Understanding **mass photometry**

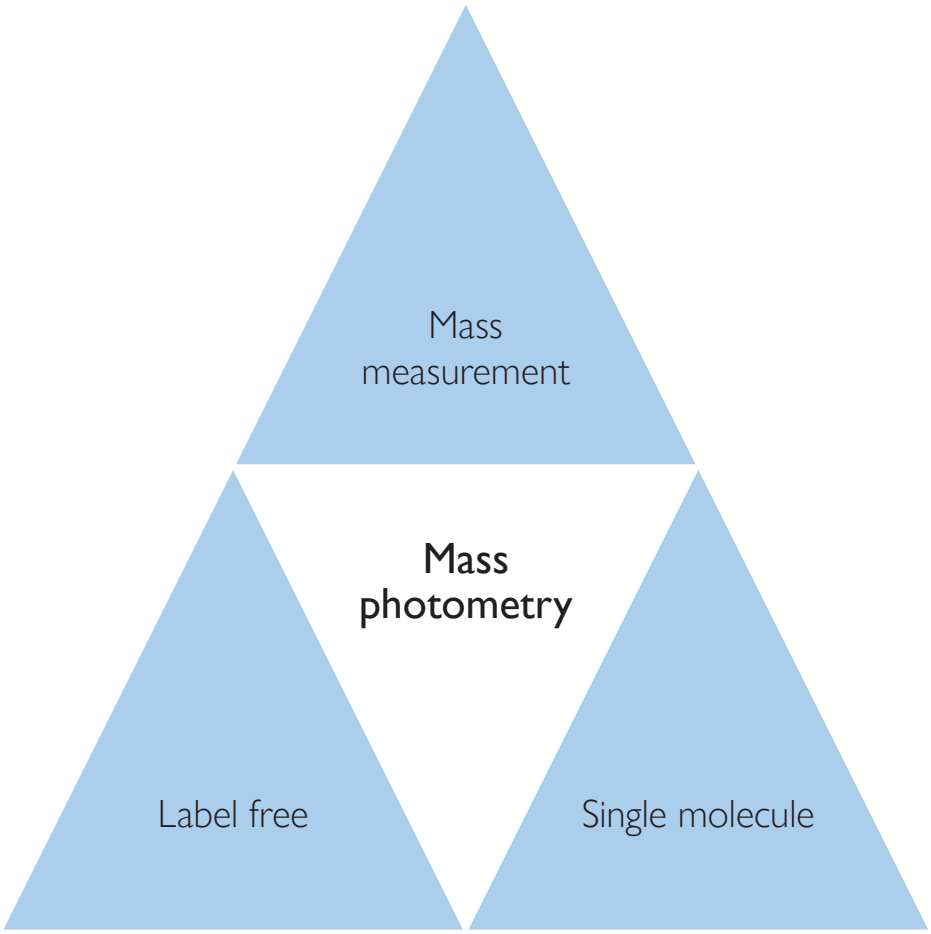
A handbook for all

REFEYN

Contents

What is mass photometry?

Mass photometry is a bioanalytical technology that accurately measures the mass of individual biomolecules or particles in solution.



With the advantages of being fast, easy to use, label-free and needing very little sample, mass photometry is being adopted for its rapid insights into oligomerization and binding, sample purity, attributes of advanced therapies, and more.

Mass photometry leverages **light scattering** – which makes the technique universal (because all matter interacts with light) and eliminates the need for labels. It **measures mass** – providing important insights into molecule identity, oligomerization, interactions and other key features. And it is a **single-molecule** technique – revealing distinct subpopulations rather than obscuring them with an average, as in bulk (or ensemble) measurements.

Why we created this handbook

Although mass photometry is simple to use and the data is straightforward to interpret, it is useful to understand how it works – and how it gives valuable and accurate information so quickly. This enables a user to choose when it makes sense to use mass photometry and how to get the most out of this unique technology.

This handbook aims to explain how mass photometry works, what its strengths and limitations are, and how it is used, while also answering frequently asked questions about the technology.

Measurement basics

The mass photometry measurement concept

The concept of how mass photometry measurements work is simple and is illustrated in Fig 1. Imagine that you have a single protein in solution, shine light on it and can detect its shadow. If there were two proteins, that shadow would be twice as strong; if there were four, it would be four times as strong, and so on.

In general, the greater the mass, the stronger the signal is. This is also the concept of a mass photometry measurement.

Mass photometry shines light on particles and measures the light that comes back. From that measurement, the particles' mass can be determined.

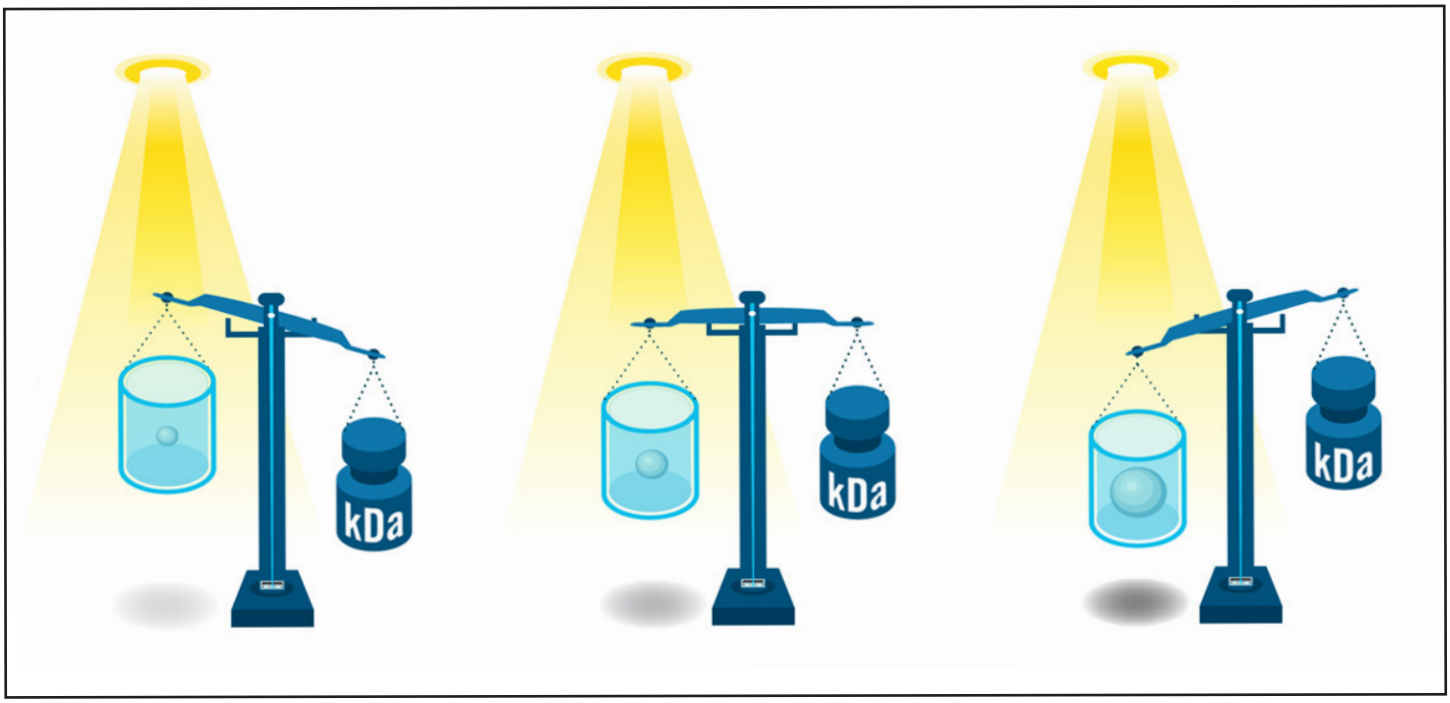


Fig. 1 Mass photometry concept.

