Refeyn TwoMP: CMI Guide to Measuring Nucleic Acids

Introduction

Mass photometry allows quantification of the mass distribution of biomolecules in solution, calculated by using light scattered as individual biomolecules adsorb to a glass coverslip. Both DNA and RNA are negatively charged in solution and adsorb poorly to an untreated glass surface. Coating coverslips with positively charged poly-lysine (PLL) enhances detection by allowing adsorption to the coverslip surface and reducing unbinding events.

Required Supplies

- Refeyn sample carrier slides, or cleaned high precision coverslips (Thorlabs-CG15KH) treated with 0.01% poly-lysine (PLL)
- Soft-tipped tweezers
- Aqueous buffer
- Recommended calibration standard
    - Invitrogen low DNA mass ladder: Invitrogen 10068013 (117.5 ng/µL)
    - Millennium RNA Marker: ThermoFisher AM7150 (1 µg/µL)

Experimental Design Tips

- Use PLL-coated coverslips for DNA and RNA samples.
- Choose your image size according to the expected sample size. Always calibrate with the same image size that you plan to collect data.
    - In normal measurement mode (used for proteins and nucleic acids), the Refeyn TwoMP has three pre-defined image sizes: small, regular, and large.
    - Regular is the default and is recommended for most samples with particle masses below 1 MDa because it allows for greater mass sensitivity. This is recommended for DNA samples with fewer than 1000 bp.
    - Large is recommended for samples with high particle mass, as it allows for the detection of more events in a single frame. While increasing the image size improves statistics, it also decreases the signal-to-noise ratio, thereby decreasing mass sensitivity for smaller particles. This is the recommended view for DNA samples with greater than 1000 bp and for most RNA samples.
    - Small has the greatest mass sensitivity and can be used to improve peak separation in samples with low particle masses.
    - The recommended maximum event counts for each image size are as follows:
        - Small: 3,000 events
        - Regular: 6,000 events
        - Large: 30,000 events

DNA/RNA Calibration Standards

- Invitrogen low DNA mass ladder: the CMI provides aliquots of working stock (diluted 1:5 in 10mM TrisCl pH 8.5 before freezing) in the -20˚C freezer below the MP. Pipet 1 µl working stock into 15 µl buffer droplet.
- Millennium RNA Marker: dilute 1:10 in your buffer to make a working stock. If using a large image size, pipet 2 µl working stock into 18 µl buffer droplet. If using a regular image size, pipet 1 µl working stock into 19 µl buffer droplet.
- There is currently no option in the DiscoverMP software to change the units of the x-axis from kDa to base pairs. Once you have applied a calibration curve that was created using Length in bases or bp, your samples will be measured in these units. The x-axis will still be labeled in kDa and units will need to be adjusted when generating graphs of your data.
- When measuring DNA and RNA standards, there are often fragments that create "noise." These appear in the first peak and cannot be used for calibration. In normal or small images, the noise peak is often sizeable. In a large images, it appears as only a small peak or shoulder of a larger peak. For RNA measured using a large image size, the noise peak appears as the shoulder of a larger peak, or may not be evident at all.

Coating slides with poly-L-lysine (PLL) for Nucleic Acid Samples

1. Place two carrier slides over a clean, lint-free surface such as a Whatman lens tissue. Label the upper left corner of each coverslip "PLL" with a permanent marker.
2. Place 10 µl of 0.01% PLL in the center of one coverslip.
3. Flip the second coverslip upside down and place on top of the droplet, perpendicular to the first coverslip so that the two labeled sides are touching each other. The PLL should spread to the entire overlapping area.
4. Incubate for 30 seconds, then separate the coverslips.
5. Using soft-tipped tweezers, dip each slide into a beaker with MilliQ water to remove excess PLL.
6. Using a squirt bottle, wash twice with Milli-Q water stream directed at the treated surface.
7. Hold the slide from bottom with the soft-tipped tweezers or gloved hand and dry coverslip with clean, pressured air.
8. Use immediately, or store in a clean container for up to one week.

