# Technical Guide

In-Process Particle Characterization of Suspensions and Emulsions

## **Advancing Beyond Turbidity:** Selecting Appropriate Technology for Monitoring and Control of Complex Particle and Droplet Systems

**This guide seeks to explain the options available for real-time in-process monitoring and control of suspensions and emulsions, and provides direction for selecting the right technology for the work being performed.**

From the production of high value chemicals and pharmaceuticals to large scale wastewater treatment, the handling and processing of suspensions and emulsions is of major industrial importance. In general, multiphase systems tend to be complex with many parameters, including overall process efficiency and economic profitability, being directly affected by the ability to consistently deal with the production, separation, and/or removal of solid particles and liquid droplets in suspension.

The ability to monitor and even measure particles and droplets – in process and in real time – helps to ensure that the process proceeds according to plan. Disturbances can be minimized and sometimes eliminated, and product quality and yield can be optimized through the use of *in situ* particle system measurements such as turbidity, Focused Beam Reflectance Measurement (FBRM®), and in-process imaging. Choosing the right in-process measurement should take into consideration the complexity of the particle or droplet system being monitored and the increased value that can be added to the process by each available measurement.

METTLER TOLEDO

## General Overview of In-Process Particle Monitoring

There are many different technologies for offline and at-line characterization of suspensions and emulsions, each providing the ability to characterize various physical properties of the particles or droplets in suspension. An investigation into these various techniques is outside the scope of this guide, which is focused on simple-to-use real-time monitoring technologies – primarily considering the complementary applications of turbidity, FBRM®, and in-process imaging.

*In situ* measurement, at full process concentration, provides an immediate measurement of the particles and droplets as they actually exist within the current process or in their natural environment. In many cases, this provides specific advantages including:

- Avoiding errors due to sampling and dilution for offline analysis
- Avoiding physical changes in the material due to the effects of physical removal from the process and subsequent transport, storage, sample preparation, and flow through the offline measurement instrument
- Continuous, real-time monitoring to track process dynamics with higher resolution for more accurate modeling and control
- The impact of disturbances and intentional process changes can be directly monitored for a clearer understanding of process interactions

It should also be noted: other techniques, such as ultrasonic attenuation and various spectroscopic methods, have potential applications for monitoring slurries in real time, providing information related to particle size and other properties. At this time however, such instruments tend to require considerable method development and modeling to develop usable algorithms. As a result, these methods are not typically considered easy-to-use and were considered outside the scope of this guide.

The fact remains that there are three widely used technologies that are relatively easy to install and use for in-process monitoring and control from laboratory to industrial scale. These include turbidity, FBRM®, and in-process imaging – which respectively represent increasing complexity of information provided in the measured data. As a general rule of thumb, turbidity is a trending tool for simple particle systems in which the bulk particle shape and size distribution remain constant or follow a highly predictable trend. FBRM® (see Appendix A – Focused Beam Reflectance Measurement for a detailed description), on the other hand, provides the ability to measure changes in complex

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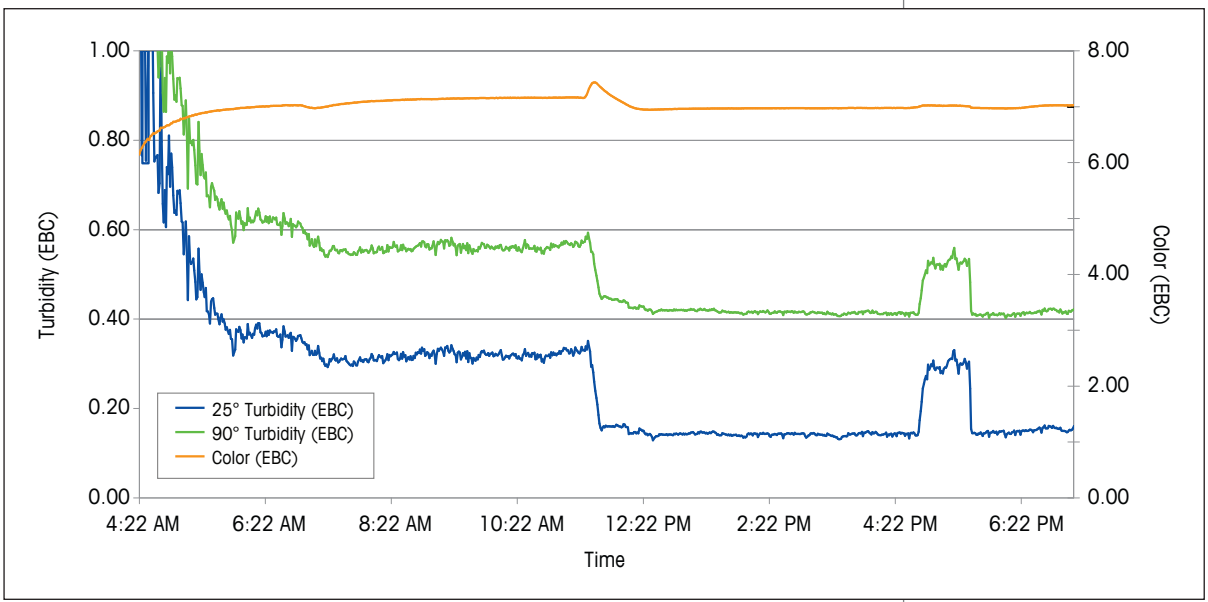



