DBY Molecular Weight Measurement

Quick Start Guide

Brookhaven Instruments NanoBrook OMNI · Particle Solutions Software

Revised: April 2026

Table of Contents

Background

The DBY Molecular Weight Measurement is a single-angle static light scattering (SLS) technique performed on the Brookhaven Instruments NanoBrook. It applies a simplified form of the Zimm equation to determine the weight-average molecular weight (M_w). This approach, the Debye Plot, assumes angle-independent scattering and is most reliable for particles with a radius of gyration R_g < 12 nm.

Instrument Setup

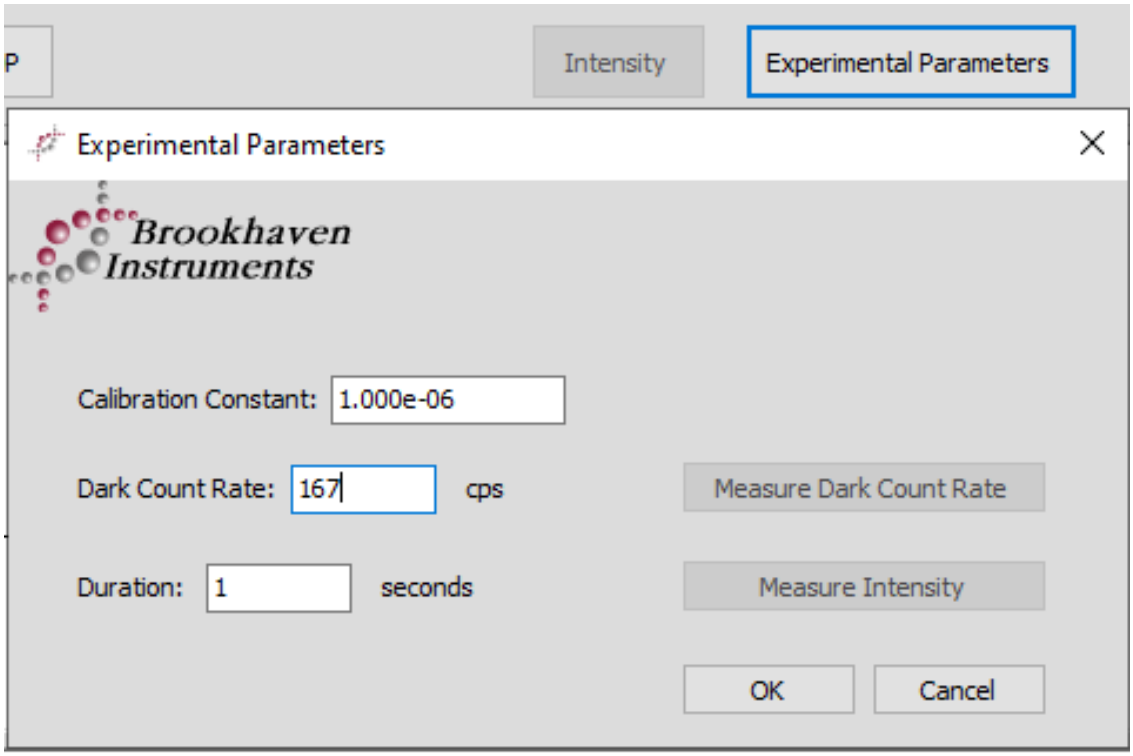
i. Verify Dead Time Correction for APDx Detector

Before running measurements, confirm the dead time correction is set correctly. Navigate to Setup > Hardware Configuration. Under Dead Time Correction, confirm APDx is selected with a value of 25 ns. If incorrect, update before proceeding, an incorrect value will produce inaccurate intensity data and erroneous molecular weight results.

ii. Dark Count Rate

Place the flow cell into the sample area with the windowless wall facing right (laser entrance). Select New > DBY Molecular Weight Debye Measurement in Particle Solutions, then click Experimental Parameters.

