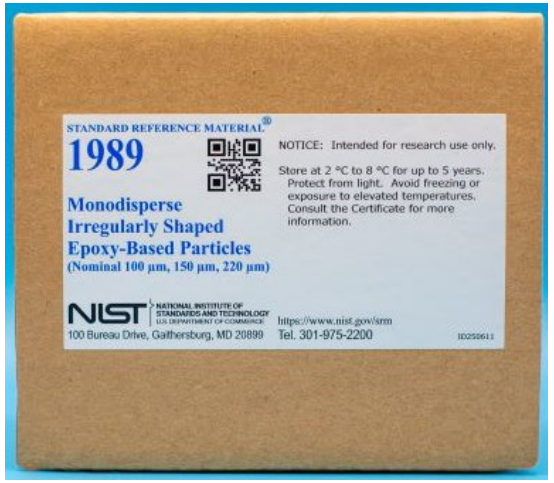
Step 1: Optimize Photolithographic Particles (SU-8) Production in the NIST Nanofab Facility (M. Carrier)

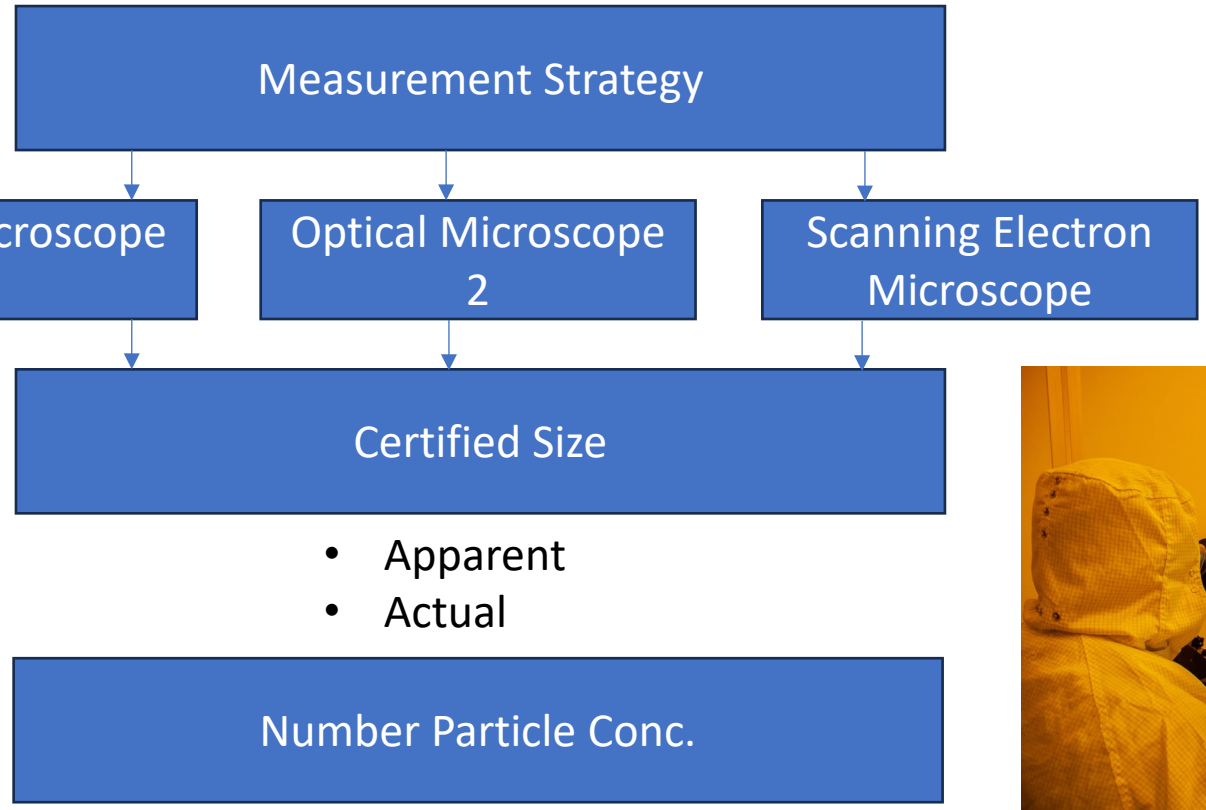


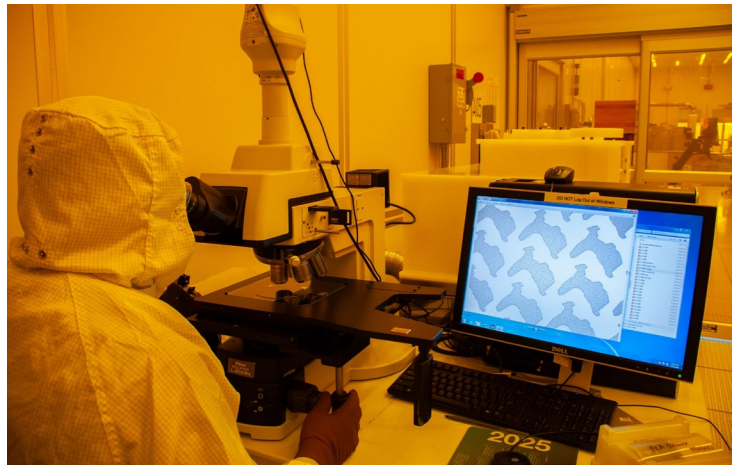
1. Coat wafer with the release layer (Omnicoat) followed by SU-8 2005
2. Expose areas of wafer to UV light with noncontact exposure tool
3. Post exposure bake at 95°C to crosslink the UV exposed areas
4. Remove unexposed SU-8 in developer solution
5. Dissolve Omnicoat layer and release particles into solvent

Solvent exchanges,
Purification,
etc.

Measurement Strategy for SRM 1989







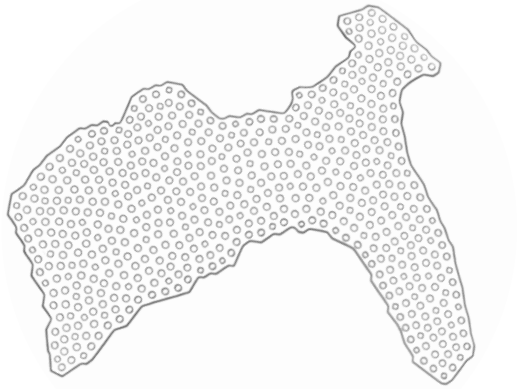
NIST SP 260-255

Ensure 1) Consistency in methods 2) Batch homogeneity 3) Stability of size over time

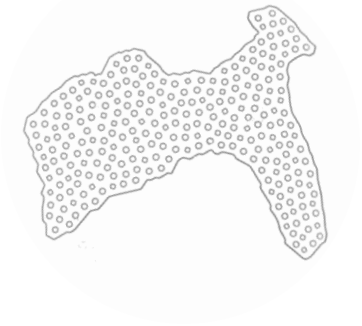
The certified size & uncertainties characterized in more rigorous detail than probably required for user community.

Sizing of SRM 1989

1 unit of SRM 1989 contains 3 particle sizes (3 vials)



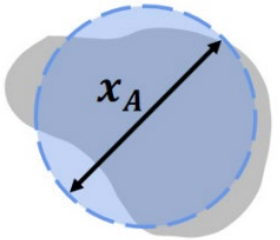
220 µm ABD or
335 µm MFD



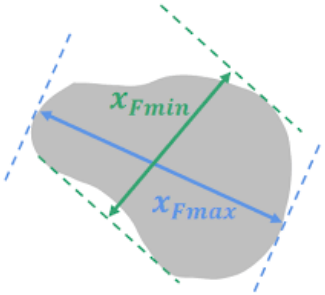
150 µm ABD or
225 µm MFD

100 µm ABD or
150 µm MFD

Area Based Diameter



Max Feret Diameter



ABD - diameter of a circle with the same number of pixels as a particle in a binary image (equivalent to ECD).

MFD - maximum distance between two parallel planes touching a particle border but not intruding into the particle interior.

Certified dimensional values are traceable to the Standard Meter through the use of a NIST-calibrated stage micrometer, which provides a length reference that is traceable to the SI.

