RE FEYN

TECH NOTE

Macro mass photometry basics

Macro mass photometry is a revolutionary new technology for analyzing large vectors, such as adenoviruses (AdV) or 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.

What does macro mass photometry measure?

Macro mass photometry analyzes individual particles, providing data on two parameters simultaneously: Particle scattering contrast (a proxy for mass) and size (diameter). By measuring these parameters for every particle, the technique provides an overview of the size-contrast distribution for the sample (Fig. 1). This multiparametric data makes it possible to identify and characterize multiple populations within a sample.

What is 'contrast' in macro mass photometry?

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. 1). The scattered light interferes with the reflected light, generating an optical contrast proportional to the 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.

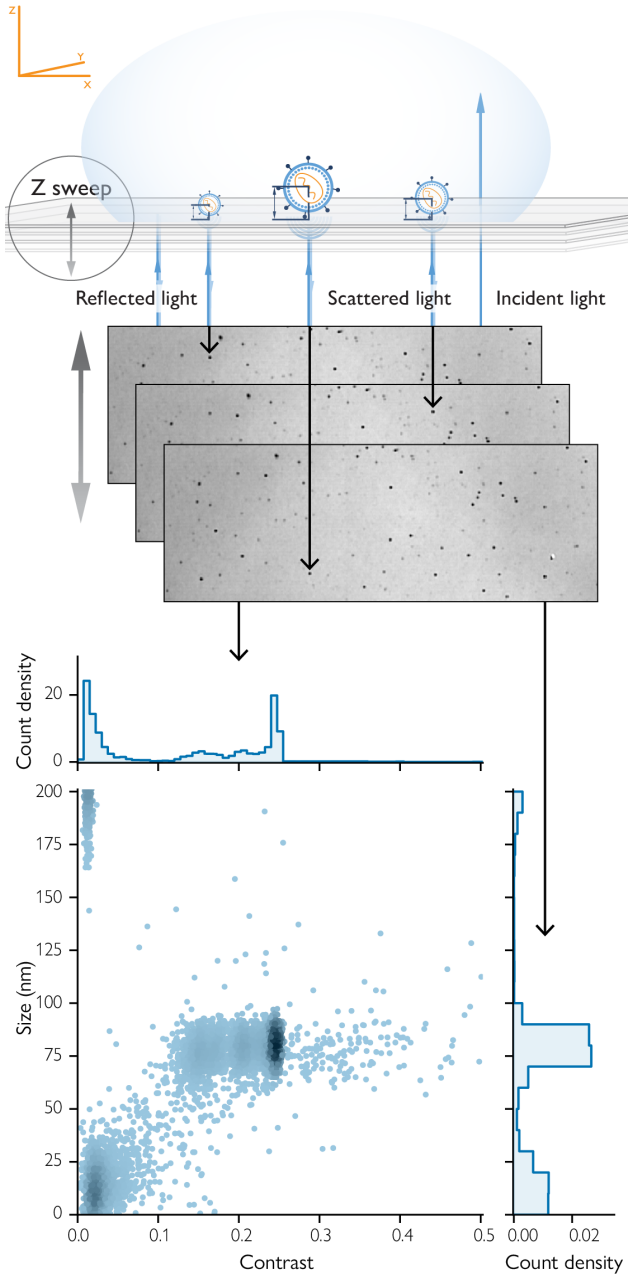

Figure 1. Macro mass photometry analysis characterizes particles based on their size and mass. During each measurement, a droplet of sample on a sample carrier slide (top) is illuminated from below and imaged while being moved vertically. This process, with associated analysis, returns the size-contrast distribution for the sample particles in the field of view (bottom). Shown here is a sample of adenovirus vectors analyzed on the Karitro™MP.

References

[1] Young et al., Science 2018

[2] Cole et al., ACS Photonics, 2017

[3] Verschueren, J. Cell Sci. 1985

[4] Ortega-Arroyo and Kukura, Phys. Chem. Chem. Phys. 2012

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. 2), 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. 1).

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.

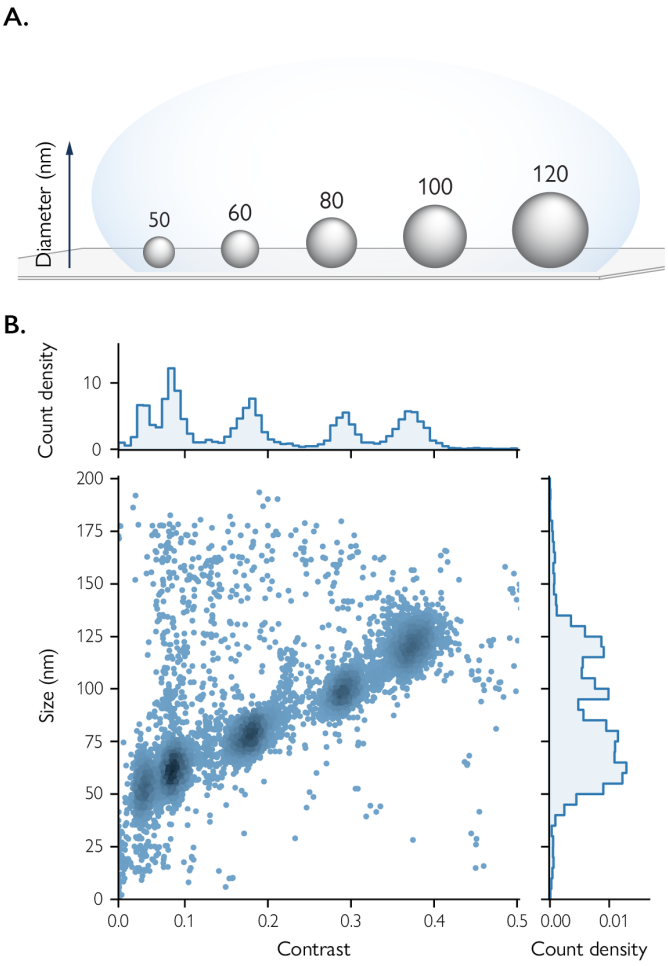

Figure 2. In macro mass photometry, contrast (a proxy for mass) can be used to resolve particles of similar size. The size calibrant SizeFerence™ is an equimolar mixture of silica beads of five diameters Analysis of a SizeFerence sample shows the five populations of beads could not be resolved based on size alone (due to their overlapping peaks), but were resolvable using contrast. Analyzed using the KaritroMP.

Macro mass photometry vs. mass photometry

Macro mass photometry builds on the analytic technology mass photometry[1,2], which uses light to measure the mass of biomolecules.

Similarities

Both technologies operate in solution without the need for labels and provide data at the level of single particles. In addition, both technologies are based on the same underlying physical concepts: The principles of interference reflection microscopy[3] and interferometric scattering microscopy (iSCAT)[4].

Differences

In mass photometry, contrast can be converted directly to mass; in macro mass photometry, it is a proxy for mass. Also, while both techniques measure the contrast, only macro mass photometry measures particle size. Finally, mass photometry is suitable for measuring smaller particles (typically proteins, nucleic acids and AAVs), while macro mass photometry measures larger particles such as AdVs and VLPs.

	Mass photometry	Macro mass photometry
Particle type	Biomolecules (proteins, nucleic acids, etc.), AAVs	Large vectors (AdVs, 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

RE•FEYN

Unit 9, Trade City, Sandy Lane West, Oxford OX4 6FF, United Kingdom

orders@refeyn.com
refeyn.com
Refeyn
@Refeyn

V2 May-2025