

# Monitoring of Microbial Growth Curves by Laser Nephelometry

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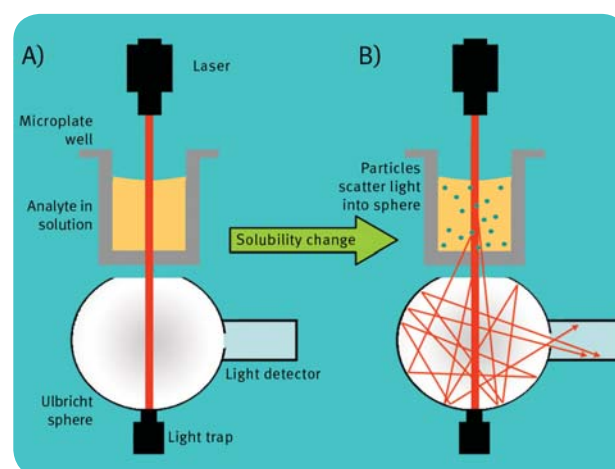
## Introduction

Different analytical approaches in clinical immunology and drug discovery take advantage of two closely related techniques based on light scattering: Turbidimetry is the measurement of light transmitted through a suspension of particles. It requires relatively higher concentrations of particles and obeys Beer's Law. In contrast, nephelometry is a direct method of measuring light scattered by particles suspended in solution at right angles to the beam, or preferably, at a forward angle. In dilute solutions, where absorption and reflection are minimal, the intensity of the scattered light is a function of the concentration of scattering particles. The most common application of laser-based nephelometry in microplate format is the fully automated solubility screen in HTS laboratories. Determining aqueous compound solubility has become an essential early measurement in the drug discovery process to avoid time-consuming and costly ADME screens of low solubility compounds. In clinical chemistry, nephelometry is used to determine e.g. serum immunoglobulin. Protein precipitation of globular proteins refers to the formation of protein aggregates by adding salt, organic solvent, organic polymer or trichloro-acetic acid. In contrast, immunoprecipitation allows for a given protein to be precipitated selectively via an antibody-antigen reaction. In organic chemistry, nephelometry is used to quantify macro-molecules, e.g. monitoring of a polymerisation reaction. Here we describe the monitoring of microbial growth curves with BMG LABTECH's microplate reader NEPHELOstar.

## Materials and Methods

As an alternative to the common cuvette based instruments BMG LABTECH offers the first laser-based microplate nephelometer, the NEPHELOstar, which detects particulate matter within microplate wells via forward light scattering (optical design is described in figure 1).

The light source of the NEPHELOstar is a red laser diode (633 nm) featuring adjustable intensity and beam diameter to reduce meniscus effects and optimized sensitivity allowing to measure even in 384-well plate format. Instrument flexibility is further enhanced by three built-in reagent injectors, precise temperature control, multi-mode shaking capabilities, automatic gain adjustment, and a robotic plate carrier.



**Fig. 1:** Schematic diagram of the measurement principle of the NEPHELOstar: A clear solution with minimal scattering results in low signal (A). A solution with particles scatters light and results in higher signal (B).

A fast and sensitive method of analyzing growth phase regulation of the *Corynebacterium glutamicum* wild type and different deletion mutants by continuous monitoring of microbial growth on a medium supplemented with different sulphur sources is described below.

*Corynebacterium glutamicum* is a Gram-positive, non pathogenic and fast growing soil bacterium which is used for industrial amino acid and vitamin production. Cells were precultured on modified minimal medium E (MMES) supplemented with different suitable sulphur sources at 30°C in a rotary shaker (300 rpm), and the optical densities of these cultures were measured at 600 nm.

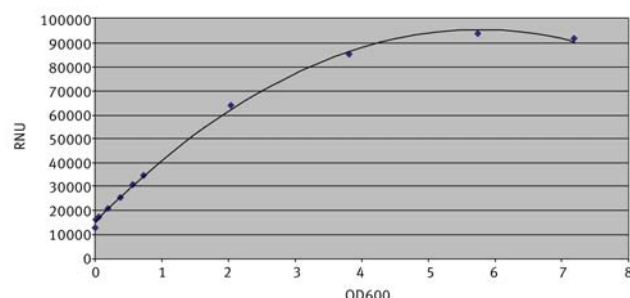
These precultures were used to inoculate 100 µL of growth media (MMES + 0.1 mM sulphur source, start OD of 0.01) using the microplate Cellstar, 96 Well Suspension Culture Plate (GreinerBioOne, Cat. No.655185).

