

# Achieving high sensitivity at different scattering angles with different optical configurations



PARTICLE SIZE

## Introduction

The sensitivity and versatility of a dynamic light scattering system depends heavily on the optical configuration of the system. Laser power, measurement angle, detection volume, and attenuation all affect the quality of the signal and subsequently the sensitivity of the instrument.

The Zetasizer range of instruments uses two different optical setups to achieve their sensitivity. They use either the classical measurement angle of  $90^\circ$  or the backscatter angle of  $173^\circ$  (figure 1).

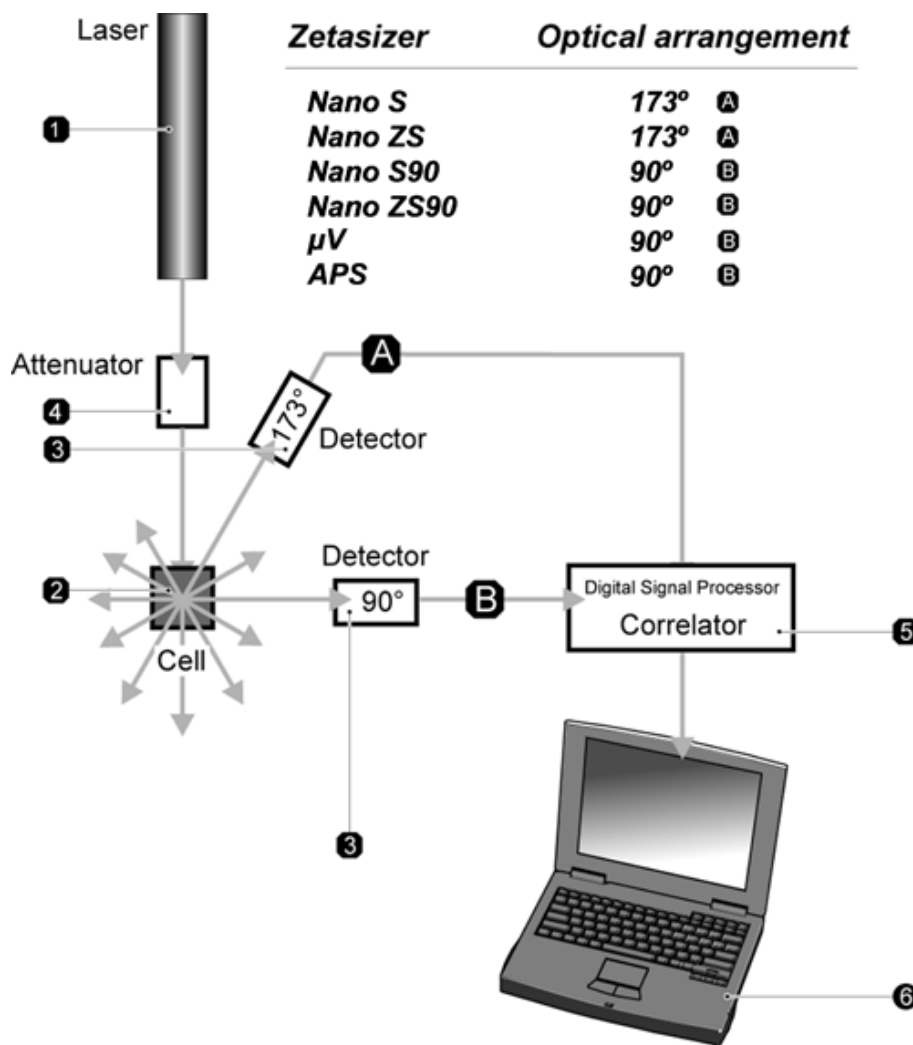


Figure 1: Optical configurations used in the Zetasizer range.

This technical note describes the different optical configurations in use in the Zetasizer range and how each optimizes sensitivity for small and dilute samples.

## Zetasizer Nano

The patented non-invasive backscatter (NIBS) system is used in the Zetasizer Nano ZSP, ZS and S. This measures the light scattered by the sample at a backscatter angle of 173°. This setup affords the instrument a number of advantages.

The first of these is that the volume of sample illuminated by the laser, which is subsequently measured by the detector, is greatly increased. The increase in measurement volume is approximately 8 fold compared with a 90° instrument, with a corresponding increase in sensitivity (figure 2). The use of fibre detection optics ensures that the signal quality is maintained. Simply increasing the measurement volume using classical optics will simply reduce the signal quality in proportion to the increase in count rate, and nothing would be gained.