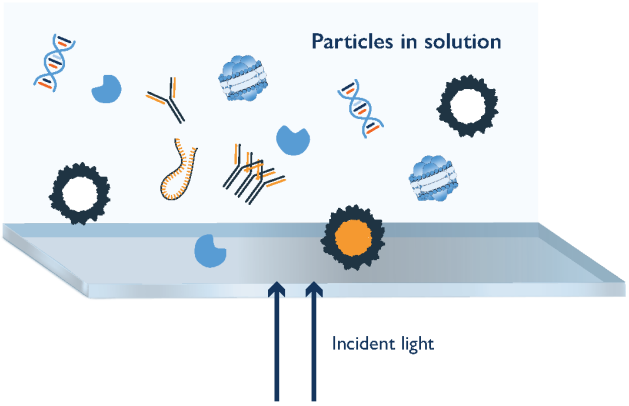
A biomolecule (light blue) that is exposed to light (yellow) in a mass photometer generates a light-scattering signal (grey circles beneath the biomolecules). The signal's intensity correlates directly with the biomolecule's mass.

What happens during a mass photometry measurement?

To begin a mass photometry measurement, a droplet of sample is pipetted onto a glass slide into a small well, and then the instrument lid is closed. At this point, the measurement can begin and takes one minute.

During that one-minute measurement,

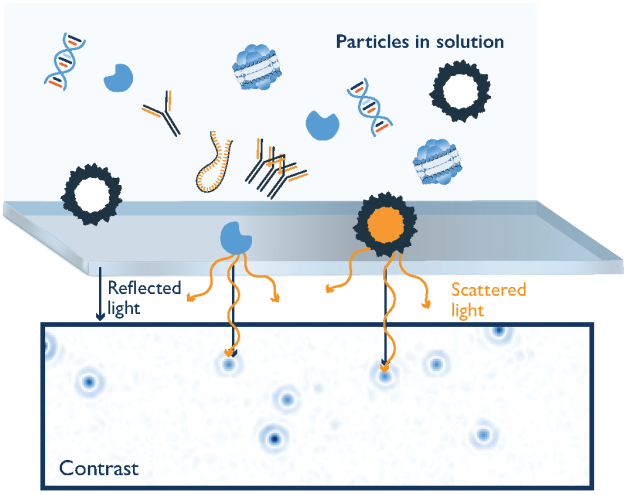
1. **Particles in the sample are landing on the glass surface while a laser illuminates the sample from below.**



2. **Some of the laser light reflects off the glass and some is scattered by the particles. The particles with greater mass scatter more light.**

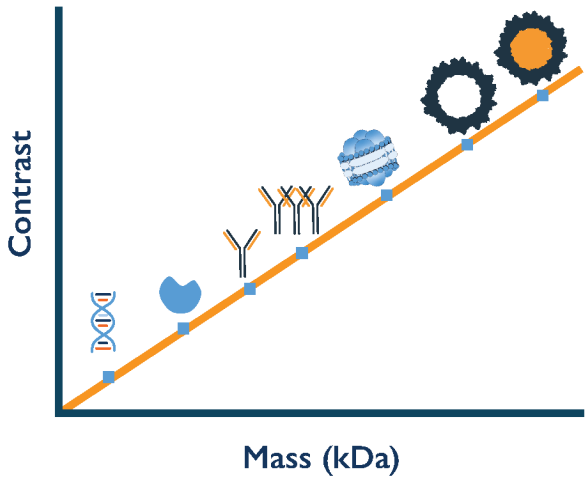
   The scattered light interferes with the reflected light, creating a signal known as the contrast. The changes in contrast over time are captured in a series of images.

   Whenever a particle lands on (or moves away from) the glass, it appears as a spot on the image.



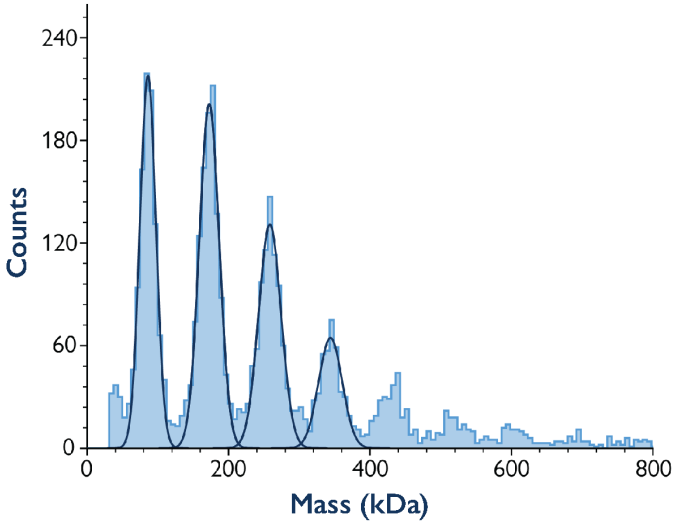
3. **The intensity of each spot (the contrast) is proportional to the mass of the associated particle, as demonstrated by the landmark publication that introduced mass photometry (Young, et al. 2018).**

   Mass photometry can be used to measure a range of different particles, from nucleic acids to proteins – including antibodies and antibody aggregates – and small viral particles.



4. **During a single, one-minute measurement, the contrast of many particles will be measured and they can be plotted as a histogram.**

   Calibration with a particle of known mass enables the contrast to be converted to mass, resulting in a mass histogram for the sample. Shown here is the mass histogram for Refeyn's protein calibrant MassFerence™ P1, measured on the TwoMP mass photometer.



For example, if a sample contained particles with all the same mass, the histogram would show a large single peak at one contrast value.

If those particles were protein monomers that existed as monomers as well as forming dimers and tetramers, then a monomer peak would be expected, along with a dimer peak (at 2x the monomer contrast) and a tetramer peak (at 4x the monomer contrast).

A calibration measurement would make it possible to convert the contrast values of each peak (in arbitrary units) to mass values (in kilodaltons).

What mass photometry data looks like

Mass photometry data is simply a series of images (Fig. 2). For each image in the series, the average of N frames is taken and divided by the average of the N subsequent frames to reveal how much the signal changed when a particle landed on the glass surface. The signals from particles landing on the glass surface contribute to the mass histogram.

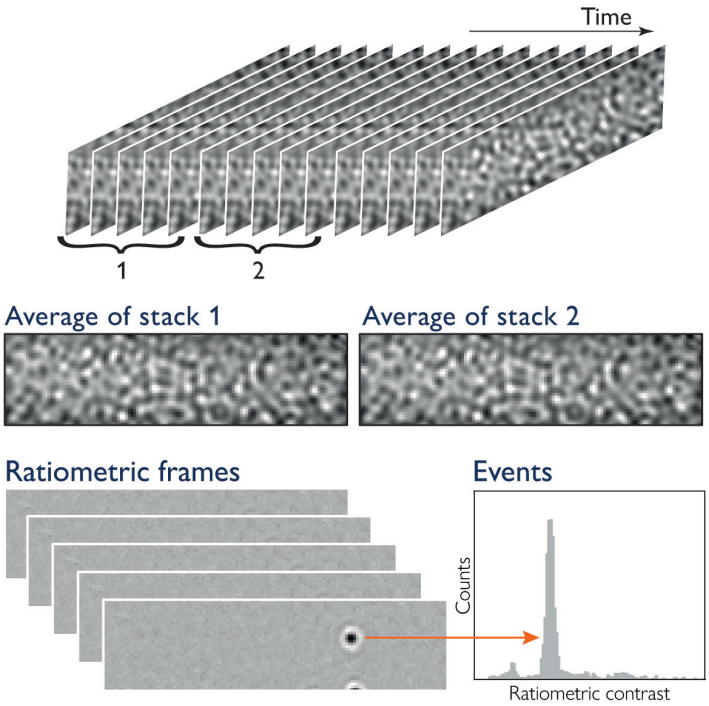


Fig. 2 Generation of the mass photometry signal.

Images of the glass surface, taken over time, are divided into two stacks of N consecutive frames (typically N=5). These stacks are averaged to calculate a single ratiometric frame. The process is repeated for stacks of frames shifted by one frame at a time, generating a ratiometric movie that shows when particles land on the glass surface as well as their contrast signal.

Single-molecule detection

When molecules and other small particles land on the glass surface, they produce features in the ratiometric images. Molecules are much smaller than the wavelengths of light and diffraction limit of an optical microscope, so they are represented as a point-spread function (PSF) (Fig. 3). The PSF describes the imaging system's response to a point source – and depends on the imaging wavelength and numerical aperture (NA) of the objective lens. The shorter the wavelength and the higher the NA, the smaller the PSF of the device.