**Figure 1.** Turbidity and color are monitored at the outlet of an industrial beer filter using the METTLER TOLEDO InPro 8600.

particle systems with multi-dimensional measurement of particle number, dimension and shape. In-process imaging is well suited for qualitative understanding of particle and droplet systems in their natural environment.

Turbidity is defined as the cloudiness or haziness of a fluid caused by particles or droplets (Appendix B – Turbidity Overview provides additional information on turbidity and nephelometry measurements). Turbidity measurement can be effectively used for contaminant detection and monitoring changes in solids concentration when changes in the particle size or shape are of minimal importance. Figure 1 plots the turbidity of the outlet from a beer filter, during different filter operation steps. Disturbances, such as filter breakthrough of yeast, can be immediately detected with online turbidity measurement to ensure process integrity and product quality. Turbidity is also effectively used to monitor growth of cell mass in bioreactors when the individual cells remain consistent and uniform in size and shape.

Focused Beam Reflectance Measurement (FBRM®) is designed for complex particle systems such as crystallization[1,2], flocculation[3,4], and aggregation[5] where changes in particle numbers, size and shape may be occurring simultaneously. The multi-dimensional FBRM® measurement permits relatively easy interpretation of complex particle systems by sensitive measurement of both the number and dimension of particles or droplets in the system under investigation.[6] FBRM® is applied inline to measure and optimize the particle distribution and can be related directly to process efficiency parameters – such as batch endpoint or filtration rates – or product quality parameters – such as bulk density or flowability or bioavailability. For example, in a crystallization system, increasing solids concentration results in complex changes in the particle system in the form of both an increase in the number of particles present (nucleation) and increases in the dimensions of existing crystals (growth). Agglomeration, breakage, and changes in crystal shape are other factors influencing the particulate product that can be simultaneously monitored with FBRM®.

In-process imaging is another technique used for characterization of slurries and emulsions. In-process imaging provides unparalleled qualitative understanding of what the particles and droplets look like in the vessel or pipeline. From that standpoint, in-process imaging is actually the most information-rich of the three technologies. However, the FBRM® data is statistically more robust and more easily applied to monitoring and control. For applications including the before mentioned cell mass growth and crystallization see figures 2 and 3, which highlight the information and understanding provided by images captured inline at full process concentration using *in situ* PVM® (Particle Video Microscopy) technology.

Offline image analysis, where the particle sample is diluted, prepared, and presented in a controlled optimized manner, has proven itself as a reliable and repeatable method

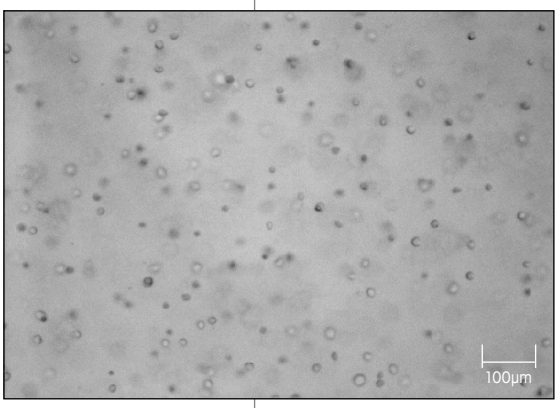



**Figure 2.** Increase in cell mass, with consistent cell size distribution and no aggregation, can be effectively monitored with turbidity (METTLER TOLEDO in-process PVM® image of mammalian cells shown)

