Refeyn Mass Photometer TwoMP Standard Operating Procedure

version 1.0

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Approved date: 02/2026 Created Date: 11/2025

1. Purpose

1.1 Measure the mass of individual molecules to obtain molecular mass distributions of proteins and other biomolecules in solution

2. Supplies & Equipment

- ❏ PPE (BSL-1)
  - ❏ Laboratory Coat
  - ❏ Nitrile Gloves
  - ❏ Goggles / Safety Glasses
- ❏ Chemicals/Reagents
  - ❏ Isopropanol
  - ❏ Immersion oil
  - ❏ MassFerence P1 Calibrants
  - ❏ Sample buffer
  - ❏ Sample
- ❏ Pipette and Tips
- ❏ Blunt/soft tip tweezers
- ❏ Gaskets
- ❏ Cover slips
- ❏ Lens paper

3. Procedure

3.1. Turn on the photometer

3.1.1. Examine the objective lens (OL) and clean up any oil residues or smudges.

a. Cut a small square piece of lens paper stored in drawer under the mass photometer
b. Pipette 20 uL of isopropanol onto the lens paper
c. Wipe the objective lens in a circular motion starting from the middle and moving outward
d. Repeat a-c until the isopropanol evaporates from the objective immediately
e. It is imperative that the objective is clean, so do it thoroughly!
f. Close the lid

! NEVER use a Kim wipe to clean the objective

3.1.2. Turn on the instrument by pressing the switch on the back. Once the instrument is on, the laser light should be on.

3.1.3. Turn on the antivibration table.

3.1.4. Open ***AcquireMP***. If you don't hear a high-pitch noise, check the cables. **Let the instrument warm up for at least an hour**.

3.1.5. Hint and tips, and user manual are in the ***Help*** drop-down

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3.2. Prepare your sample

3.2.1. While the instrument is equilibrating, prepare your samples, buffer, and dilutions—the ideal measurement concentration is 10 nM (acceptable range: 5–10 nM; maximum 50 nM). A 10X stock at 100 nM works well to start. Use the software's concentration calculator for assistance.

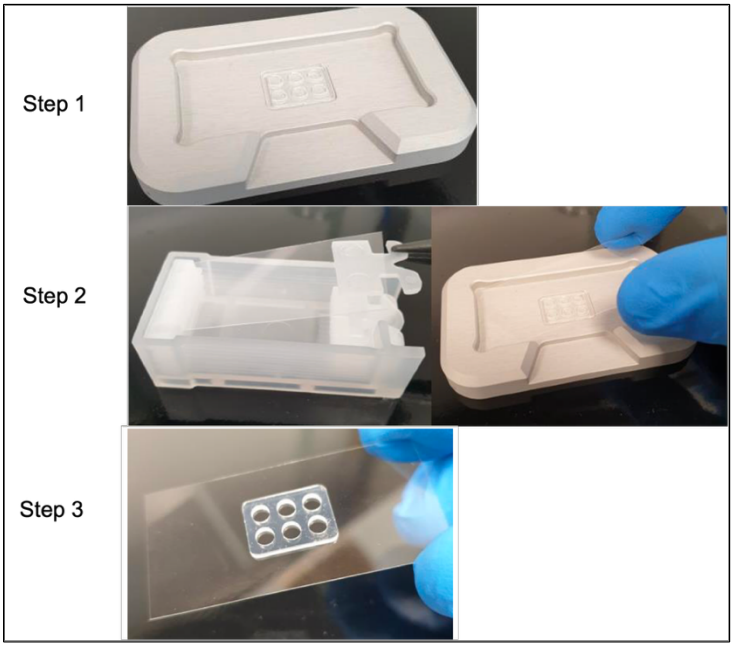
3.2.2. Fresh made/filtered buffers are always recommended.

3.2.3. The measurement is sensitive to temperature; therefore, it is advisable to maintain the working solution, such as the buffer, at room temperature.

3.3. Assemble the coverslip and gasket

3.3.1. Clean the surface of the alignment tool using Kim wipe. Grab the edge of the silicone gasket (with 6 wells), and place it on the alignment tool.

3.3.2. Pick up a cover slip, ensuring you handle it by the edges to avoid contact with the interior surface. Place the cover slip carefully over the gasket, then gently tap its corners to secure positioning.