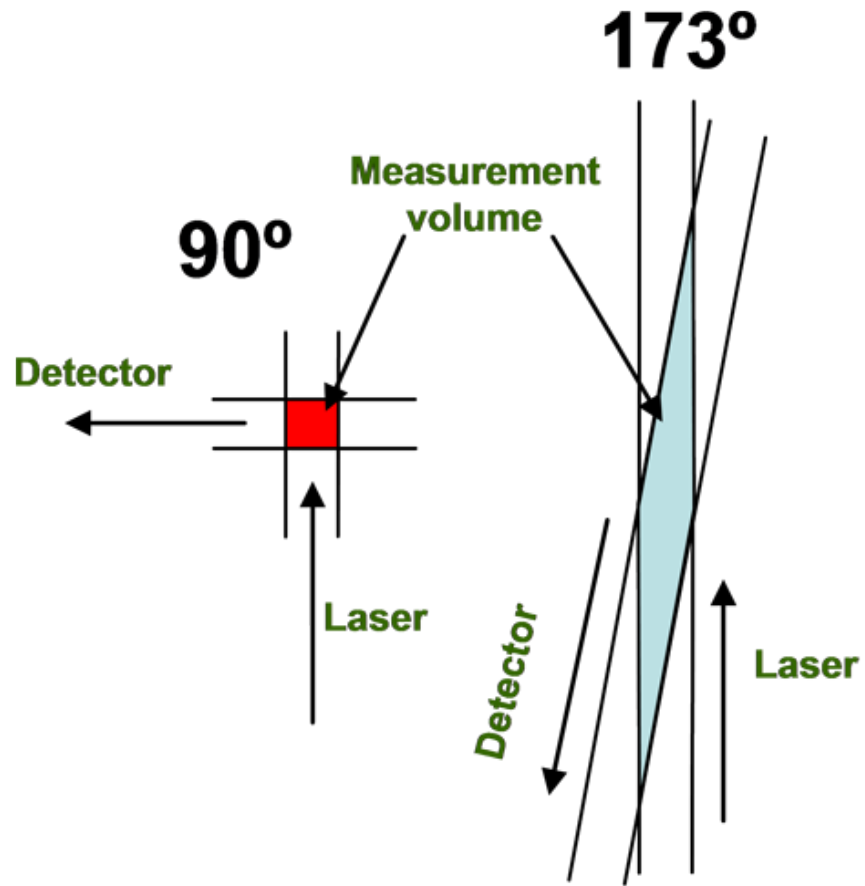
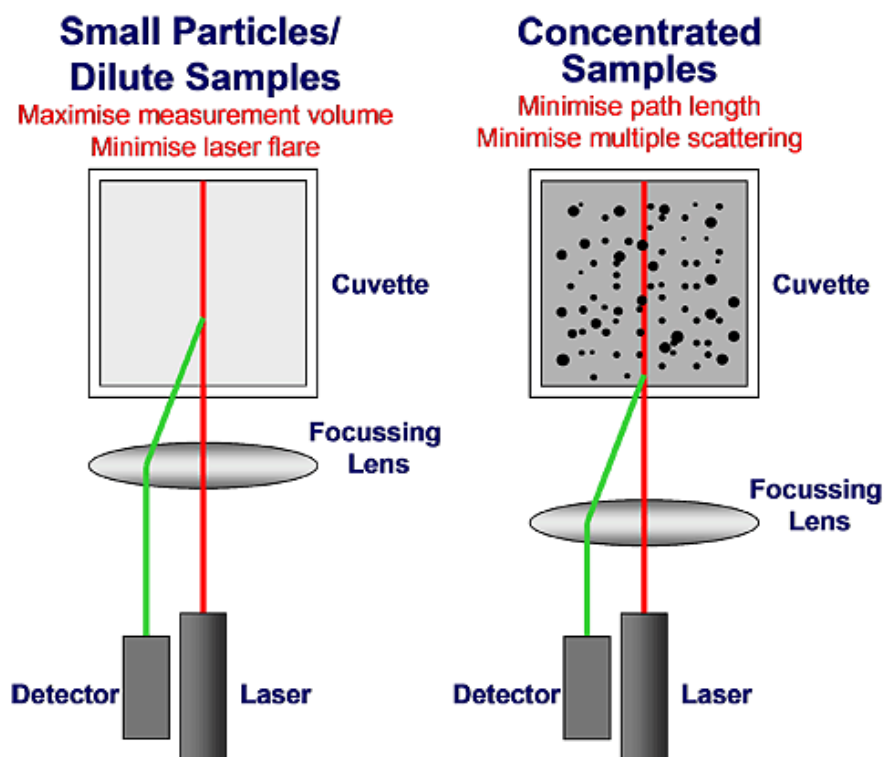


Figure 2: Increased measurement volume at 173° degrees compared to 90°.

Secondly, larger particles primarily scatter light at forward angles. Therefore, backscatter detection will be less sensitive to the presence of large particles. This means that scattered light from smaller and more weakly scattering samples will not be masked.

Thirdly, by moving a focusing lens between the laser and the sample, it is possible to move the measurement position within the cell. Moving to the center of the cell allows the maximum volume to be measured. By measuring far from the cell wall, the effect of laser flare is minimized to increase the signal to noise ratio and improve sensitivity, particularly to weakly scattering and dilute samples.

For concentrated samples, where signal strength is high, multiple scattering becomes significant and this has the effect of reducing the apparent size of the sample. To minimize multiple scattering the measurement position can be moved close to the cell wall. This reduces the distance that the light must travel through the sample within the cell (figure 3).



**Figure 3: Measuring dilute and concentrated samples using the NIBS system**

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Multiple scattering is thus minimized so accurate measurements can be made of large and concentrated samples.