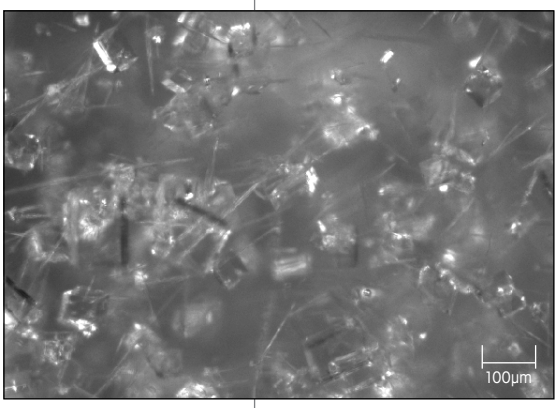
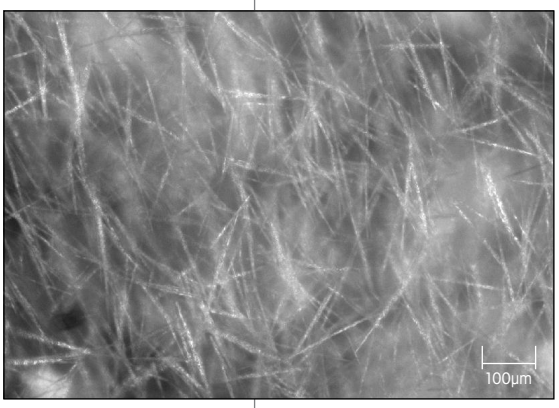



**Figure 3.** Crystallization often involves simultaneous changes in particle count, dimension and shape. PVM® *in situ* images are shown before and after a phase transformation of the same chemical compound.

3a. Anhydrous form of carbamazepine (CBZ)




3b. Hydrated form of carbamazepine


[1] Nguyen, T.N.P. et al. Kinetic study on hemipenta hydrate risedronate monosodium in batch crystallization by cooling mode

[2] Mousaw, P. et al. Crystallization Improvements of a Diastereomeric Kinetic Resolution through Understanding of Secondary Nucleation

[3] Thapa, K.B. et al. Interaction Of Polyelectrolyte With Digested Sewage Sludge

[4] Wu M.R. et al. Flocculation and Reflocculation: Interplay Between the Adsorption Behavior of the Components of a Dual Flocculant

[5] Kim, K.J. et al. Agglomeration of NTO on the surface of HMX particles in water-NMP solvent

[6] Deneu, E. et al. An In-Line Study of Oiling Out and Crystallization, Organic Process Research & Development

of measuring particle size and shape distribution information. However, capturing quantitative data from inline image analysis is difficult at an elevated concentration where overlapping particles obstruct measurements. Often its greatest value is in the excellent qualitative understanding that it provides its user – detecting and identifying information such as changes in particle shape, agglomeration, or phase changes that improve the user's ability to make informed decisions leading to more effective process optimization.

### When is Turbidity Not Enough?

For many industrial processes, turbidity is an easy-to-implement measurement that provides immediate ability to track changes in the particle system. However, it should be recognized that turbidity is a bulk measurement, tracking the overall condition or progress in a given process stream. This is adequate for disturbance detection or process monitoring of a well-characterized, stable and relatively straightforward process.

For more complex particle systems, such as crystallization, flocculation or fermentation processes where particle size, shape and concentration are changing simultaneously, there can be significant advantages to using more advanced in-process technology to fully understand and control how the particle system is evolving over time. The following discussion helps to explain how turbidity and backscattering are affected by changes in common variables – concentration, particle size, particle shape and surface properties.

### Impact of Concentration on Turbidity and Scattering

With particle size, particle shape and all other factors constant, an increase in solids concentration will increase the turbidity of the slurry. At low concentrations (approximately less than 1000ppm), the relationship between attenuation and concentration tends to be linear for both transmission and backscattering (Figure 4). This is in direct relationship to the projected area of the particles within the measurement zone.

At higher concentrations, the relationship becomes nonlinear as particles begin to "overlap". As the concentration continues to increase, the absorbance measurement saturates at the point where essentially all of the light is blocked by the slurry and the transmission reaches zero. Note that the concentration at which nonlinear behavior and saturation are reached is not fixed, and depends on factors such as the size of the particles, the width of the measurement cell (or pipeline) and the refractive index of the particles and the carrying fluid.

In terms of the backscattering properties of the particle system, the quantity of backscattered light will also increase linearly over low concentrations, but the backscattering signal can provide a linear response up to much higher concentrations before the signal becomes saturated.

