

Mass Photometry - An innovative biophysical analysis tool with numerous applications

Stop by booth 26 to learn more!









Research & Discovery

Process Development

Quality Control

Understanding biomolecular interactions and behaviours

- What are the compositions of the interacting species?
- Does the expected complex form & what is its stoichiometry?
- How does the complex assemble?
- How stable is the complex & how strong are the interactions?



Research & Discovery

Process Development

Quality Control

Optimizing products and processes

- How stable is the formulation?
- What conditions give the optimal product performance,
- What conditions are best for producing the desired protein (or other product)?

Research & Discovery

Introduction

Process Development

Quality Control

Assessing sample purity and quality

- Is the sample in the required state to progress to the next stage of analysis / development / production?
- Does the product meet its critical quality attribute requirements?
- What is the AAV empty/full ratio?
 GMP compliant soon!



Why using mass photometry?

An innovative biophysical analysis tool with numerous applications

Mass photometry delivers

rapid, accurate mass measurement of

label-free single molecules in

solution in their **native state**

- access to subpopulations to determine complex stoichic and oligomeric state
- the monitoring of **complex, multistep processes**
- detection of low abundance species
- the characterization & monitoring of sample heterogeneity





Data courtesy of Weston Struwe, Univ. of Oxford and Refeyn team

Proprietary & confidential

Introduction



Mass photometry measures different types of biomolecules







What does a mass photometry measurement look like?



Mass photometry weighs molecules with light





Introduction



How does mass photometry work?









Biomolecules approaching a glass surface are illuminated by a laser

The biomolecules scatter light, which interferes with the light reflected at the interface The resulting interface contrast scales linearly with the mass of the biomolecule A mass histogram is generated from the single molecule measurements

Mass photometry in biophysical characterization

Meet the family!

Samux^{MP} Optimized for AAV characterization GMP Compliance coming soon!





Two^{MP}Auto

Automated mass characterization

Two^{MP}

Second-generation mass photometer for single-molecule mass measurements



Proprietary & confidential



A mass photometer optimized for AAV characterization

- Easily and accurately assess empty-full AAV ratios
- Quantify partially filled & overfilled particles
- GMP compliance coming soon



Interaction studies

with rapid, single particle, serotype-agnostic

Samux^{MP}

mass measurement of capsids in their native state, using

minimal sample consumption and with low operational costs



13 December, 2022 • 13



Samux^{MP} specifications

GMP Compliance coming soon!

Mass range Optimal concentration Sample volume Resolution (FWHM) Measurement time Laser wavelength 500 kDa – 6 MDa 10¹¹ particles/mL 10-20 μL 235 kDa @ 3700 kDa < 5 minutes 488 nm





Performance





	E : F calculation (% Full)				
Sample	Cryo-TEM	AUC	Samux ^{MP}		
1	6 %	5 %	5 %		
2	18 %	28 %	18 %		
3	24 %	35 %	30 %		
4	60 %	-	53 %		

	康	龙	化	成
\checkmark	PH	ARN	IAR	ON

Performed at Pharmaron Gene Therapy, Liverpool, UK

	E : F calculation (% Empty)			
Sample	Cryo-TEM	AUC	Samux ^{MP}	
A	16 %	24 %	16 %	
В	-	100 %	95 %	

Benchmarking

- E:F ratios of multiple AAV preps of different serotypes were measured with:
 - electron microscopy (cryo-TEM)
 - analytical ultracentrifugation (AUC)
 - Samux^{MP} mass photometry
- The results from Samux^{MP} agreed with the results from AUC and cryo-TEM





A versatile mass photometer with a wide range of biophysical applications

Detect low abundance species and verify sample purity Ana Characterise sample heterogeneity and determine filled ratios com Determine oligomeric states & characterise complexes Monitor complex, multistep processes & quantify biomolecule interactions





with a rapid, single-molecule mass measurement of label-free biomolecules in their native state, over a wide mass range with minimal sample consumption





A versatile mass photometer with a wide range of biophysical applications



Mass range Concentration range Sensitivity Resolution (FWHM)

Mass precision Mass error 30 kDa – 5 MDa 100 pM – 100 nM << 1 ng of protein 25 kDa @ 66 kDa 60 kDa @ 660 kDa ± 2% ± 5% (single measurement)







Automated mass characterization of biomolecules

- Greater data confidence
- Reduced operator time
- Ideal for screening and titration assays
- + standard mass photometry applications





with rapid, autonomous measurement of multiple samples and increased data reproducibility

- Up to 1 hour of autonomous measurement possible
- Compatible with Two^{MP} systems
- Same specifications as Two^{MP*}

*or $One^{\rm MP}$ if an upgraded system

Introduction

Two^{MP} and Two^{MP} Auto applications



Mass photometry can quantify protein interactions



What is the binding affinity?

Determining the binding affinity of an antibody with another protein in a single-shot K_{d}

- 1:1 mixture of IgG & FcyRla
- ightarrow dissociation constant information



Measured on the One^{MP}

1:1 mixture of deglycosylated IgG (Fc N-glycan removal) & FcγRla

ightarrow 20-fold change in binding affinity



Soltermann et al., Angew Chem Int Ed Engl. 2020

Interaction studies

How does the ternary complex form?

- Characterization of small bifunctional molecule (PROTAC) binding •
- Ternary complex formation measured as a function of MZ1 (PROTAC) concentration ۲
 - Target protein (with binding sites 1. BD1 & BD2) + E3 ligase (VHL)

- 3. PROTAC **binds** E3 ligases to both binding sites on target protein
- \rightarrow quinary complex forms



Measured on the One^{MP}



Mass photometry enables PROTAC characterization



- PROTAC binds E3 ligase to 2. binding site on target protein
- \rightarrow ternary complex forms

- Saturation of binding sites at 4. target protein & ligase
- \rightarrow inhibition of complex formation at 1200 nM M71





Mass photometry tracks the assembly of complexes



How do the binding partners influence oligomeric assembly?

- Mass photometry was used to determine the subunit stoichiometry within the R2TP chaperone complex
- Stoichiometry of oligomeric assembly revealed by titration of binding partners



Measured on the One^{MP}

Seraphim et al., Structure, 2022

Mass photometry can quantify sample heterogeneity

Which chromatography fractions contain most of the fully assembled complex?

- Purification of the Anaphase Promoting Complex / Cyclosome (APC/C) is a multistep process
- Mass photometry provided clear information on native assembly quickly & using very little sample (15 µL of typically 5-25 nM protein)
- Perfect agreement with nsEM but easier + faster



Measured on the One^{MP}

Mass photometry measures nucleic acids



pUC18

sallata -

5000

Nucleic acid sizing

- Mass photometry can be applied to nucleic acids and other biomolecules
- Same linear dependence between ٠ measured contrast & length/mass
- Here, the coverslip was coated in PLL to enable DNA binding



DNA calibration

Unknown DNA lengths determined

Measured on the One^{MP}

Interaction studies



Mass photometry reveals protein-DNA interactions

What is the MR-DNA assembly mechanism in double-strand repair?

- Determine how many proteins bind to a DNA sequence ۲
- Small changes in protein/DNA ratio clearly observable
- It is straightforward to assess how the DNA-protein complex forms
- It is possible to study MR complex in double-strand repair ۲



Measured on the One^{MP}



Mass photometry in biophysical characterization

Single-molecule counting over a wide mass range & high dynamic range of **label-free** biomolecules in their native state

- Applicable to many biological questions
- Quick & easy to use
- Cost effective





What to learn more about mass photometry?

Come to our booth slide!



info@refeyn.com • www.refeyn.com • @refeynit