DBY Experimental Parameters

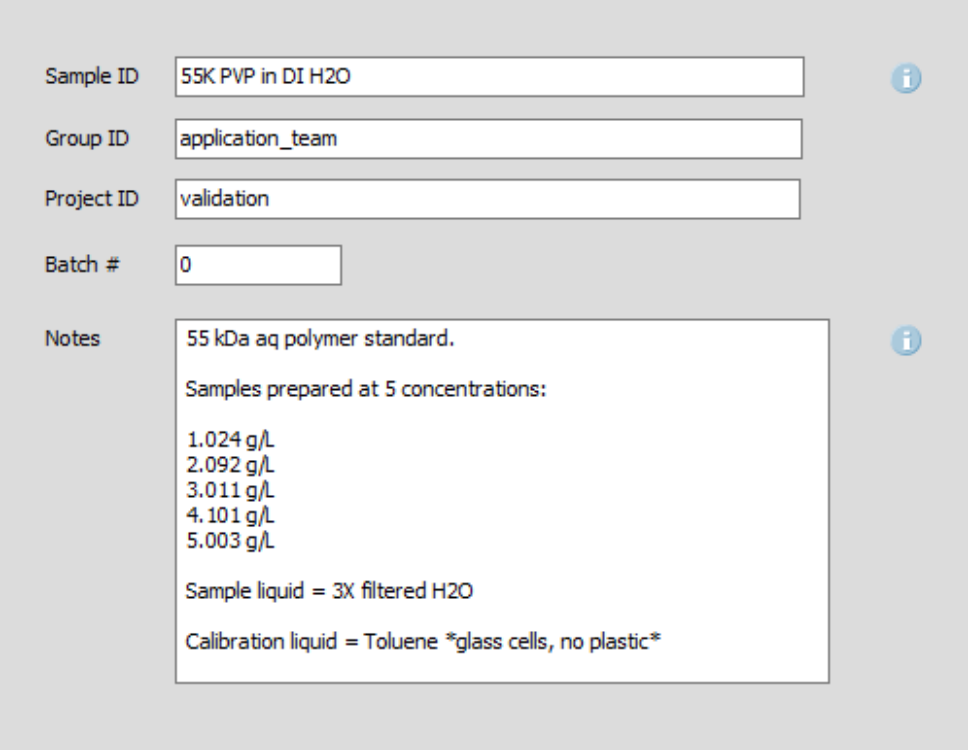


Click Measure Dark Count Rate. An acceptable value is anywhere between a few tens to several hundred counts per second (cps). Repeat a few times to confirm stability. You may increase Duration to 10 seconds; reset to 1 second before closing this window.

> **Note**
> If the value is in the kcps range, it is too high, make sure the windowless side is oriented correctly.

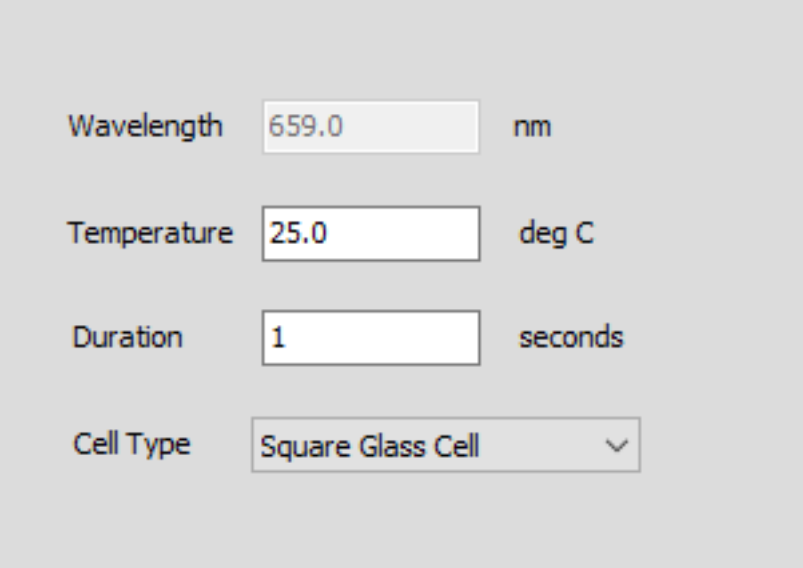
> **Note**
> The Calibration Constant is shown here and updates automatically once the measurement begins. Manual adjustment is not recommended.