Molecules of different masses measured on the same instrument will therefore have PSFs with identical shapes but varying intensities (Fig. 3). Thyroglobulin, for example, has 10 times the mass of bovine serum albumin (BSA), so its signal intensity is 10 times stronger. During a one-minute acquisition, several hundreds of molecule-glass landing events are detected, providing direct information on the mass distribution of a sample containing both types of molecules (Fig. 3).

Events need to be well separated in both time and space, otherwise fitting errors due to overlapping PSFs can obscure the results. Therefore, it is important to keep the sample concentration at a level that allows single-molecule detection (typically <100 nM), resulting in the very low sample concentrations required for mass photometry measurements.

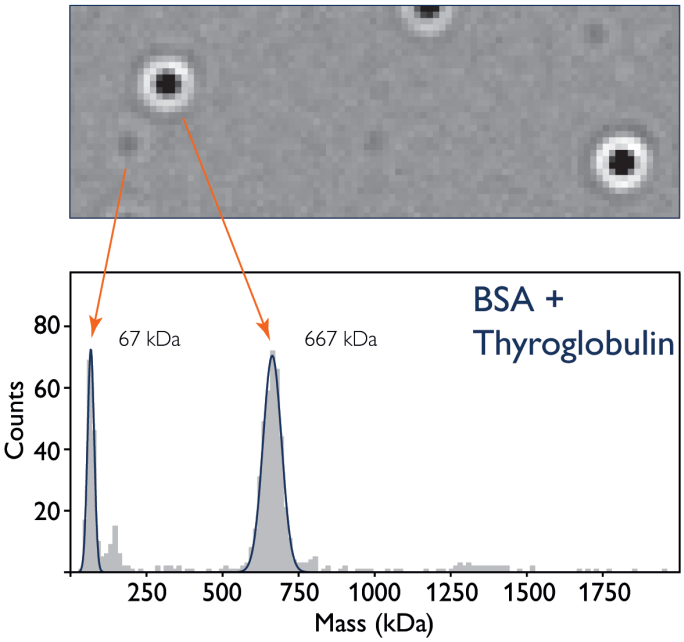


Fig. 3 Single-molecule detection with mass photometry.

Top: A single ratiometric frame of a mixture of BSA and thyroglobulin, showing PSFs with different intensities. Bottom: A mass histogram for that mixture, with the mean mass for each peak indicated. Arrows show that the PSFs with lower intensity correspond to the lower-mass species (BSA), while the PSFs with greater intensity correspond to the higher-mass thyroglobulin. A mass photometry movie recorded for one minute consists of about 3000 frames, during which several hundreds of molecules can be detected as they land on the glass surface. From the resulting series of ratiometric frames, a mass histogram can be created.

Why is contrast proportional to mass?

The linear correlation between mass and the contrast measured by mass photometry holds for a wide variety of particles – making mass photometry a universal tool for biomolecules in solution. In addition, as discussed further on p. 13, the mass photometry signal depends only on mass, not on particle size or shape.

It may at first seem surprising that there is such a consistent linear relationship between contrast and mass.

The simplest reason that the linear contrast-mass relationship holds is that, taking proteins as an example, all proteins have very similar optical properties – because they are all made of the same thing (amino acids).

Similar arguments hold for other types of particles (e.g. nucleic acids). However, while the relationship remains linear, the constant of proportionality between the contrast and mass varies by particle type. This is because the optical properties of proteins are slightly different from those of, say, nucleic acids.

A more detailed explanation is that the interferometric scattering signal is proportional to polarizability, which is a function of the refractive index and proportional to the particle volume (for particles that are small relative to the wavelength of light – which is the case for those analyzed by mass photometry).

For more details:

- The observation that protein density and refractive indices vary by only ~1% is made and discussed in more detail in Young et al. 2018, which introduced mass photometry.
- To learn more about the theory behind the calculation of the scattering signal, see Ortega-Arroyo and Kukura (2012), which describes how interferometric scattering microscopy (iSCAT) can achieve ultrafast, ultrasensitive and label-free optical imaging.

Understanding mass photometry histograms

During a mass photometry measurement, every time a molecule or other particle in the sample lands on the glass measurement surface, it produces a light scattering (interferometric contrast) signal. The typical mass photometry measurement lasts for one minute, during which thousands of landing events can be detected. Histograms are a helpful way to visualize those many individual contrast measurements (Fig. 4).

In histograms, the measurements of single-particle landing events are grouped into narrow mass ranges ('binned') to make the data easier to interpret. Each bin is represented by a vertical bar, and the height of the bar (the 'counts') tells you how many measurements fell into that particular range. If a bar is tall, it means that the mass photometer counted many landing events within that mass range.

As for most biological data, repeated measurements of molecules with the same mass will produce data with some variability that is centered on the true value. In a mass photometry histogram, such data will appear as peaks made up of several bars. Each peak can be fit by a Gaussian curve (Fig. 4), a straightforward statistical approach that is implemented in Refeyn's mass photometry data analysis software, DiscoverMP.

This fitting yields two key values:

1. The peak's mean, which corresponds to the mass of the particles captured in that peak, and

2. The peak's standard deviation, which indicates how spread out the values are – an indicator of the uncertainty in the measurement.

When a mass photometry histogram has multiple peaks, it indicates that there are multiple species present in the sample. Indeed, the single-particle nature of mass photometry means that you can characterize samples containing many different species across a broad mass range. Those species can be detected provided they differ enough in mass (the mass differences must be above the resolution of the instrument).

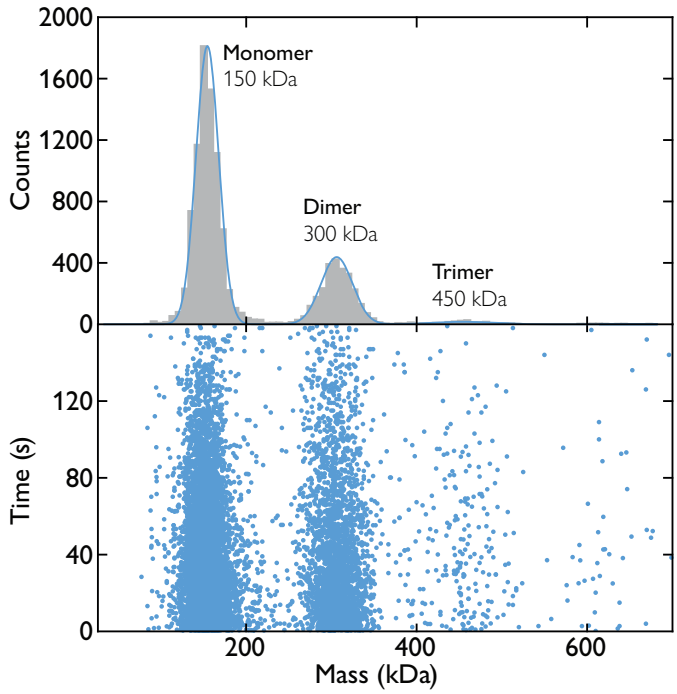


Fig. 4 Mass photometry data of a sample of the antibody 2G12.

The scatter plot (bottom) shows the mass measurements associated with the many landing events recorded over a 160-second time period. The mass photometry histogram (top) presents the data as a histogram, with the peaks fit by Gaussian curves. The peaks correspond to monomers, dimers and trimers of 2G12 IgG, a monoclonal antibody against the HIV envelope glycoprotein gp120.

The example above shows data from a sample containing the antibody 2G12, which is known to form oligomers. In the histogram, three peaks are visible, indicating that there were three species, each with different mass, in the sample. From the mean of each peak (which tells us the mass of the molecules in that subpopulation), we can conclude that the peaks correspond to 2G12 monomers, dimers and trimers (Fig. 4). From the heights of the peaks, we can see that the monomers were the most abundant, followed by dimers and then trimers. By calculating the area under the Gaussian curve, we can quantify those abundances.