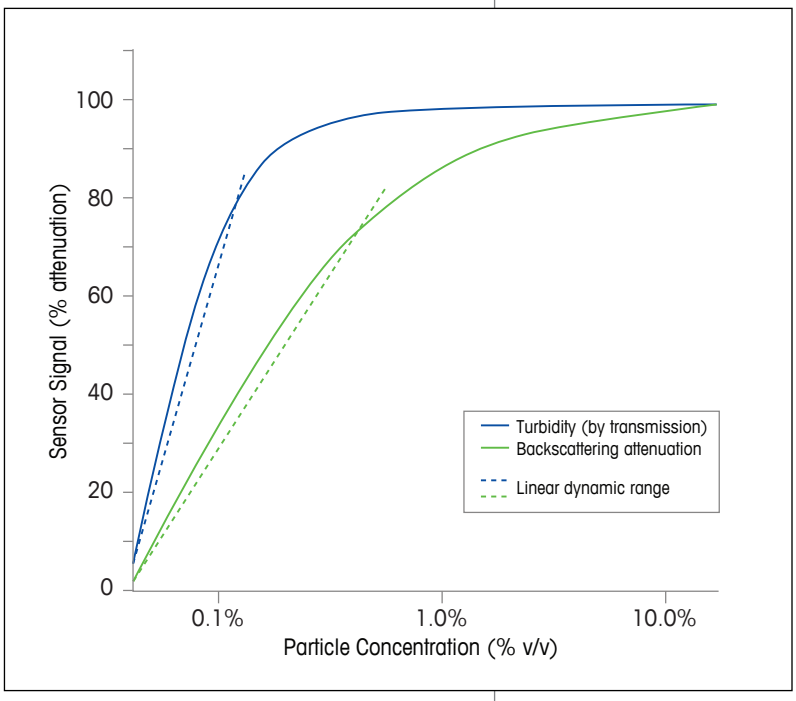



**Figure 4.** Relative measurements of turbidity by transmission and backscattering signals. (For ideal 1 micron spherical particles measured across a 1mm gap.)

## Impact of Particle Size on Turbidity and Scattering

Changes in particle size can convolute turbidity data. A larger particle size – at the same concentration of suspended solids – will actually result in a lower reading of

