Nanoparticle Tracking Analysis (NTA) – ZetaView TWIN

In a nutshell: Track the Brownian motion of individual particles to measure their size, concentration, and zeta potential. Analyze the properties of exosomes, microvesicles, viruses, and nanoparticles.

Services: Biophysics Facility offers ZetaView as an open-access instrument. First-time users must complete a short training session before using it for the first time. Training includes instrument calibration and size analysis of a standard sample.

Location: NTA is located in Building 50, room 3122.

Description:

Nanoparticle Tracking Analysis (NTA) is a method of estimating the particle size-distribution by observing the Brownian motion of particles in solution using an ultramicroscope with laser illumination. By tracking hundreds of particles, NTA can measure both their size and particle concentration. The rate of Brownian motion is described by the Stokes-Einstein equation ($D=k_BT/3\pi\eta d$) and depends on the particle size, temperature, and the viscosity of the buffer. Importantly, this rate does not depend on the particle density or their refractive index. The refractive index of the particles will, however, influence how easy it is to detect them. For example, metallic particles have a high refractive index and scatter light more effectively. This allows NTA to detect gold particles as small as 15 nm in diameter. Liposomes, with their refractive index close to water, are more difficult to detect. Typically, NTA is not able to track liposomes with the diameter smaller than 50-60 nm, unless they are fluorescently labeled.

To provide accurate size-distribution and concentration information, NTA has to analyze a large sample volume tracking as many particles as possible. The ZetaView instrument achieves this by using a sliding optics that quickly moves between 11 focal positions to sample over 30 nL of the solution volume and to track over 1,000 individual particles per minute.

NTA can track unlabeled particles in the scatter mode. It is also possible to use the fluorescence mode and two laser wavelengths to specifically detect particle populations labeled with two types of fluorescent probes. Additionally, ZetaView sample cell is equipped with electrodes to generate particle drift in an electrical field. This allows the instrument to measure the zeta potential (ζ) of particles in solution.

NTA can be compared to the dynamic light scattering (DLS), which also estimates particle size by measuring their diffusion properties. However, DLS analyzes scattered light fluctuations to measure the rate of diffusion of an ensemble of molecules. In contrast, NTA obtains this information by tracking the Brownian motion of individual particles. Consequently, DLS provides

z-averaged molecular size and intensity-weighted size distributions. Size distributions obtained from NTA are number distributions.

Typical applications:

- Unlabeled and fluorescently labeled bio-nanoparticles
- Liposomes, micelles, extracellular vesicles
- Protein aggregates
- Viruses and virus-like particles
- Nanometals, quantum dots

Basic instrument specifications:

- Illumination lasers:
    - Green: 488 nm
    - Red: 660 nm

  Either wavelength can be used in the scatter mode and both excitation wavelengths can be combined in a fluorescence mode experiment
- Cell volume: 0.5 mL (2 mL recommended for loading, 1.5 mL minimum)
- Number of measuring focal positions: 11
- Volume sampled in a measurement: 3 nL per focal position, 33 nL total
- Optics field of view: 340 x 450 µm
- Tracking rate: Over 1000 particles per minute
- Minimum detectable bio-particle size:
    - EVs: typically 30-40 nm (the broader the particle distribution, the closer this limit is to 40 nm)
    - Liposomes: 50-60 nm
- Maximum measurable particle size: 2 µm

Sample requirements and recommended buffers:

NTA is compatible with a wide range of buffers. Please prepare a sufficient volume (10-50 mL) of a well-filtered buffer for sample dilutions and for rinsing the NTA cell.

The recommended sample loading volume is 2 mL. The NTA samples are highly diluted. The typical stock dilution factor is 500-1,000x, and the final concentration is approximately 10^7 particles per milliliter. Samples must always be diluted with a freshly filtered buffer.

Minimum sample amount:

1 mL of a solution at a concentration of approximately 10^7 particles per mL. A slightly larger sample volume is recommended for a convenient loading.

Consumables:

Disposable syringes for loading (1 mL or 2 mL) and a standard solution for instrument calibration are required. Standard is provided by the core, please supply your own syringes.