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Mass photometry V.2

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Minghao Chen[1], Yuanchang Zhao[1]

[1]University of California, Berkeley

Minghao Chen
University of California, Berkeley

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Protocol status: Working
We use this protocol and it's working

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Abstract

A protocol for performing mass photometry measurements.

1 Clean the high-precision coverslips (Azer Scientific) by alternating between isopropanol and water.
Repeat the above treatment for 3 times in a bath sonicator, each 3 min.
Air dry the coverslips

2 Clean the gasket by alternating between isopropanol and water.
Repeat the above treatment for 3 times, without sonication.
Air dry the gasket.

3 (Optional) For crosslinking, add 1 µL of 0.1% (v/v) glutaraldehyde in mass photometry buffer to 9uL protein sample and incubated for 10 minutes on ice, followed by quenching the reaction with adding 10 µL of 1M Tris-HCl pH 7.4.

4 Add 19 ul of mass photometry buffer (30 mM HEPES pH 7.4, 5 mM MgSO4 and 1 mM EGTA) to the well for autofocus

5 Dilute the protein sample in the mass photometry buffer to a concentration of 5–20 nM.

6 Prior to the measurement, perform mass calibration using a standard mix of conalbumin, aldolase, and thyroglobulin. Analyze the mass photometry profiles and fit them to multiple Gaussian peaks, with
the mean, standard deviation, and percentages calculated using DiscoverMP software (Refeyn).

7 Measure the protein contrast counts using Refeyn TwoMP mass photometer, with three technical replicates.