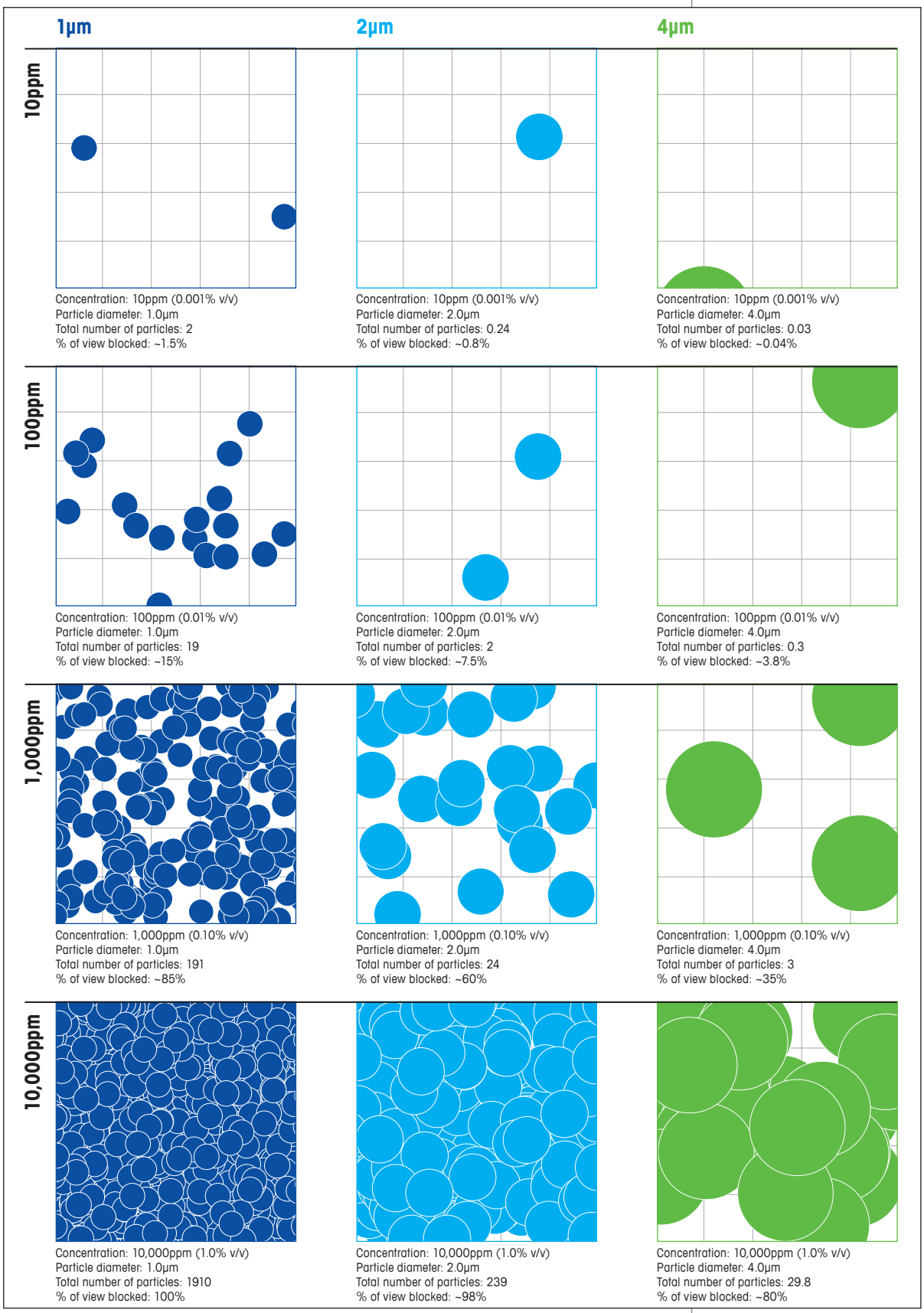



**Figure 5.** Diagrams illustrating particle systems at varying concentration and particle size

turbidity due to a lower projected surface area per unit volume. Figure 5 illustrates the effect of changes in concentration and particle diameter for ideal spherical particles in a measurement zone that is 10 microns by 10 microns across and 1mm deep. As discussed previously, turbidity measured by transmission saturates at relatively low concentrations (approximately 1000ppm) and is typically useful for detection of the presence of particles due to filter breakthrough or cloud point determination. It rarely can be extended to monitor significant changes in concentration.

An increase in concentration (with constant particle size, as shown in Figure 4) results in an increase in turbidity or backscatter. An increase in particle size at constant concentration (Figure 5) actually results in a decrease in turbidity or backscatter. Therefore, in systems where size and concentration are changing simultaneously – such as in crystallization, flocculation, or aggregation processes – interpretation of the results can be convoluted. For example, when particle size is unknown or changing at the same time as concentration – accurately relating turbidity or total backscatter to concentration becomes extremely difficult. This situation is commonly observed in crystallization processes where wide bimodal distributions occur due to nucleation and growth occurring at the same time.

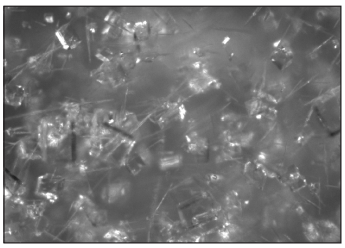
## Impact of Particle Shape on Turbidity and Scattering

Changes in particle shape, such as those due to shifts in crystal habit or flocculation and aggregation, can produce significant changes in turbidity as well.

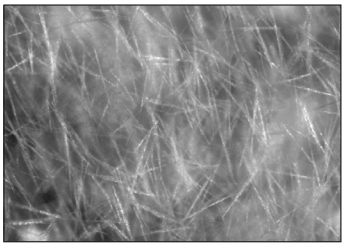
As with the impact of particle size, changes in particle shape can result in turbidity data that is difficult to interpret without qualifying information. Earlier, Figure 3 presented two possible crystal forms of the same chemical compound (carbamazepine), illustrating the impact of crystal shape on the backscatter from the particles.[7] Needle-shaped crystals have a higher surface area to volume compared to cubic or roughly spherical crystals. Typically, the result is that different crystalline forms of the same material will have very different backscattering properties, making turbidity an unreliable measurement of concentration.

Likewise, flocculation – as illustrated by the series of *in situ* PVM® images in Figure 6 – results in a very significant change in turbidity with increasing levels of flocculation. In general, a flocculated or aggregated slurry will backscatter less than a well dispersed sample of the same concentration. However, depending on the floc density – or fractal dimension – the surface area per unit mass can vary significantly as the compactness of the flocs varies as well. Therefore, as the size and compactness of the flocs vary at the same time, turbidity data can be very difficult to deconvolute – even when solids concentration is constant.

