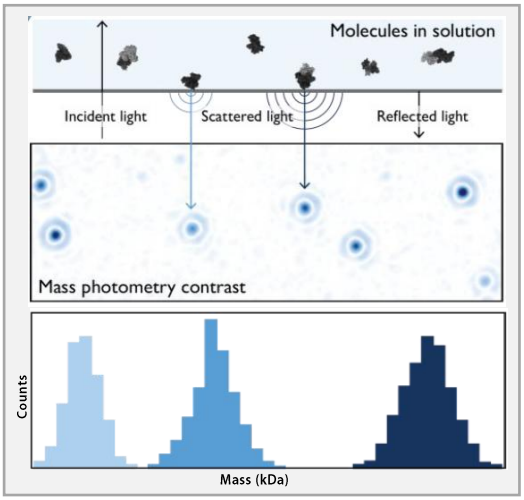
TwoMP

Mass Photometry (MP)

Mass photometry delivers a rapid, **accurate mass measurement** of **label-free** single molecules **in solution** in their **native state.** It is a simple, easy-to-use technology, which measures the light scattered by individual particles and uses the signal to count the particles and measure their mass with high accuracy.

Mass photometry is built upon the principles of interference reflection microscopy and interferometric scattering microscopy. It enables the reliable detection of single molecules and the direct measurement of each molecule's mass in solution.

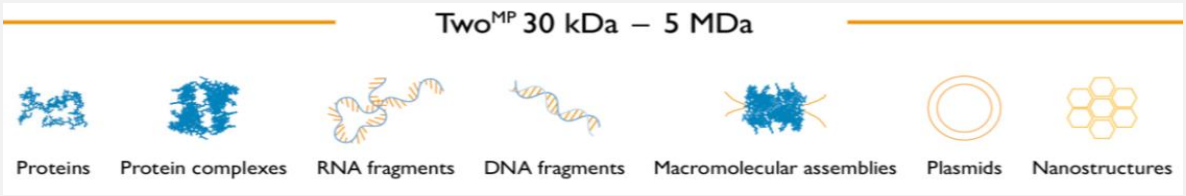


Mass photometry measurements provide the mass distribution and relative concentrations of a wide range of biomolecules in a sample. The method is suitable for sample **purity** and **homogeneity**, **protein oligomerization**, **biomolecular interactions** and **macromolecular complex assembly** determination.

MassFluidix HC is an add-on for Refeyn's TwoMP mass photometer and includes a central unit with fluid controls and a rapid dilution chip.
MassFluidix HC, significantly expands the range of sample concentrations amenable to investigation by mass photometry, by raising the upper sample concentration limit from the nanomolar to the micromolar range. This enables applications such as the **characterization of low-affinity interactions.**

MP Method can be used for:

- Proteins / Glycoproteins
- Nucleic Acids
- Adeno-associated viruses (AAV)
- Antibodies
- Virus-like particles (VLPs)
- Membrane proteins



CEITEC – Central European Institute of Technology
Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
www.ceitec.eu

Technical specifications

Instrument: TwoMP, MassFluidix HC (Refeyn)

TwoMP:

- Measurement at RT only
- Mass range: 30 kDa – 5 MDa
- Resolution (FWHM):
  ±25 kDa for 66 kDa particle,
  ±60 kDa for 660 kDa particle
- Mass error: ±5%
- Concentration range: 100pM – 100nM
- Wavelength: 488 nm

MassFluidix HC:

- Measurement at RT only
- Mass range: 50 kDa – 5 MDa
- Concentration range 100nM - 50µM
  Measurement of low-affinity and transient interactions
- Rapid dilution 37 ms

CEITEC – Central European Institute of Technology
Masaryk University
Kamenice 753/5, 625 00 Brno, Czech Republic
www.ceitec.eu

Sample requirements

- Samples of **protein origin** (or protein complexes with other biomolecules) are measured directly on special glass (MassGlass) and evaluated based on provided calibrant (MFP1 or MFP1 complemented with BSA).

- Measurement of **other biomolecules** needs the special glass coating and measurement set up.

- Measurement is suitable in broad range of commonly used **buffers**. High (above 500mM) **ionic strength** is inconvenient, **glycerol** concentration shouldn´t exceed 5%, **sucrose** concentration shouldn´t exceed 0.1%. **Detergent** presence can be very problematic and need to be validated at intended measurement. Buffer needs to be **free of particle impurities**; the best approach is to filter the buffer through 10 kDa concentrator membrane.

- Recommended initial sample concentration for TwoMP measurement is **20nM.** For final droplet dilution, use 2 – 10 ul of 50 – 200 µM sample solution. Be aware that for **TwoMP**, samples are diluted manually, and in the case of interactions, only **high-affinity** complexes can be detected. For mid or low-affinity interactions, measurement on MassFluidix chip should follow.

- The samples used in **MassFluidix** must have at **least 20 µL volume**. Sample concentration must be in range **100 nM - 50 µM**.

SOP

- To ensure the most accurate results, Refeyn Ltd. recommends **turning the mass photometer on** and starting AcquireMP **1 hour before.**

- Be aware that Mass Photometry measurements are temperature sensitive. Use and keep **buffers and samples at RT**. **Calibration** needs to be done at least ones per measurement or every 2 hours. **Never use old calibration!**

- Objective needs to be carefully **cleaned by 100% isopropanol** before instrument leaving.

Established methodologies and provided services

Mass photometry delivers a rapid, accurate mass measurement of label-free single molecules in solution in their native state, enabling:

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

Instrument operation

Operational mode:

- TwoMP is operated independently by users after the obligatory initial training
- MassFluidix HC measurements are performed only by CF staff

Provided services:

- Initial training MP
- Data evaluation user training
- Mass photometry - MassFluidix HC
- Consulting/assistance

Data evaluation SW:

- DiscoverMP software is available on the TwoMP (2.23) and Evaluation (2.21) computers

It is recommended to discuss the project and the details of the experiment (sample preparation, sample requirements) with the Core Facility members in advance.

Contacts

Biomolecular Interactions and Crystallography Core Facility

bic@ceitec.cz

Core Facility Leader: JOSEF HOUSER

josef.houser@ceitec.cz

Main Responsible Person: MONIKA KUBÍČKOVÁ

monika.kubickova@ceitec.cz

Instrument Location:

CEITEC MU Campus Bohunice, pavilion C04/2.23 laboratory, Kamenice 5, 62500 Brno