DBY SOP Identification



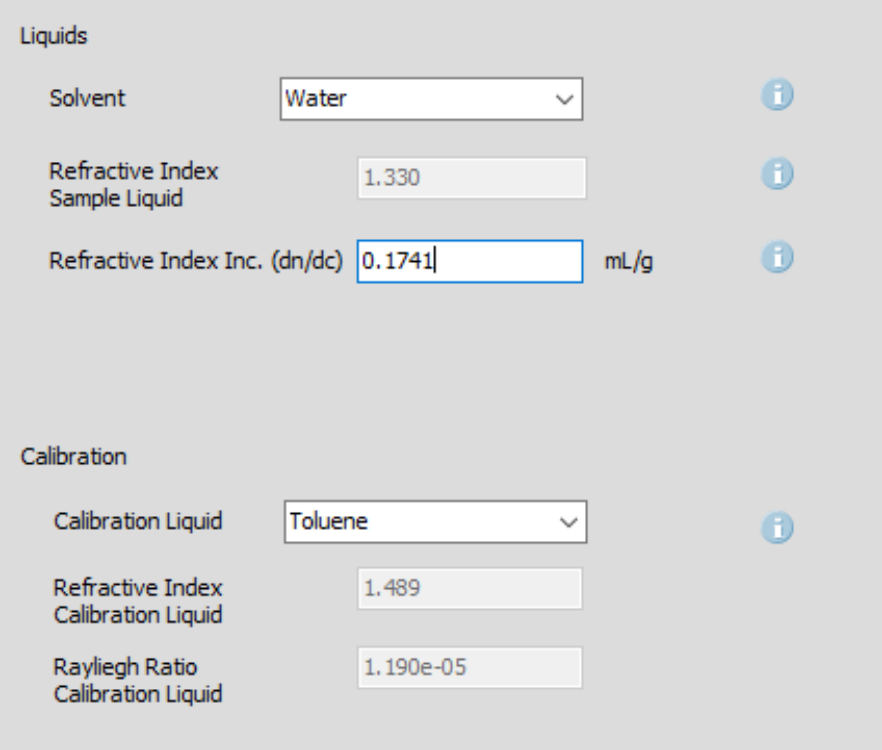
Enter the SOP editor and select the Identification panel. Enter the Sample ID, Group ID, Project ID, Batch number, and any preparation notes. This panel is used for general record-keeping and does not affect measurement parameters.

DBY SOP Instrument Parameters



Select the Instrument Parameters tab. Select either Square Glass Cell (SGC) or Flow Cell Quartz. Do not use plastic cuvettes with organic solvents. If using SGC, ensure all cuvettes, including the calibration cuvette, are physically identical. Confirm wavelength is set to 640 nm and temperature to 25.0 °C.

DBY SOP Measurement Parameters



Select the Measurement tab. Choose your sample solvent and fill in the dn/dc value for your sample in that solvent. This value is critical for an accurate M_w and A_2. Confirm the Calibration Liquid shows Toluene. Calibration will not begin until the measurement is started.

> **Note**
> The calculated Mw is extremely sensitive to dn/dc. This value is wavelength-dependent; if using a literature value, verify it was measured at 640 nm.

Supplies

Kit Contents (90PDP)

- 10 mL glass syringe
- Flow cell, three glass-windowed sides; one windowless side
- 30 cm orange PEEK inlet tubing (0.5 mm I.D.) with Luer-lock fitting
- 1 m white/clear Teflon outlet/waste tubing (0.75 mm I.D.), trim to length

Additional Supplies Required

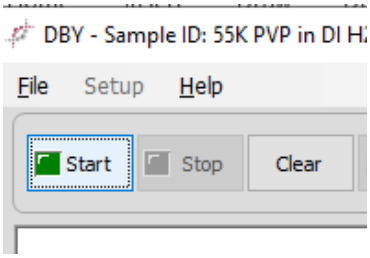
- HPLC-grade toluene (calibration liquid)
- Deionized (DI) water
- Intermediate solvent: acetone, methanol, or ethanol (HPLC methanol preferred)
- Aqueous syringes: 3, 10, or 20 mL; aqueous syringe filters 0.2 µm
- Solvent-resistant or glass syringes: 3 mL or larger
- Solvent-resistant syringe filters: 0.1 µm for toluene
- Waste containers
- Sample: minimum 3 concentrations (5 preferred), known to 4 decimal places (e.g., 4.6205 mg/mL); at least 10 mL per concentration; 4- to 10-fold concentration range