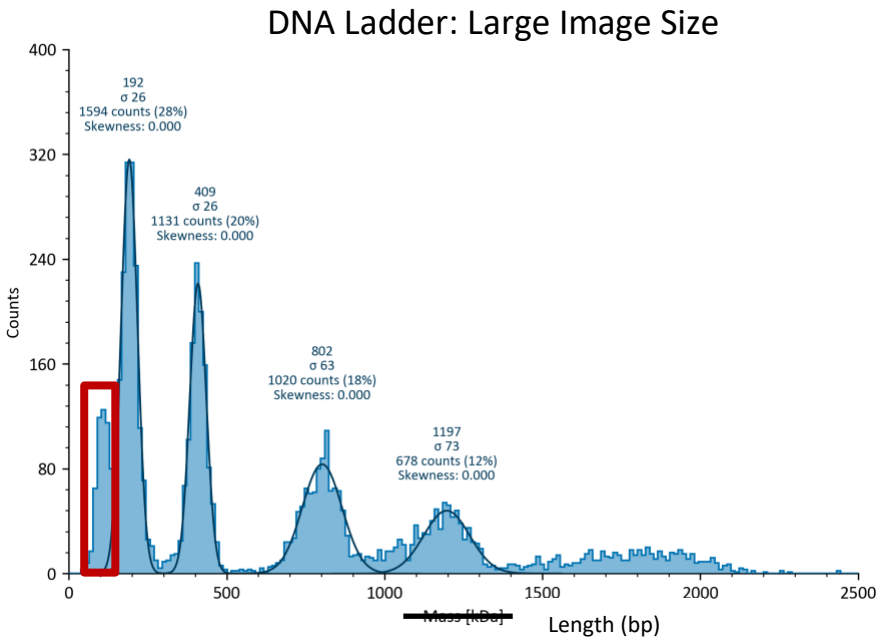
Data Collection

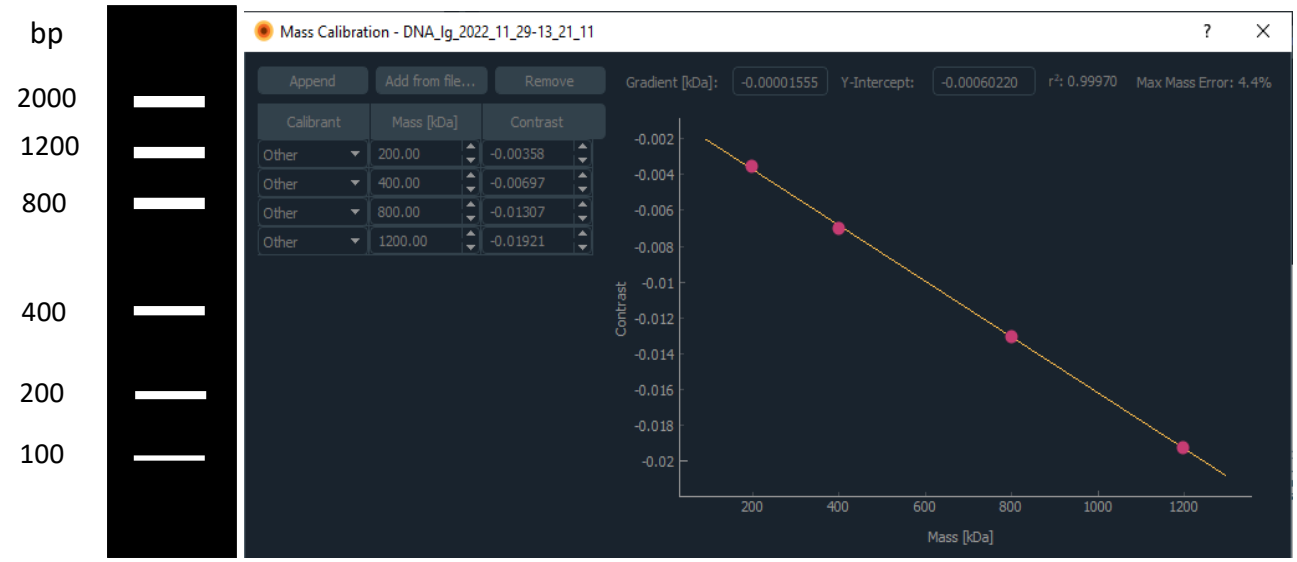
1. Collect data following standard protocols outlined in the CMI Refeyn TwoMP Mass Photometry Getting Started Guide.
2. Adjust image size if needed.
   a. The selected Measurement Mode should be "Normal."
   b. Select an image size: small, regular, or large.
   c. Save.