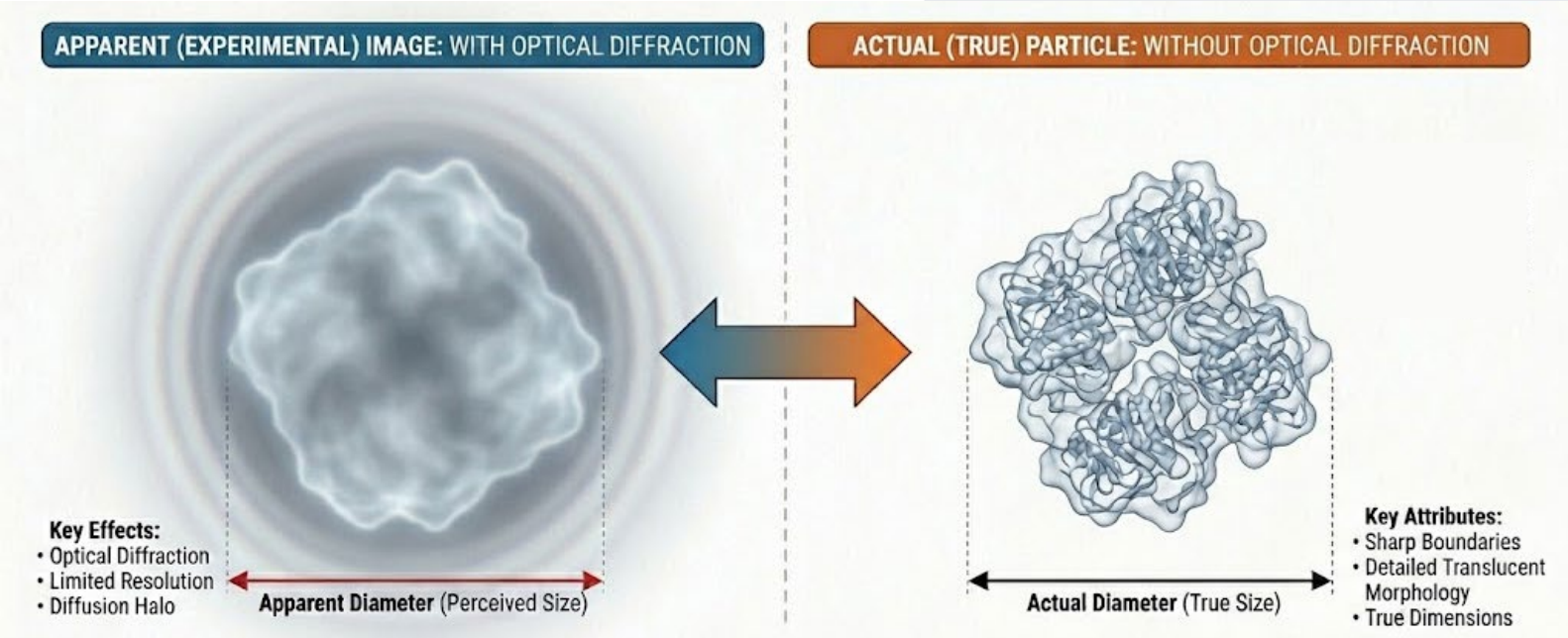
Apparent vs. Actual Size

Apparent Size (Optical)

- Based on optical image analysis (experimental)
- General Method: 1) Optically image particles on two optical microscopes; 2) Adjust for background and threshold; 3) Software to size particles
- ABD & MFD

Actual Size (True)

- True particle diameter
- Corrected for optical diffraction effects
- Higher accuracy representation
- ABD & MFD



For most applications, this differentiation is probably **NOT** important.

NIST SP 250-255

Image generated by Gemini

NIST Special Publication 260
NIST SP 260-255

Certification of Standard Reference Material® 1989

Monodisperse Irregularly Shaped Epoxy-Based Particles (Nominal 100 µm, 150 µm, 220 µm)

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David Newton
Christopher Montgomery
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Michael Halter

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.260-255

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U.S. DEPARTMENT OF COMMERCE

Date of Issue:
1 May 2025

Standard Reference Material® 1989

Epoxy Based Monodisperse Photoresist Particles for Particle Size and Morphology

CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for use as a particle size standard to validate the precision of sizing methods of irregular shaped particles over an approximate size range of 100 µm to 220 µm. SRM 1989 has been developed to mimic the optical properties of visible aggregated proteinaceous particles.

Description: A unit of SRM 1989 comprises 3 vials, with each vial containing approximately 20 mL of an aqueous suspension of SU-8 photoresist particles in a solution of 0.02 % sodium azide and 0.01 % surfactant [4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol] by mass concentration. The SU-8 photoresist particles were prepared by fabrication of particles onto a sacrificial silicon wafer using a photolithographic process, followed by release from the wafer, purification, and dilution.