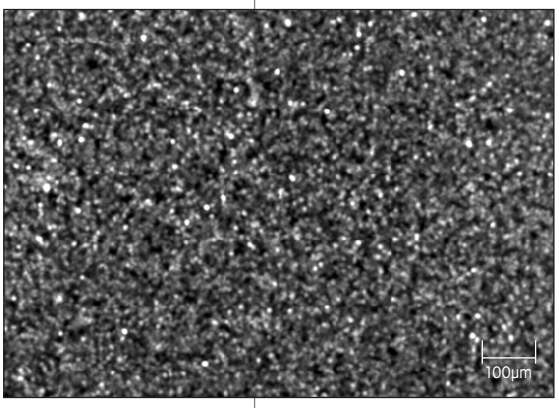
**Figure 3.** Also see page 3



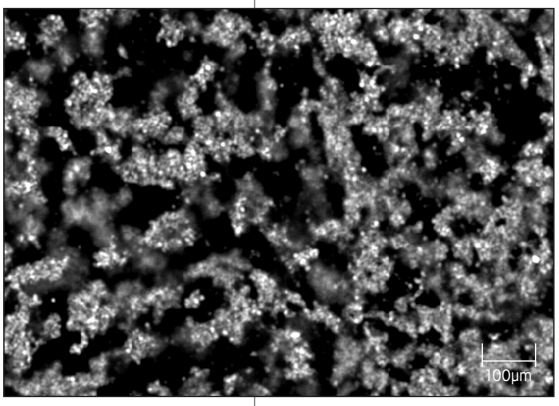
3a. Anhydrous form of carbamazepine (CBZ)



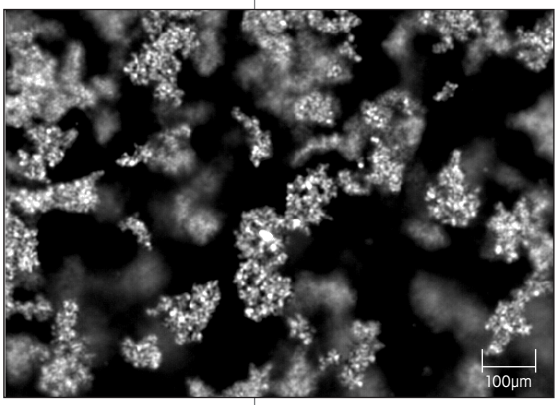
3b. Hydrated form of carbamazepine




6a. 0 min




6b. 90 min




6c. 180 min

**Figure 6.** Inline PVM® images showing flocculation of a fine powder after elapsed time

[7] Liu, W. et al. An Investigation of the Transformation of Carbamazepine from Anhydrate to Hydrate Using *In Situ* FBRM® and PVM®

## Focused Beam Reflectance Measurement (FBRM®) for In-Depth Analysis

While turbidity and nephelometry are limited in the information they provide in complex systems, FBRM® provides multi-dimensional information for deeper understanding of complex particle and droplet systems. The basic measurement of FBRM® (Appendix A) provides a measurement that is sensitive to changes in particle shape, size, and concentration. The ability to independently track changes in different size ranges of the particle population – such as the "fine" (small particle) range or "coarse" (large particle) range – makes it possible to differentiate between increases in concentration, dimension, and even shape. FBRM® is a probe-based, inline technology that is implemented in laboratory or manufacturing environments with no sampling or sample preparation necessary.

The fundamental measurement of FBRM® is the Chord Length Distribution (CLD) – a measurement of the count and dimensions of particles and particle structures as they actually exist in the process environment at full concentration. The CLD provides a direct fingerprinting of the particle system (Figure 7) that can be used to quantitatively measure changes in particle count and particle dimension.

For complex particle systems – where particle dimension is changing at the same time as particle concentration – FBRM® is able to differentiate between these competing physical properties to better understand the particle system. This can be as simple as identifying and optimizing size regions which can be used to track different parts of the particulate system independently. Figure 8 illustrates this concept with the simple selection of two chord length ranges to track both the capture of fine particles and the formation of larger floc particles[1]. In addition, basic statistics such as average dimension (in