3.4. Add immersion oil on the objective lens and set positions

3.4.1. Add a small amount of the immersion oil on the objective lens. **Tip**: use the spatula to dip the oil and leave it to drip 1-2 drops. When the third drop is forming, touch the droplet on the objective lens.

3.4.2. **DO NOT** touch the objective lens with the plastic tip of the spatula, this could damage the objective lens.

3.4.3. If the oil is thick and won't drip, let the staff know.

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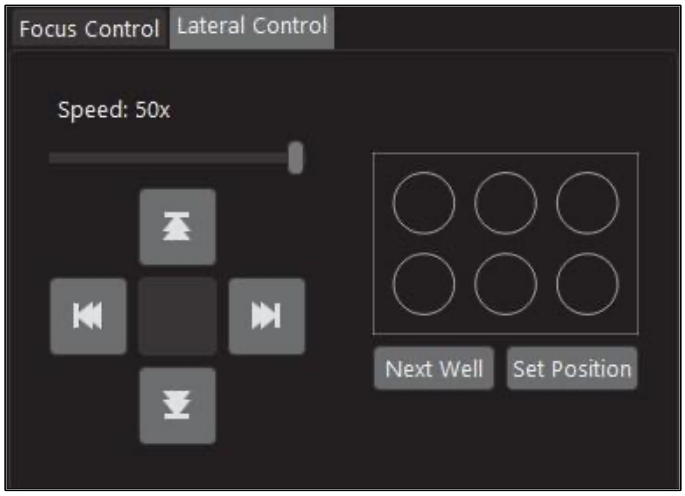
3.4.4. Grab the assembled gasket and the cover-slip on the side using the soft-tip tweezer, and place it on top of the objective lens. Make sure the gasket is on the top and the glass is on the bottom.

3.4.5. Adjust the position of the slide by moving it gently to the bottom left corner. Secure slide with magnets on the upper left and bottom right corner.

3.4.6. Use the lateral position panel on the ***AcquireMP*** to move the OL inside the first well. Change Speed to 50X.

3.4.7. Highlight the first well and Set Position.



3.5. Add buffer

3.5.1. Pipette 10 ul of buffer into the first well without touching the glass slide. Each well can hold up to 20 ul.

3.5.2. Select ***Native mode*** and click on ***Droplet Dilution***. Observe any super bright spots, which may indicate the presence of dirt. If dirt is detected, move the stage at speed 1X to avoid the affected area.

3.5.3. Check the Autofocus ring. It should not have gaps. If there are gaps or bubbles are detected, unlock the stage and wiggle the slide gently to remove bubbles.

3.5.4. Switch to ***Ratiometric*** mode, and Click ***Record*** (60 seconds are recommended)

3.5.5. Save the movie under proposer directories per site specific requirement.

3.5.6. Open ***DiscoverMP***, and load the buffer movie

3.5.7. Examine if there are any peaks except the two mirror peaks (one from the left of zero and one from the right) from the buffer.

3.6. Create the calibration plot

3.6.1. Pre-dilute the MassFerence P1 calibrant to 50 X in the buffer

3.6.2. Move stage to the next well. Add 18 ul of buffer, and click ***Droplet Dilution***. Check the Autofocus ring.

3.6.3. Add 2 ul of diluted MassFerence P1 calibrant on the top of the buffer droplet. Mix it a few times.

3.6.4. Switch to ***Ratiometric*** mode, and Click ***Record***.

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3.6.5. In ***DiscoverMP***, load the calibrant movie.

3.6.6. Create calibration: choose Mass Calibration for proteins. DNA and RNAs are different, use the single strand or double strand nucleic acids respectively.

3.6.7. Assign the peaks to their respective masses based on the reference standards. An R^2 value of 0.999 or higher and a percent error below 5% are required to ensure that the calibration curve is reliable.

3.6.8. You should re-calibrate any time you switch buffers (calibration standards need to be made in the buffer your experimental samples are in).