The plates were sealed with transparent, hydrophobic and gas permeable plastic films (Breathe-Easy, Roth, Cat. No. T093) during incubation and measurement to avoid evaporation and condensation.

The growth curves were recorded by monitoring the turbidity of the cultures using the NEPHELOstar. The laser intensity was adjusted to 50% and the laser beam focus to 2.5 mm. Incubation temperature was 30°C and the plate was orbitally shaken with a shake width of 3 mm between measurement cycles.

## Results and Discussion

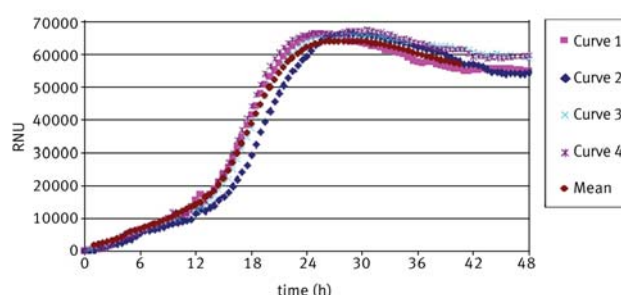
In earlier experiments the comparability of the data obtained by optical density methods was assured using a transmission reader with data obtained by measuring the scattering of light using a nephelometer. A serial dilution of the *C. glutamicum* culture was made and turbidity determined by measuring both the optical density and the forward scattered light. Fig. 2 shows a good correlation between the two methods, which is almost linear up to 4 OD.



**Fig. 2:** Correlation of the OD values (absorbance at 600 nm, FLUOstar Galaxy, BMG LABTECH) with the nephelometric approach (NEPHELOstar, BMG LABTECH), using a serial dilution of the *C. glutamicum* culture

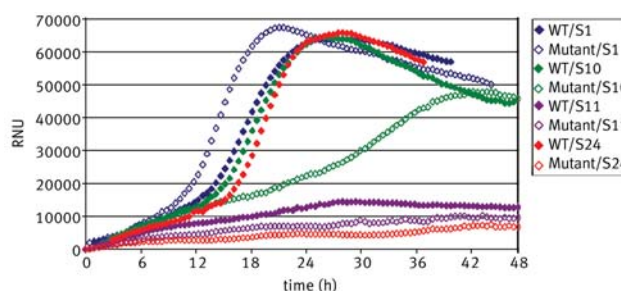
The great reproducibility of the nephelometric assay is demonstrated in figure 3, which shows four independent growth curves of a *C. glutamicum* culture. In this graph six replicates were used for each curve plotted against time and including the mean of these four growth curves.

Finally, figure 4 demonstrates a concrete biological application of nephelometry in bacterial growth



**Fig. 3:** Four independent growth curves of a *C. glutamicum* culture monitored with the NEPHELOstar

regulation. It shows the growth curves of the *C. glutamicum* wild type and a deletion mutant, which has a deleted gene for a regulatory protein. Both strains are cultured on minimal medium supplemented with different sulphur sources (S1, S10, S11, and S24).



**Fig. 4:** Growth curves of the *C. glutamicum* wild type and a deletion mutant cultured on minimal medium supplemented with different sulphur sources

The plotted growth curves show that the deletion mutant, compared to the wild type, grows better on S1 but worse on S10 and no more on S24.

## Conclusion

The described application reveals that laser nephelometry is a reliable technique for monitoring microbial growth besides the classical applications like compound solubility testing and immunoprecipitation.

These studies show that the nephelometric assay, compared to the turbidimetric assay, is not only comparable, but clearly superior regarding sensitivity.

The key advantage of nephelometry is the ability to detect scattered light, even if the concentration of scattering particles is very low, which is the case during the lag phase and beginning of the log phase.

Using the NEPHELOstar, instead of a traditional transmission reader, this early part of the growth curve can be monitored much more accurately.

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