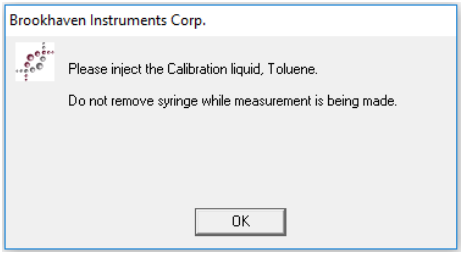
Running the Measurement

Use a well-characterized standard for the first trial. Narrow-distribution polystyrene standards or lysozyme (14 kDa, dn/dc = 0.18 mL/g) are recommended. A minimum of 3 concentrations are required; 5 are strongly preferred, with a 4- to 10-fold concentration range, each known to 4 decimal places.

i. Calibration Procedure

Reorient the flow cell so the windowless side faces the back of the instrument. Click Start. The software will prompt for calibration.



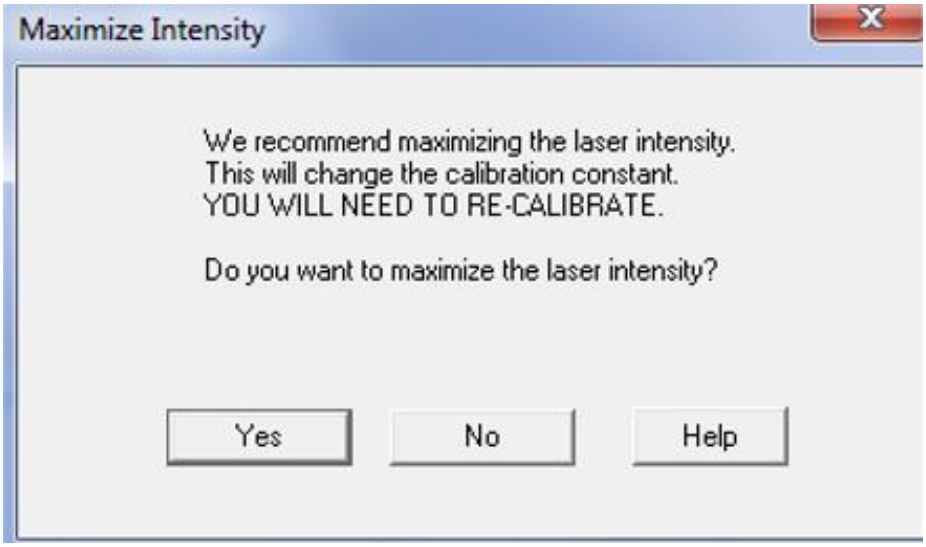


Fill a solvent-resistant or glass syringe with HPLC toluene. Attach a 0.1 µm filter and connect to the inlet tubing. Flow toluene until it drips from the outlet. Once the signal stabilizes the software will display the calibration constant.

> **Note**
> If the cell previously contained another solvent, flush with an intermediate solvent before introducing toluene.

> **Note**
> If calibrating for the first time, record the calibration constant, press Esc rather than OK, and repeat 2–3 times to confirm repeatability.