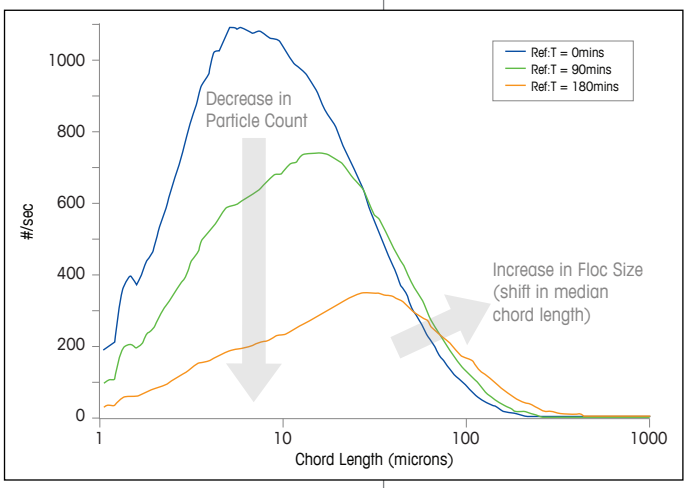



**Figure 7.** Chord Length Distributions showing change in number and dimension (FBRM® data shown relates to PVM® images from Figure 6).

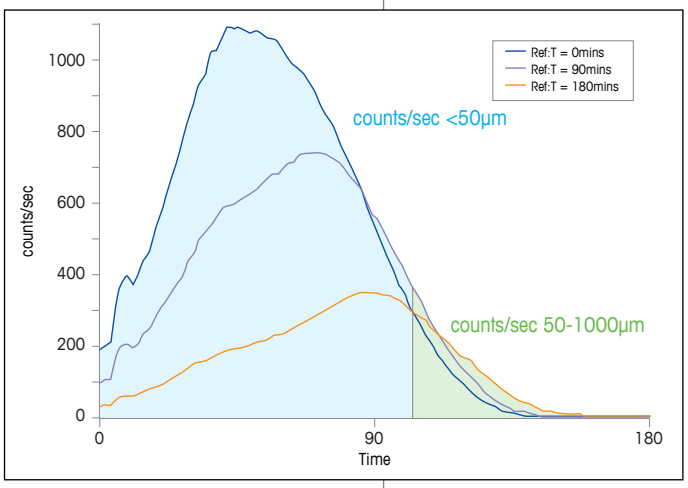
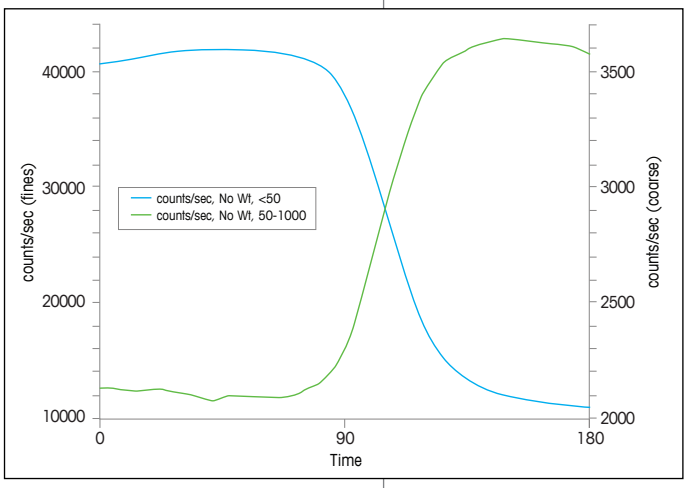
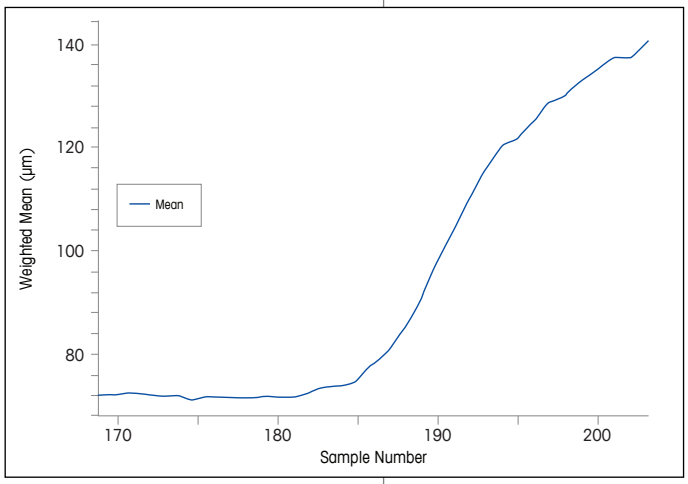



**Figure 8.** Selecting appropriate chord length ranges allow simultaneous measurement of multiple particle properties:

8a. Chord Length Distributions showing selection of isolated ranges




8b. Trend lines showing decrease in numbers of small particles and increase in larger flocs




8c. Weighted mean tracks growth of flocculated particles

this case a weighted mean), can provide additional measures of critical changes in the particle system, or the direct effects of changes in operating conditions that cannot be provided with basic turbidity or backscattering measurements.