In summary:

- Mass photometry data are typically presented as histograms, where each peak of the histogram represents a population with a particular mass.
- Analysis of the peaks yields the mass of the subpopulation, along with the uncertainty of that mass measurement and relative abundances of that species.

Key considerations

Do particle size and shape affect mass photometry measurements?

Mass photometry only measures mass; it is not sensitive to particles' size or shape. The scattering signal measured by mass photometry depends on the mass of the particle being measured, and not on its hydrodynamic radius or structure. Insensitivity to particle size and shape is one of the key differences between mass photometry and other techniques that measure light scattering, such as dynamic light scattering (DLS).

Whether a protein is folded or unfolded, its mass remains unchanged – and so does its mass photometry readout. The insensitivity of mass photometry to a protein's folding status is illustrated in measurements of the enzyme enolase during native and denaturing conditions (Fig. 5). Under native conditions, enolase forms mainly homodimers (~93 kDa mass) and some monomers (~46 kDa mass). In denaturing conditions (urea), the dimers separate into mostly unfolded monomers. However, the mass of the unfolded monomers, as measured by mass photometry, was identical to the mass of the folded monomers.

One important application of mass photometry's sensitivity to mass but not size is the measurement of empty vs. full AAV capsids (Fig. 6). Empty AAV capsids are the same size as full ones, but have different mass.

By measuring individual capsids' mass, mass photometry can quickly quantify whether a sample contains mostly full capsids or a high number of undesirable empty (or partially filled or overfilled) capsids – a valuable insight during the production of AAV therapeutics.

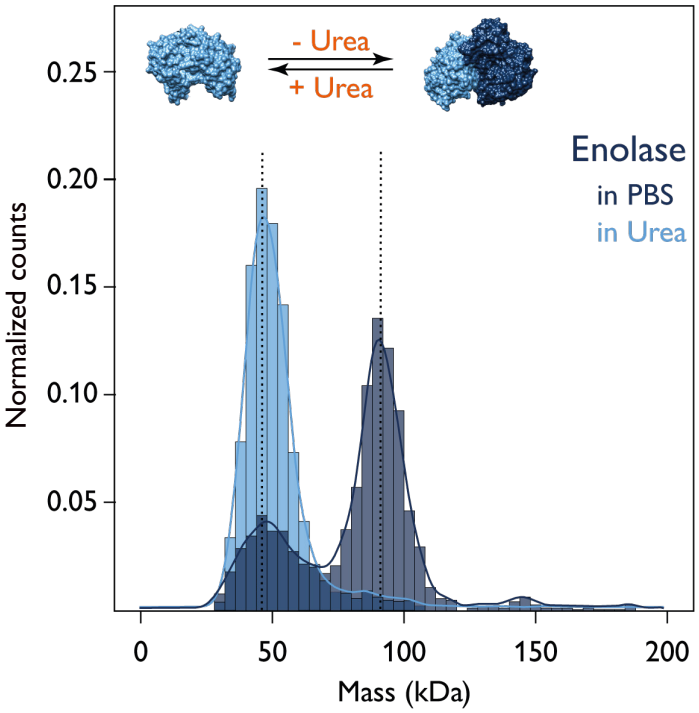
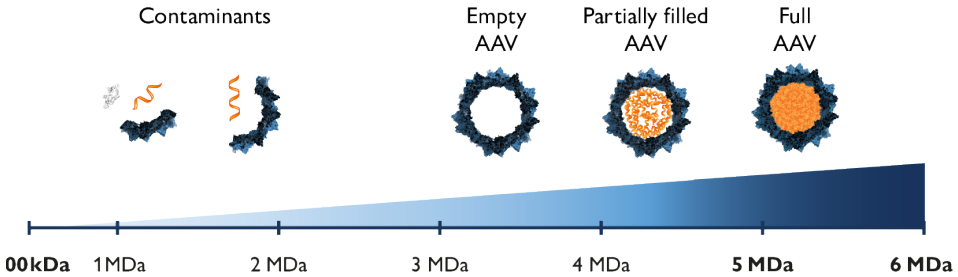


Fig. 5 Mass photometry is not sensitive to folded vs. unfolded status.

Enolase, measured by mass photometry in PBS (dark blue) and 5.4 M urea (light blue) shows more monomers but no mass changes in denaturing conditions. The denatured sample was diluted to <0.05 M urea prior to measurement. Measured on a OneMP mass photometer.

Fig. 6 Mass photometry analysis of AAV capsid loading.

Because mass photometry is sensitive to mass but not size, it can readily assess whether AAV capsids in a sample are empty, partially filled or full.



The need for calibration

Converting contrast measurements to mass requires calibration with an appropriate standard of known mass. This is because, as mentioned above, the precise linear relationship between the contrast and mass may differ for each class of particle, due to differences in optical properties of different types of particles.

A protein calibrant is therefore needed when measuring proteins, a DNA calibrant is needed for measuring DNA, etc. Small differences may also occur from day to day and in different buffers, so regular calibration is needed for maximum accuracy.

Accuracy and precision

Mass photometry measures molecular mass with high accuracy. However, as with all analytical methods, you must be aware of possible experimental error.

A single mass photometry measurement comes with a measurement error of up to ± 5% (for Refeyn mass photometers), meaning that the molecular mass measured by mass photometry might deviate from the expected molecular mass by ± 5% (Fig. 7). The peaks in a mass photometry histogram can be fitted using Gaussian approximations, with the peak center indicating the measured mass of the species. This experimental error arises from a combination of sources, including sample measurement error, calibrant measurement error and error in fitting a Gaussian curve to the raw data. The error can be reduced by taking the average of repeated measurements.

The mass precision (or reproducibility) of Refeyn's mass photometers is consistently within 2%, meaning that there is very little variability when a sample is measured more than once.

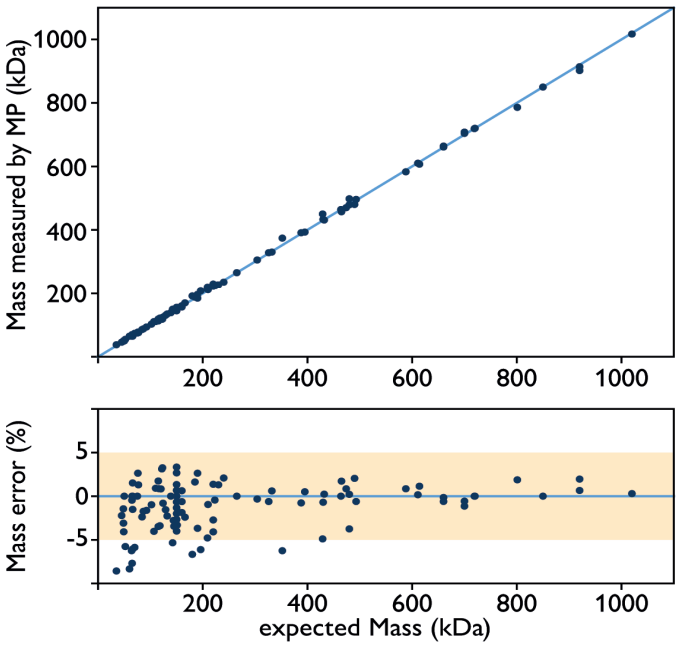


Fig. 7 Accuracy of mass photometry.

Top: Correlation of expected vs. measured molecular mass (in kDa) for a set of proteins across the 60 – 1000 kDa mass range. Bottom: Mass error shown as a percentage of the expected mass (N=150). Measured on a OneMP mass photometer.

Detection range

Mass photometry can detect and quantify molecules across a broad mass range (detection range). The range covered by mass photometry is exemplified by measurements of Protein A (42 kDa) and an AAV particle (~3.7 MDa), which, having nearly 100 times greater mass, generates a signal (the contrast) with almost 100 times greater intensity (Fig. 8).

The ability to accurately detect and quantify PSFs depends on the noise levels in the ratiometric image. While shot noise cannot be eliminated, averaging more frames to calculate each image in the ratiometric movie reduces the shot-noise-induced fluctuations in each ratiometric image – increasing the signal-to-background ratio and enabling detection of molecules with lower mass.