3.6.9. Fresh calibration is required for every three hours.

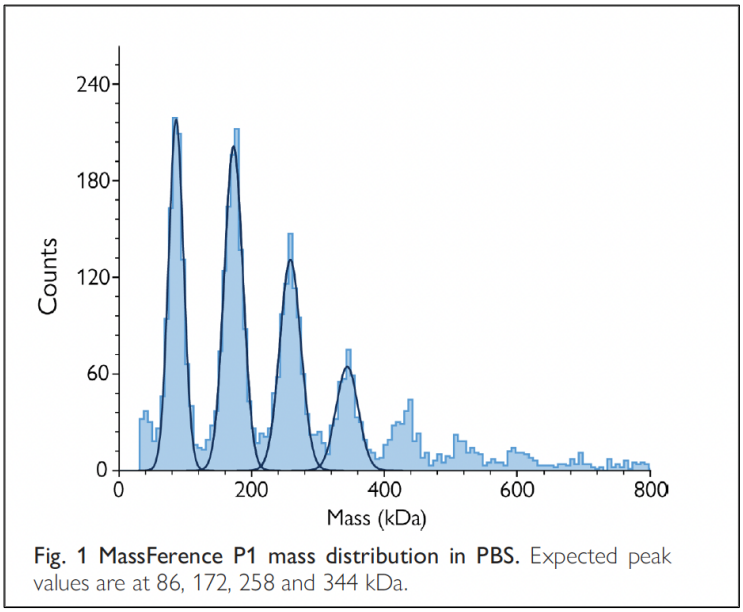


Fig. 1 MassFerence P1 mass distribution in PBS. Expected peak values are at 86, 172, 258 and 344 kDa.

3.7. <u>Measure sample</u>

3.7.1. Switch to next well to measure the sample of interest.

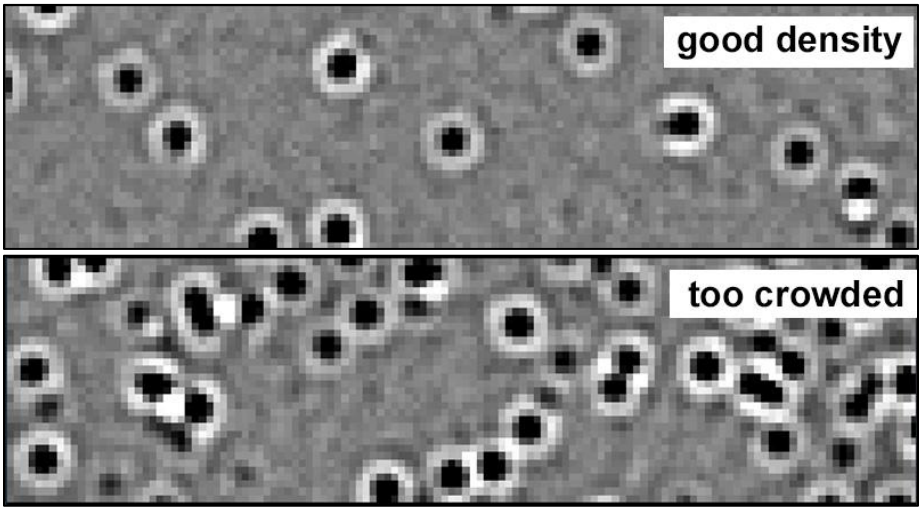
3.7.2. Add 18 ul of buffer, and click ***Droplet Dilution***.

3.7.3. Add 2 ul of sample from the 100 nM stock (the final concentration is 10 nM). Mix it a few times.

3.7.4. Switch to ***Ratiometric*** mode, and Click ***Record***.

3.7.5. Open the recorded data in ***DiscoverMP***, and double click the saved calibration to assign mass (kDa).

3.7.6. Repeat steps 3.7.1-5 for more measurement.



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3.8. Clean up

3.8.1. Unlock the focus 

, and center the stage.

3.8.2. Remove the magnets and the slide from the stage

3.8.3. Fold the cleaning tissue into a thin rectangle and clean the oil from the objective lens. Repeat until no excess oil is left on the lens

3.8.4. Pipette 20uL of isopropanol into another clean lens tissue, wipe the objective lens (beginning in the center) in a circular motion to the edge of the lens. **Note**: clean the lens thoroughly until it is mirror shine and free of oil smudges.

3.8.5. Close the software and turn off the instrument.

4. Waste Disposal

4.1. Follow facility procedure for proper disposal of biohazardous and chemical waste **(see site specific instructions).**

4.2. Biohazardous waste will be collected in designated bins lined with red biohazard bags.

4.3. Chemical hazardous waste will be segregated by hazard class (e.g. flammable, corrosive) and state (e.g. solid, liquid), appropriately labelled, and placed in the laboratory's hazardous waste cabinet.

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