Sample DNA/RNA Standards

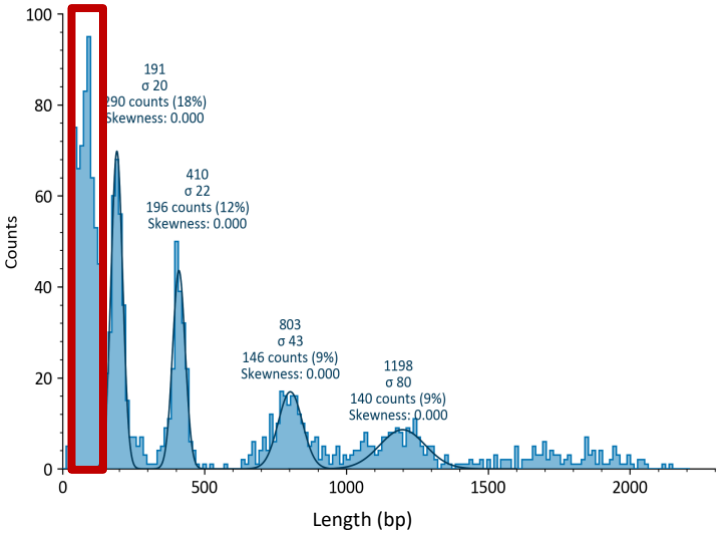
Example Data: Invitrogen Low DNA Mass Ladder

Highlighted peaks (in red) are the noise peaks seen in measurements taken at all image sizes; these peaks should not be used as calibration points.