The downside of averaging more frames, however, is a reduction in temporal resolution. Protein concentrations may need to be decreased to prevent PSFs from overlapping. Additionally, longer averaging times may impair data quality due to lateral drift. Measurements of molecules with mass close to the detection limit may therefore require averaging more frames, with attention to drift. Larger molecules yield a stronger signal and so require less frame averaging.

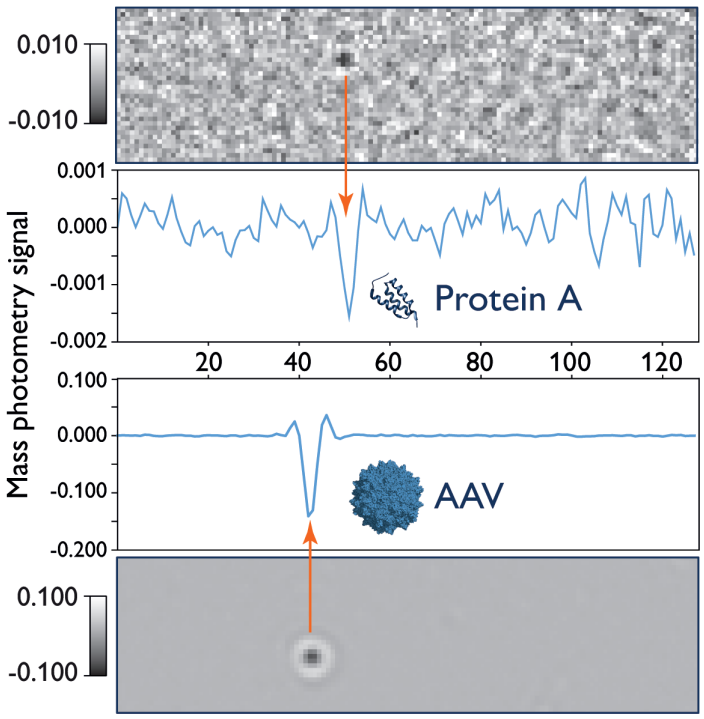


Fig. 8 Mass photometry detects particles across a broad mass range

Signal traces of landing events for protein A (42 kDa, upper panel) and an adeno- associated virus (AAV) particle (3.7 MDa, lower panel) are depicted.

Resolution

Resolution in mass photometry is the smallest difference in mass that you can detect in a mass photometry measurement. In other words, it is the smallest difference in mass that resolves as two distinguishable peaks.

There is no single value that defines the resolution of mass photometry because it depends on several factors, including:

- The mass range of the particles of interest,
- The purity of the sample, and
- The relative concentrations of species in the sample.

We can take the TwoMP mass photometer as an example to illustrate how the minimum separable distance increases for species with greater mass. At the lower end of the mass range, the resolution is ± 25 kDa for a measurement of a 66 kDa biomolecule (defined as the FWHM or Full Width of the peak at its Half Maximum value). This means you could identify other species present in the sample if they were smaller than 41 kDa or larger than 91 kDa (Fig. 9, top). These different species would be visible as distinguishable peaks in the mass histogram. On the other hand, if all species in the sample were within the range 41 – 66 kDa (or 66 – 91 kDa), only one broad peak would be observed, and it would be made up of counts from all the different species.

At the higher end of the mass range, for example around 660 kDa, the resolution is ± 60 kDa FWHM. This means that you could distinguish 660 kDa particles from others if their mass were ≤ 600 kDa or ≥ 720 kDa (Fig. 9, bottom).

Resolution also depends on sample purity, as well as on the relative concentrations of the species in the sample. For resolution, it is optimal to have equal peak heights. In this case, two peaks can be resolved when the separation between their centers is larger than the sum of half their full width at half maximum (FWHM), as described above. But when one species is much more abundant than another, the minimum separation distance increases. Where impurities are present within the mass window of interest or there is significant sample heterogeneity, these factors can also negatively affect the resolution in mass photometry.

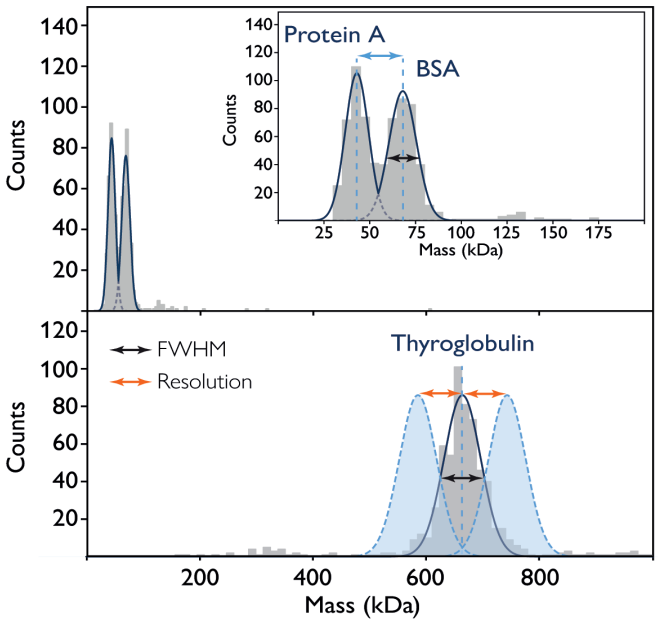


Fig. 9 Resolution and full width at half maximum.

Top: The mass histogram for a 1:1 mixture of protein A (42 kDa) and BSA (66 kDa). Inset: The FWHM (black arrow) indicates the resolving power allowing for clear peak separation (blue arrow). Bottom: Thyroglobulin (670 kDa, glycosylated) with FWHM (black arrow) indicated. Theoretical Gaussians (blue) indicate possible resolution (orange arrows), assuming similar peak heights (i.e. numbers of events).

Samples

What concentrations can you measure?

Mass photometry measures the mass of single particles as they land on a glass measurement surface. To ensure that the landing events are well separated in space and time, it is essential to prepare samples to the appropriate concentration. Mass photometry can be performed with sample concentrations ranging from 100 pM to 100 nM, with the optimal range being 5 – 20 nM for proteins or nucleic acid molecules, and 10^{11} particles/mL for adeno-associated viruses (AAVs).

Being able to run mass photometry experiments at such low sample concentrations can be a great advantage when limited sample is available. However, when higher concentrations need to be used, such as if you are studying weak biomolecular interactions of molecular species, a rapid-dilution microfluidics add-on (Refeyn's MassFluidix™) can make it possible to measure samples at up to the tens of micromolar.

What types of molecules and particles can you measure?

Because mass photometry simply measures light scattering, any particle within the appropriate mass range should be measurable. The technique has been successfully applied to samples containing a range of different types of particles, including:

- Proteins
- Glycosylated and biotinylated proteins
- Membrane proteins in membrane mimetics (e.g. detergents, nanodiscs)
- Nucleic acids
- Adeno-associated viruses (AAVs)

Mass photometry can also analyze samples containing a mix of different types of particles, enabling studies of protein-DNA interactions, for example.

Mass photometry can measure the mass of particles in the 30 kDa – 6 MDa mass range, depending on the mass photometer (30 kDa – 5 MDa with the TwoMP, 500 kDa – 6 MDa with the Samux), as illustrated in Fig. 10. To characterize samples of larger particles, such as adenovirus capsids, the technology macro mass photometry can be used (see p. 23).

The reason for the lower limit is that the weak signal produced by smaller particles can be difficult to differentiate against shot noise – which arises from random fluctuations of photons and limits image quality. For particles above 5 – 6 MDa, the linear contrast-mass relationship may not hold and the signal may saturate the detector.