The multiple benefits of this system mean that it has a very high sensitivity to dilute and weakly scattering samples while also reducing multiple scattering from large and very concentrated samples. This makes the NIBS system the most versatile DLS system for a range of samples. However, the large probe volume means that the smallest sample volume is 12  $\mu\text{l}$ .

## Zetasizer $\mu\text{V}$ and APS

The Zetasizer  $\mu\text{V}$  and APS use a  $90^\circ$  measurement angle. They are, however, able to achieve the same sensitivity as the backscatter systems.

The Zetasizer  $\mu\text{V}$  and APS have 60 mW diode lasers. The amount of light scattered is proportional to the intensity of the incident light so by using a laser that is 10 times more powerful than the Zetasizer Nano, sensitivity can be increased similarly. This also overcomes the fact that using a longer wavelength of 830 nm (compared to 633 nm in the Zetasizer Nano) will also slightly reduce intensity of the scattered light. The fibre detection optics also ensures that the signal quality is optimized.

In a  $90^\circ$  system, the measurement volume is small compared to the NIBS systems. To increase the amount of light scattered, the laser is focused into a small measurement

volume. This further increases the amount of incident light reaching the sample to increase the scattered light correspondingly (figure 4).

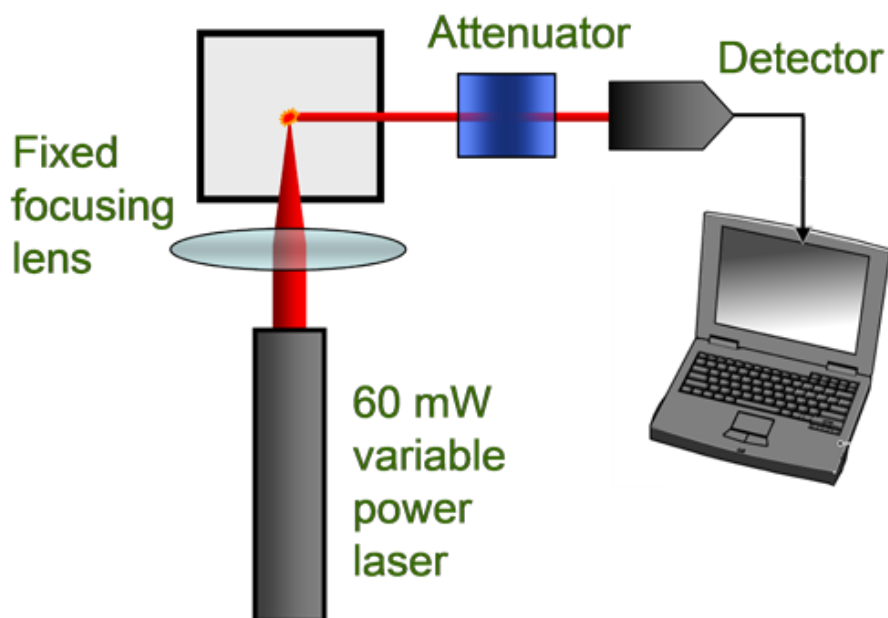


Figure 4: Optical setup in the Zetasizer  $\mu$ V and APS

Furthermore, using the scattering angle of  $90^\circ$  reduces laser flare to a minimum so that there is no interference caused by this effect.

Dual-attenuation, by varying the laser power and the attenuator allows the signal intensity to be carefully modulated to optimize sensitivity.

This combination of high powered laser focused on the small measurement volume has the advantage that it enables this sensitivity to be achieved using a small sample volume, in the case of the Zetasizer  $\mu$ V, as low as  $2 \mu\text{l}$ .

The fixed optics of the  $\mu$ V and APS mean that high-concentration samples, which are turbid, will be affected by multiple scattering, giving an apparent smaller size. This is an unimportant effect for small materials such as proteins that can be measured at high concentrations. The largest measurable size is also limited to approximately  $1 \mu\text{m}$  in radius due to the small detection volume.

## Conclusion

Two methods are used to enhance instrument sensitivity in the Zetasizer range. Using the NIBS system, the Zetasizer S and ZS increase sensitivity and can also measure large and concentrated samples. Alternatively, the  $\mu$ V and APS use a high-powered  $90^\circ$  system allowing sensitive measurements of small sample volumes. Table 1 summarizes the specifications of the instruments.

Table 1: Technical specifications of the Zetasizer S, APS and  $\mu$ V

Parameter	Specification		
	Zetasizer S	Zetasizer APS	Zetasizer $\mu$ V
Size range (diameter)	0.3 nm - 10 $\mu$ m*	0.3 nm - 2 $\mu$ m*	0.3 nm - 2 $\mu$ m*
Concentration range	0.1 mg/mL 15kDa protein - 40% w/v	0.1 mg/mL 15kDa protein - optically clear samples	
Minimum Sample volume	12 $\mu$ L	20 $\mu$ L in 96-well plate	2 $\mu$ L
Laser	4 mW He-Ne Laser (633 nm)	60 mW diode laser (830 nm)	
Measurement angle	173°	90°	
Attenuation	Discrete attenuation	Dual continuous attenuation	

\*sample dependent



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