Mass Photometer SOP (Calibration)

Calibrant choice (Excerpts from Brenda Watt Refeyn®)

For calibrants, it is not recommended the use a 30kDa calibrant. This molecular weight calibrant requires very clean slides and buffer to get a good measurement of 30kDa protein where the contrast is not over-represented (i.e., you might only be counting/measuring the larger-end particles of a population, and thus the contrast of that Gaussian is skewed to larger (more negative) than the true contrast of that population.

Range of Masses covered: The excellent linearity of contrast: mass in mass photometry allows a measure of proteins outside the range of masses used for calibration. Thus, it is more important to get an accurate linear calibration curve (i.e., where you know you are capturing the true contrast of a population of known size without background noise or other similar mass populations merging and thus affecting the true contrast value) than getting a calibration that encompasses all possible masses.

Best Standards: A mix of beta-amylase and thyroglobulin (56-670kDa coverage) works well for samples even as large as AAVs (empty AAVs are 3700kDa). To measure small proteins, it is recommend running beta amylase alone.

About concentrations: The best is to run lower concentrations of calibrants to ensure particles land separate from each other in time and space. Maintain the total counts under 3000 in a 1min movie in regular image size. One can measure at higher concentrations, but for best accuracy, especially with samples that have multiple populations where it is desirable to get the best baseline resolution possible, having fewer than 3000 counts will still produce fittable populations (you normally need at least 200 counts in a population to fit a gaussian) that are accurate and where the background signal is not so high that you effectively raise the limit of detection.

Catalogs number of common standards:

1. Beta-Amylase from sweet potato: Sigma A8781

(freshly prepared produces peaks at 56kDa (monomer), 112 and 224kDa

1. Thyroglobulin from bovine thyroid: Sigma T9145

(freshly prepared produces dimer predominantly at 670kDa but can have some low mass contaminant peaks of unreliable mass)

Standards Concentration: final droplet concentration of 5-10nM beta amylase monomer and 1-3nM thyroglobulin dimer.

It is expected to see all four peaks above any buffer noise peaks, that there is not too much thyroglobulin and associated low mass contaminants to skew the beta amylase monomer peak, and that the background is clean without particles landing right next to each other in time and space. Therefore, consider only using the beta-amylase monomer peak if it is well resolved from buffer noise peaks.