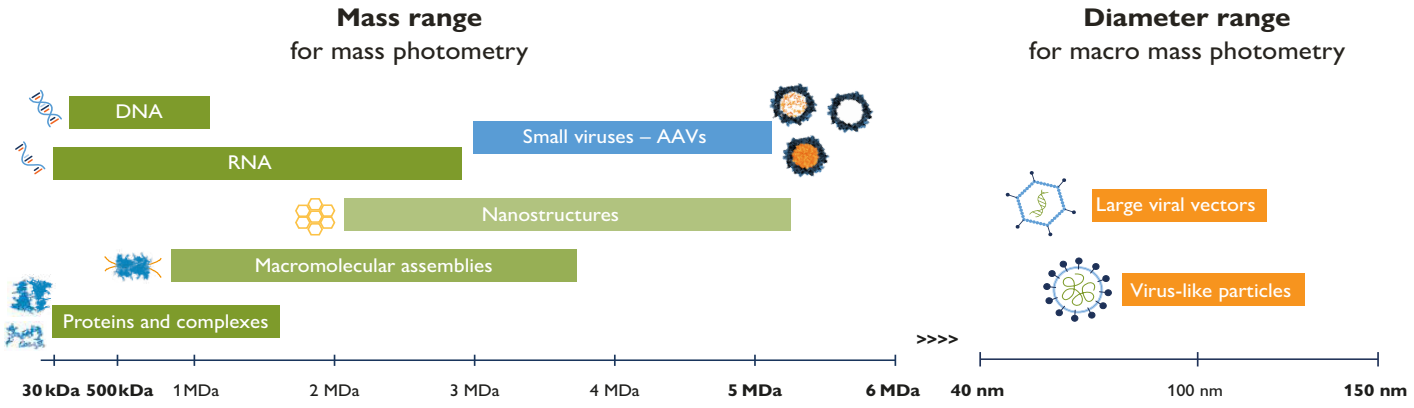


Fig. 10 Mass range for mass photometry and macro mass photometry.

Comparison to other light scattering techniques

Because mass photometry measures a light scattering signal, a common question is how it compares to other light scattering techniques.

Mass photometry vs. DLS

DLS is a label-free technique that is used to analyze samples of proteins, nucleic acids and complexes in solution (Lorber et al., 2012). It enables detection of aggregation and binding. It measures the hydrodynamic radius of particles in solution based on how they scatter light as they move due to Brownian motion. Fluctuations in the intensity of scattered light enable calculation of the diffusion rates of the particles in the sample.

DLS is an ensemble technique that measures scattering as a property of the solution as a whole – as opposed to the scattering of each particle individually, as in mass photometry.

In DLS, the hydrodynamic radius is taken as a proxy for size, with the assumption that the particles measured are spherical (which most biomolecules are not).

Key differences

- Mass photometry measures mass while DLS measures the hydrodynamic radius (a proxy for particle size).
- Mass photometry is a single-molecule measurement, whereas DLS provides an ensemble average (and is very sensitive to aggregates and larger-size particles, which dominate the signal and so tend to be overrepresented).

Mass photometry vs. SEC-MALS

SEC-MALS is a label-free technique that measures the size and mass of particles in a sample in solution (Hong et al., 2012). First, particles are separated by size and shape in a chromatography column from which the largest particles elute first.

Next, a MALS detector measures light scattering from multiple angles, enabling calculation of particle mass and size (radius of gyration) provided that UV detection is used or information on the refractive index is also available.

Key differences

- SEC-MALS requires an initial chromatographic separation step, and possibly column optimization steps. Mass photometry does not require this.
- Mass photometry is a single-molecule measurement, whereas SEC-MALS provides an ensemble average for each elution peak. When different populations fall within the same elution peak (have similar size and shape), only a single averaged mass value is obtained.
- For measuring interactions, mass photometry measures the solution at equilibrium as no separation step is required, which could disrupt interactions such as oligomerization or aggregation.

Mass photometry also uses significantly less sample than SEC-MALS and takes less time.

A quick note on mass spectrometry (MS) vs. mass photometry

MS and mass photometry have similar names, but they are very different. They differ in terms of how they work, what they measure and when they would be used.

In particular:

- Mass photometry measures the mass of molecules in solution, in their native state, from their light scattering. MS measures the mass-to-charge ratio of ionized molecules in the gas phase.
- MS is typically used to sequence a protein, by measuring the mass of protein fragments after digestion, whereas mass photometry is used to measure the mass of intact proteins in native conditions, analyze non-covalent interactions (e.g. oligomerization) and characterize the mixture of species present in a sample.
- With MS, the exact mass can be determined (with accuracy within <1 Da). Mass photometry provides a mass measurement within 5% of the true value.
- MS is much more complex to use than mass photometry, and requires more sample and time.
- One application of MS, native MS, does not disrupt complexes. A comparison of native MS and mass photometry for AAV analysis has shown similar accuracy (Ebberink et al., 2022), although native MS is more complex to use.

The use of mass photometry

Why mass photometry is useful

Several valuable benefits set mass photometry apart from other bioanalytical techniques:

1. **It measures true molecular mass.** Mass photometry does not infer the mass indirectly from a different physical parameter. This is different from other techniques, such as dynamic light scattering (DLS) and size-exclusion chromatography (SEC), which infer molecular size from the hydrodynamic radius. Instead, the mass photometry signal measured is directly correlated with the true molecular mass, enabling measurement of the mass of molecules in the range 30 kDa to 6 MDa.

2. **It shows sample heterogeneity.** Mass photometry measures the mass of each molecule or particle on the measurement surface. These single-molecule measurements make it possible to detect subpopulations of species or detail the heterogeneity – aspects of a sample that are invisible to methods that use bulk measurement (such as DLS, SDS-PAGE and SEC).

3. **It works in solution.** Mass photometry measurements are performed in solution, and mass photometry is compatible with water as well as a wide range of buffers. Measuring biomolecules in an environment that mimics the intracellular aqueous environment allows their true native behavior to be studied.

4. **It uses very little sample.** Volumes as little as 10 µL can be enough for a single mass photometry measurement, and the recommended concentration range is 100 pM – 100 nM. The low concentration range means that biomolecules can be studied at concentrations that are physiologically relevant – again helping to mimic the intracellular environment and observe native behavior.
   A rapid-dilution microfluidics device enables measurement of samples at higher concentrations – up to the tens of micromolar.

5. **It requires no modification of the sample.** Mass photometry does not require any labelling of the molecules or particles under investigation. This simplifies sample preparation and eliminates the risk of labels interfering with native behavior.

6. **It provides results rapidly.** Mass photometry workflows are very quick, with both sample preparation and measurement together usually taking just minutes.

7. **It is easy to use.** It takes less than half a day to learn how to do a mass photometry measurement and the data is intuitive to interpret. The instruments also fit conveniently on a lab benchtop.

An overview of applications

With mass photometry, you can measure the mass of single biomolecules, oligomers, macromolecular assemblies and small viral capsids (of e.g. adeno-associated viruses, AAVs). Macro mass photometry, meanwhile, extends applications even further, as it can be used to analyze larger viral vectors.

Here, we present a brief overview of published applications of mass photometry, illustrating the many different ways the technology is being used.

One of the most frequent applications of mass phtometry is to quantify the **oligomerization and aggregation** of biomolecules (Naftaly et al. 2021, Balakrishnan et al. 2024) and characterize sample heterogeneity (Olerinyova et al. 2021, Sonn Segev et al. 2020). It can be used to monitor the **stability** of sample components (Nuber et al. 2021) and study the effects of molecular or experimental modifications on **sample integrity** (Bertosin et al. 2021).

Mass photometry is especially valuable for studying biomolecular interactions – including **protein-protein interactions** (Higuchi et al. 2021; Soltermann et al. 2020) and **protein-nucleic acid interactions** (Hickman et al. 2020; Acharya et al. 2021). Mass photometry makes it possible to determine **stoichiometries** in biochemical reactions (Xu et al., 2024) and quantify **affinities** and rate constants in molecular interactions (Wu and Piszczek 2020; Soltermann et al. 2020).

Highlighting the versatility of mass photometry, it is also suitable for work with nucleic acids – such as in **characterization of mRNA and dsRNA** (Schmudlach et al. 2025, De Vos et al. 2024).

Other key applications include **assessing antibody affinity and aggregation** (den Boer et al. 2021, Cramer et al. 2023), characterizing **membrane proteins** (Olerinyova et al. 2021, Dodge et al. 2024) and supporting **structural studies** (Vasquez et al. 2023, Crowe et al. 2024).

Mass photometry is also widely used to characterize **AAV** samples, quantifying proportions of **empty and full AAV** capsids and resolving also partially filled capsids (Wu et al. 2022, Wagner et al. 2023, Wagner et al. 2024, Ebberink et al. 2024).