DBY Sample Injection



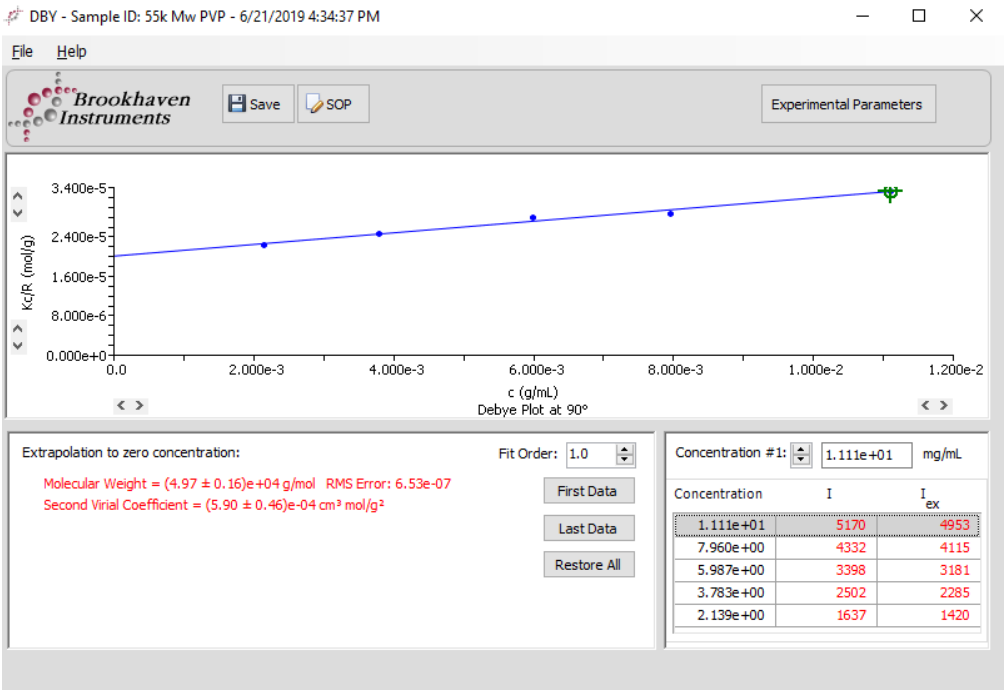
ii. Sample Injection

After calibration, flow your sample solvent until the background signal is stable (horizontal trace), then click OK.

Inject samples from lowest to highest concentration. Select a filter pore size that passes your analyte but retains aggregates and dust, 3–4 mL syringes with 13 mm filters are typical. Flow each concentration until stable, then click OK to collect data. Repeat for all concentrations as prompted.

> **Note**
> If prompted to maximize laser intensity, accepting this will change the calibration constant and require recalibration before proceeding.

Results, Calculated M_w



Once all concentrations are measured, the software performs an extrapolation to zero concentration and reports M_w (g/mol), A_2 ($cm^3 \cdot mol/g^2$), and the RMS fitting error. If dn/dc needs to be corrected, update it in the SOP editor and the software will recalculate and save an updated M_w.

> **Note**
> The Debye Plot is only strictly valid for angle-independent scattering, typically Rayleigh scattering particles with R_g < 12 nm.

Troubleshooting Data Quality and Stability Issues

If the measurement fails to converge, exhibits high noise, or produces an unstable Debye Plot, work through the following steps.

i. Verify Dark Count Rate

Navigate to Intensity mode and run a 1-second acquisition with a blacked-out cuvette as a beam block. Confirm the dark count is in the range of tens to hundreds of cps. An elevated dark count indicates stray light entering the detector.

ii. Adjust Trend/Noise Settings

If the software will not accept a measured intensity as stable and advance to the next concentration, the Trend and Noise limits may be too tight for your sample. This is most likely to occur with weakly scattering or noisy measurements, particularly during dark count, solvent/background, or dilute sample acquisitions. Navigate to Setup > Trend/Noise and loosen the settings as needed. Stability Points sets how many consecutive stable data points are required before the software accepts the measurement and moves on. Trend and Noise limits define the acceptable thresholds for signal drift and variance respectively.

Parameter	Recommended Setting
Stability Points	10
Trend Limit	15
Noise Limit	15

> **Note**
> Only loosen these settings as needed, starting with the recommended values below and adjusting further if the software continues to stall.

Clean Up and Storage

Proper flushing of the flow cell and tubing after each session is essential to prevent clogging from sample residue or precipitates.

- If sample solvent was DI water: flush with fresh DI water, then methanol.
- If sample solvent was organic: flush with an appropriate intermediate solvent, then methanol.
- After solvent flushing, push a syringe full of air through the system to expel residual liquid.
- Store the flow cell and tubing in a clean, dry location.

> **Note**
> If you do not flush the tubing and flow cell, sample and other precipitates can clog the cell or tubing and it can be difficult to clean in the future.