Certified Values: The certified particle size in Area Based Diameter (ABD) and Maximum Feret Diameter (MFD) and their related expanded uncertainties were measured on SRM 1989 through optical and electron microscopy methods. Area Based Diameter is the diameter of a circle whose area contains the same number of pixels as a particle in a binary image. Maximum Feret Diameter is the maximum distance between two parallel planes touching a particle border but not intruding in the particle interior. These certified dimensional values are traceable to the Standard Meter through the use of a NIST-calibrated stage micrometer, which provides a length reference that is traceable to the SI. Table 1 below reports both the Actual physical size (the true diameter of the particle) and the Apparent size of the particle, as determined by optical methods. The Apparent size is larger than the Actual size because of optical diffraction effects. Apparent size was measured on an optical microscope using Köhler illumination with green light (≈ 530 nm wavelength), a 0.30 numerical aperture objective, and particle edge defined as the perimeter where the image intensity is halfway between the background intensity and the darkest portion of the imaged particle border. Actual size was determined by correcting the Apparent size for optical diffraction effects.

Table 1. The certified Apparent and Actual Size, in Area Based Diameter (ABD) and Maximum Feret Diameter (MFD), of the 3 nominally sized particle suspensions that comprise 1 unit of SRM 1989.

Nominal Particle Size (µm)	Apparent ABD (µm) [a]	Actual ABD (µm) [a]	Apparent MFD (µm) [a]	Actual MFD (µm) [a]
100	100.65 ± 2.15	98.38 ± 2.20	149.11 ± 1.37	147.69 ± 1.40
150	150.16 ± 2.21	147.92 ± 2.30	223.08 ± 1.51	221.76 ± 1.55
220	222.39 ± 2.14	219.76 ± 2.20	333.33 ± 1.66	331.67 ± 1.74

SRM 1989 Quality

APPROVAL OF QUALITY MANAGEMENT SYSTEM

APROBACIÓN DEL SISTEMA DE GESTIÓN DE LA CALIDAD

SIM SISTEMA INTERAMERICANO DE METROLOGÍA

National Institute of Standards and Technology (NIST) – USA

Certified Reference Materials for Biomolecular Measurement – Nucleic Acid-Based, Particle-Based, Protein-Based Materials

The Quality Management System supporting measurement services, as defined in a motion of the Quality System Task Force (QSTF), has been reviewed and found to be in conformance with the requirements of ISO 17034:2016 under the mandate of the Inter-American Metrology System in support of the expectations of the CIPM Mutual Recognition Arrangement.

El Sistema de Gestión de Calidad en que se apoyan los servicios de medición, según se define en una moción del Grupo de Trabajo del Sistema de Calidad (QSTF), ha sido revisado y se ha determinado que cumple con los requisitos de ISO 17034:2016 bajo mandato del Sistema Interamericano de Metrología en apoyo de las expectativas del Acuerdo de Reconocimiento Mutuo del CIPM.

Sally Bruce, Quality System Task Force Chair

Javier Arias, President, SIM

Approval date: 6 October 2025

Expiration date: 6 October 2030

- **International recognition of quality**: Our QMS is formally approved by Inter-American Metrology System (SIM QSTF), confirming compliance with key ISO standards and validating the rigor of our SRM processes.
- **Enables global acceptance**: This approval is essential for recognition of Calibration and Measurement Capabilities (CMCs) under the CIPM Mutual Recognition Arrangement, supporting worldwide comparability of measurement results.
- **Demonstrates excellence and traceability**: The certificate verifies our commitment to high-quality reference material production, strengthening confidence in our SRM portfolio and its metrological traceability.

Potential Applications of SRM 1989

Training of visual inspection analysts with regards to “protein-like” particles

Determining sensitivity of inspection/probability of detection studies

Qualification of semi-automated or automated methods

Status: Product released in 2025

Acknowledgements

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Dean Ripple (retired)
Michael Carrier
Kristen Steffens
Christopher Montgomery
Kurt Benkstein
Sean Lehman
Wyatt Vreeland
Kyle Anderson
Dick Cavicchi
David Newton
Anthony Asmar
Michael Halter
Nicholas Ritchie
Z.Q. John Lu

Thank you!


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