Macro mass photometry (see p. 23) is a newer technology that builds on the principles of mass photometry. It characterizes samples of larger viral vectors (e.g. **adenovirus**), virus-like particles (**VLPs**) and **lipid nanoparticles** (Wu et al. 2025).

Although mass photometry was introduced relatively recently (in 2018), its range of applications has expanded rapidly as users discover the technology and its versatility.

For an up-to-date list of publications citing mass photometry, visit Refeyn's database of mass photometry publications.

What mass photometry users say

"**Compared to SEC-MALS, mass photometry is fast and provides robust and reliable data.** We're also very much impressed by the software used to control the mass photometer and evaluate the data – it's very intuitive and easy to operate."

Michael Ploug, University of Copenhagen Biotech Research & Innovation Centre

"We're generating data to compare mass photometry with gold standards like AUC and cryoTEM. Early indications show that it's much quicker and equally accurate. Partners who've compared mass photometry to other methods themselves vouch for its accuracy. **It's a game changer in AAV characterization, for us as well as our clients**."

Quentin Bazot, Head of Innovation and Development at ABL

"**It was very difficult to study the BoNT/Wo M-PTC using size-exclusion chromatography.** The complex interacted with the column material and we believe that the strength of this interaction was pH dependent, resulting in uninterpretable results... **We instead utilized mass photometry**... [which] revealed that the BoNT/Wo-NTNH/Wo complex is stable under acidic conditions and may dissociate at neutral to basic pH."

Košenina et al. (2024). The cryo-EM structure of the BoNT/Wo-NTNH complex reveals two immunoglobulin-like domains. *The FEBS Journal*

"Consistent measurements of mRNA lengths were observed with calculated errors of less than 2.3%... demonstrating the robustness and accuracy of **MP as a reliable tool for accurately determining mRNA length across a wide range of sizes**."

Camperi et al. (2024). Comprehensive Impurity Profiling of mRNA: Evaluating Current Technologies and Advanced Analytical Techniques. *Analytical Chemistry*

"One of the routine things that we are doing now is, after solubilizing the protein and purifying it, **we put it into the mass photometer to check if there is a single peak of the right size before we... do the expensive cryoEM experiments**."

Philip Kitchen, BBSRC Discovery Fellow. Aston University

"The quality and information content of the MP data, combined with simple and fast measurements and low sample consumption makes MP a new **preferred method for measuring strong protein-protein interactions**."

Wu and Piszczec (2020). Measuring the affinity of protein-protein interactions on a single-molecule level by mass photometry. *Analytical Biochemistry*

"I feel that **mass photometry is really reporting on my actual sample as I have it in my Eppendorf tube.** That's a huge benefit. In particular, you can parse out compositional heterogeneity independent of conformational variability, which is a difficult thing to get with other techniques."

John Zinder. Senior scientist at Odyssey Therapeutics

"Due to the availability and simple process of MP which is a label-free and light scattering-based technique, **we were able to measure the binding of S2A9 to S2 in PBS buffer without any biosensors...** The K_D was calculated to be 590 nM based on the binding curve."

Buffington et al. (2023). Identification of nurse shark VNAR single-domain antibodies targeting the spike S2 subunit of SARS-CoV-2. *The FASEB Journal*

Macro mass photometry

A brief overview

Macro mass photometry is a revolutionary new technology for analyzing large viral vectors, such as adenoviruses (AdV), and virus-like particles (VLPs). Returning results in minutes, it offers fast, simple, qualitative analysis to inform process development and optimization in the development of cell and gene therapies as welll as vaccines.

Macro mass photometry analyzes individual particles, providing data on two parameters simultaneously:

1. Particle scattering contrast (a proxy for mass) and
2. Size (diameter).

By measuring these parameters for every particle, the technique provides an overview of the size-contrast distribution for the sample (Fig. 11). This multiparametric data makes it possible to identify and characterize multiple populations within a sample.

Particle scattering contrast ('contrast' for short) is a proxy for particle mass. The contrast provides a way to differentiate particles that would not be resolvable based on size alone, so a distinct strength of macro mass photometry is that it measures both contrast and size.

To measure contrast, first, the sample (consisting of particles in solution) is illuminated from below by a laser. A small portion of the light is reflected at the slide-sample interface, while some of the light transmitted into the sample is scattered by particles in the sample (Fig. 11). The scattered light interferes with the reflected light, generating an optical contrast proportional to the

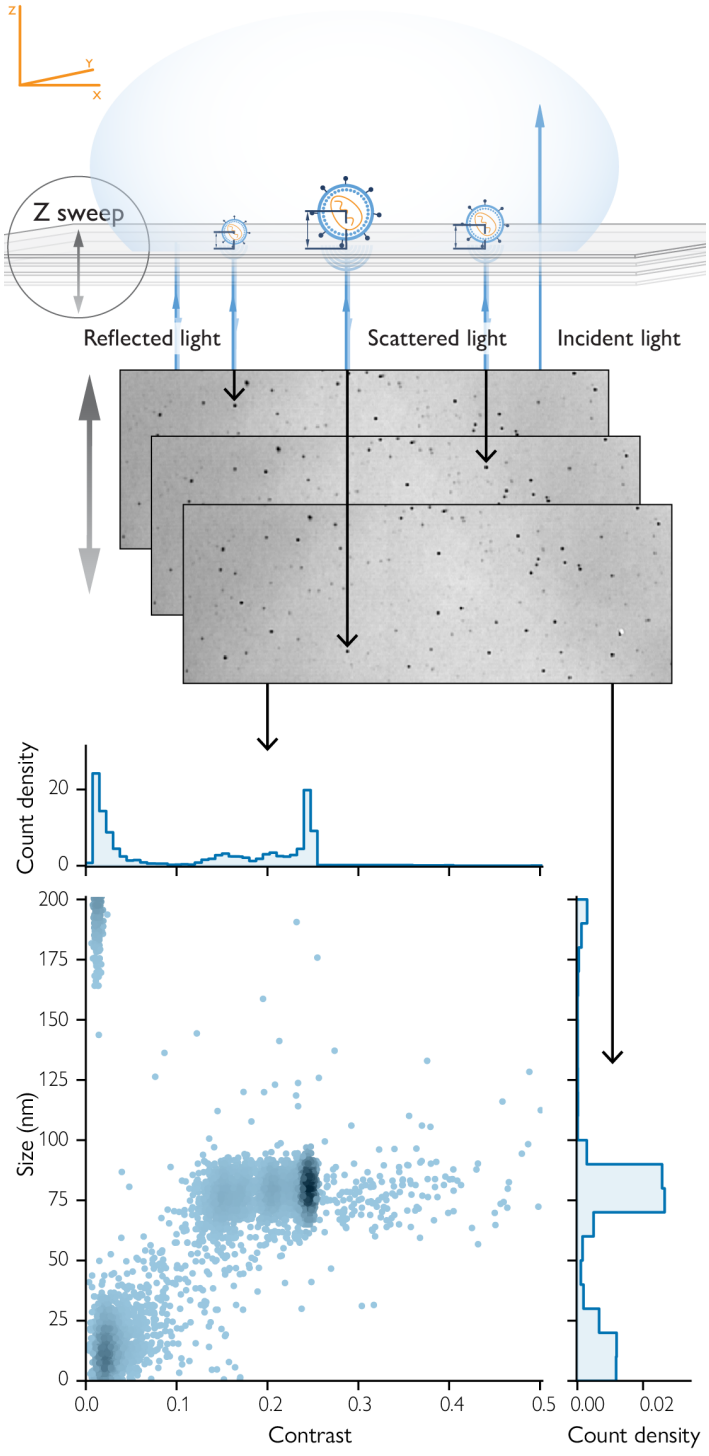


Fig. 11. Macro mass photometry analysis characterizes particles based on size and mass. During each measurement, a droplet of sample on a sample carrier slide (top) is illuminated from below and imaged while being moved vertically. This process, with associated analysis, returns the size-contrast distribution for the sample particles in the field of view (bottom). Shown here is a sample of adenovirus vectors analyzed on the Karitro™ macro mass photometer.

amplitude of the light scattered.