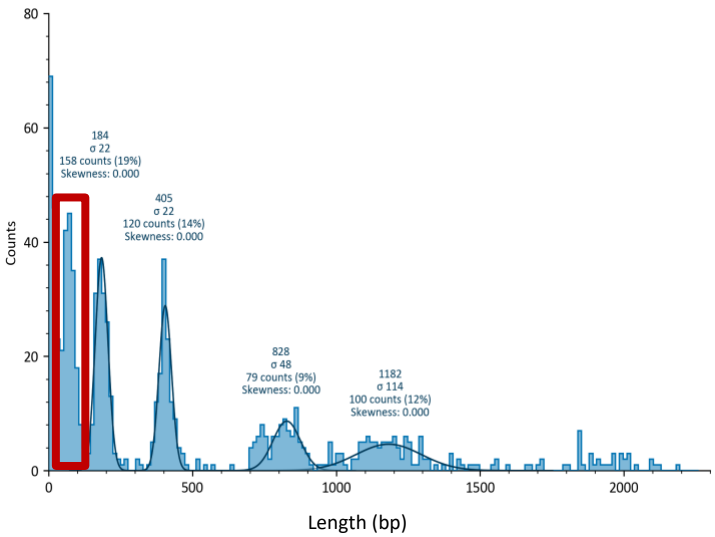




DNA Ladder: Regular Image Size

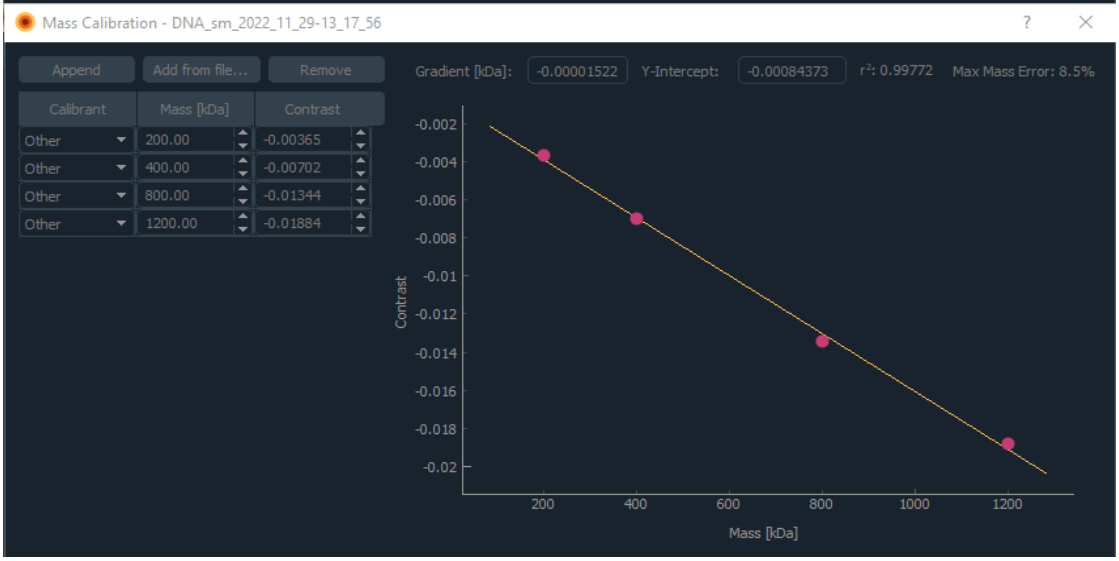


DNA Ladder: Small Image Size

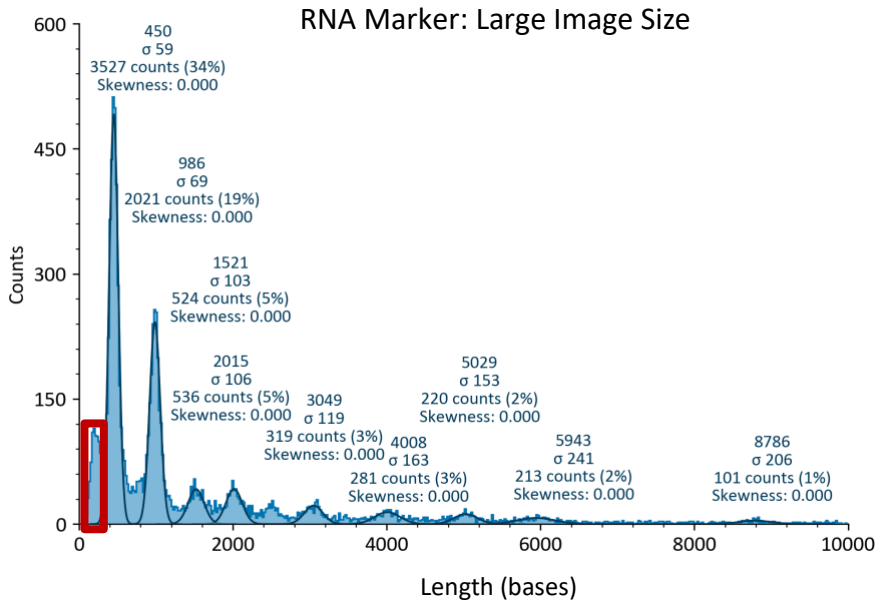


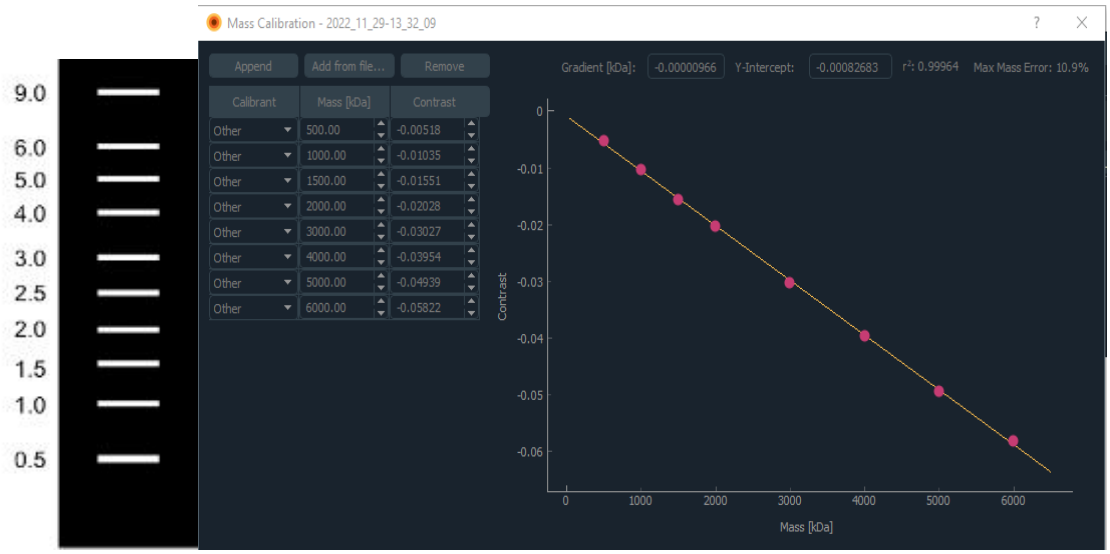
Regular Image Size

Small Image Size



Example Data: Millennium RNA Marker





Contact cmi@hms.harvard.edu with questions.

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