The scattering signal depends on the particles' size and refractive index, so a particle with greater contrast would be i) larger than another with the same refractive index or ii) have a higher effective refractive index than another of the same size or iii) both. In all cases, the particle with greater contrast would have greater mass, meaning that contrast is a proxy for mass. The contrast cannot, however, be easily converted into mass, owing to the complex composition of vector particles and the corresponding variability of the optical properties within a given particle.

How does macro mass photometry work?

First, a sample (consisting of particles in solution) is loaded onto a sample carrier slide, where the particles are immobilized to the slide's surface via non-specific binding. A series of contrast images are then recorded as the sample stage sweeps vertically (along the *z* axis). The vertical sweep enables the contrast to be quantified for particles of different sizes – which reach their maximum contrast at different planes.

The vertical sweep also enables the size measurement. Each particle's size (the distance between its center of mass and the measurement surface) is proportional to the position in the *z* sweep at which the particle exhibits maximum contrast. With a straightforward calibration using particles of known size (Fig. 12), the sizes of other particles can be readily calculated. The data acquired in the vertical sweep enables the diameter of each particle to be measured and correlated to its contrast (Fig. 11).

The *z* sweeps are repeated for multiple fields of view across the sample. This measurement process, in tandem with automated analysis, returns the sample's overall size-contrast distribution.

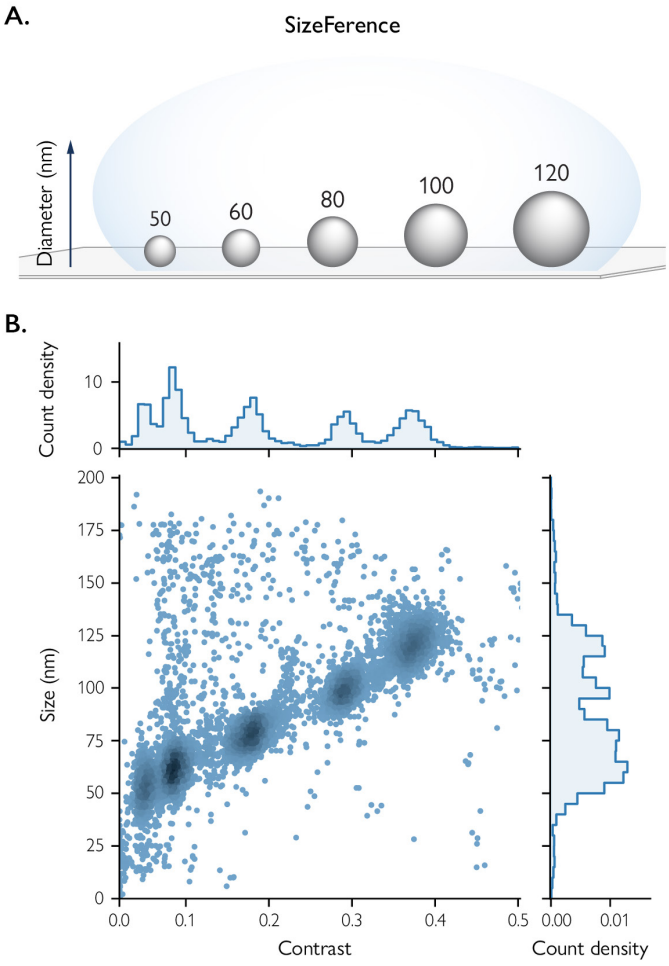


Fig. 12. In macro mass photometry, contrast (a proxy for mass) can be used to resolve particles of similar size.

A. The size calibrant SizeFerence™ is an equimolar mixture of silica beads of five diameters.

B. Analysis of a SizeFerence sample shows the five populations of beads could not be resolved based on size alone (due to their overlapping peaks), but were resolvable using contrast. Analyzed using the KaritroMP.

How does macro mass photometry compare to mass photometry?

Macro mass photometry builds on mass photometry, which uses light to measure the mass of biomolecules.

Key similarities

- Both technologies operate in solution without the need for labels and provide single particle analysis.
- They are both based on the same underlying physical concepts: The principles of interference reflection microscopy and interferometric scattering microscopy (iSCAT).

Key differences

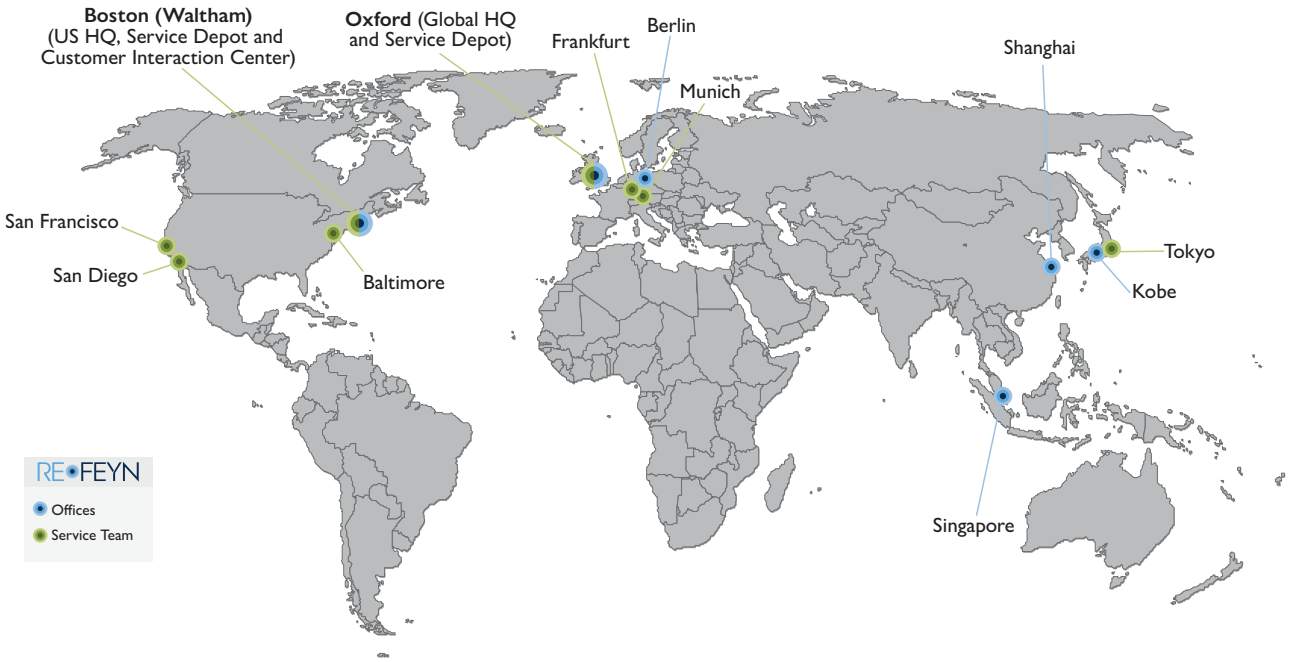
As detailed in the table below:

- In mass photometry, contrast can be converted directly to mass; in macro mass photometry, it is a proxy for mass.
- While both techniques measure contrast, only macro mass photometry measures particle size.
- Mass photometry is suitable for measuring smaller particles, such as proteins or adeno-associated virus (AAV) while macro mass photometry measures larger particles, such as adenovirus vectors (AdV).

	Mass photometry	Macro mass photometry
Particle type	Biomolecules (proteins, nucleic acids, etc.) and AAVs	Large viral vectors (AdV) and virus-like particles (VLPs)
Particle range	30 kDa – 6 MDa mass, << λ of light (Rayleigh regime)	40 – 150 nm diameter, ≈ λ of light (towards Mie regime)
Parameters measured	Mass (directly proportional to contrast)	Contrast, Diameter
What is visualized	Particles landing (ratiometric approach)	Immobilized particles (non-ratiometric approach)
Data outputs	Mass histogram	Contrast histogram, Size histogram, Size-contrast scatter plot

Our vision is to accelerate discovery through innovation, empowering the latest scientific breakthroughs in basic research and transforming biotherapeutic development and manufacturing.

Get in touch to speak with one of our mass photometry experts.



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