### In-Depth Analysis Provides Added Value

Side by side comparisons of turbidity and FBRM® have further illustrated the limitations of turbidity in particle systems with phase changes which can drastically change the backscattering properties of the suspended material.[8] For example, in the crystallization of organic compounds, it is not uncommon for a phase separation resulting in a dispersion of droplets within another liquid phase. Turbidity will often detect this phase separation – or oiling out – as a nucleation event which effectively saturates the turbidity measurement (Figure 9). The end result is that when the crystals do begin to form, the turbidity may not reflect it – or may actually drop only momentarily as the droplet phase disappears and the crystal phase appears simultaneously. FBRM® – by monitoring multiple parameters simultaneously – can detect both the oiling out and crystallization phenomena.

The ability to differentiate between changes in particle number and particle dimension provides a fundamentally better understanding of the particle system in question. The information-rich FBRM® measurement provides the ability to fingerprint the process and monitor it in real time – enabling a higher degree of process understanding and optimization to assure batch to batch repeatability or process consistency.

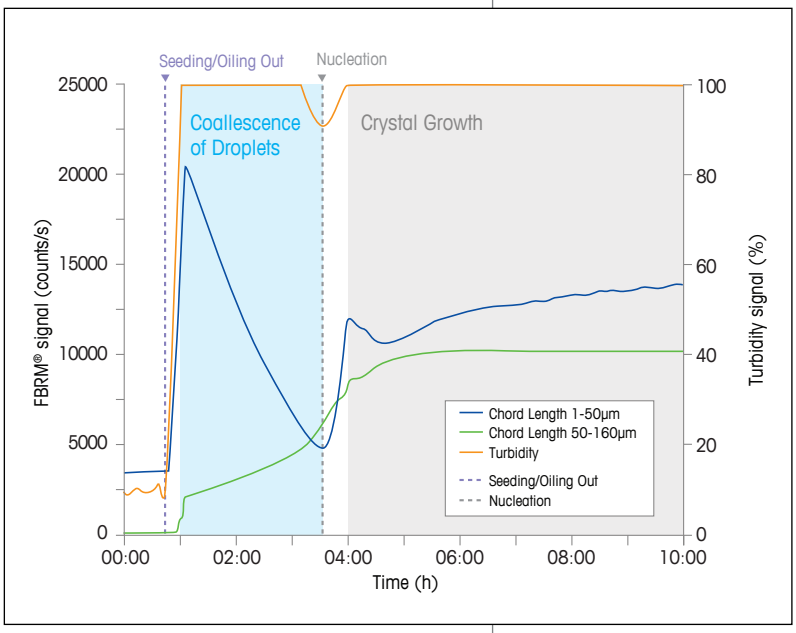



**Figure 9.** Turbidity and FBRM® compared for a crystallization process in which a phase separation (oiling out) occurs before crystallization begins[8]

[8] Lafferrère, et al., *In Situ* Monitoring of the Impact of Liquid-Liquid Phase Separation on Drug Crystallization by Seeding

## Conclusions and Recommendations

An understanding of complex particle and droplet processes requires detailed knowledge of how the particle distribution changes over time – which often includes simultaneous changes in concentration, particle dimension, and shape. As a result, complex particle systems – such as processes involving crystallization, flocculation and aggregation – require an information rich tool to understand, characterize, and control.

FBRM® provides a robust fingerprint of complex particle systems that can be used for real-time monitoring of changes in particle dimension, changes in particle count, and changes in particle shape.

In-process measurement of the particle system provides information that cannot be achieved through offline sampling and analysis. Real-time monitoring of dynamic changes in the particle system enables faster screening of operating conditions for optimized process design, and provides the fundamental knowledge required for scale-up and operation of a robust, controllable process.

FBRM® and PVM® provide the immediate ability to optimize and control the particle distribution in a process to ensure batch or process consistency and maximize process efficiency. By understanding the particle performance, engineers can link upstream process control parameters (such as addition rates, flow and mixing rates, temperature) to downstream process efficiency (such as filtration/separations efficiency, product dissolution rate) and optimized product quality (such as yield, purity, and bulk density).

Selecting the appropriate technology for monitoring complex particle and droplet systems can be a difficult decision. METTLER TOLEDO Technology and Application Consultants can provide full support of your efforts to characterize and optimize your crystallizations, suspensions and emulsions.

FBRM® provides immediate insight into the particle system enabling chemists and engineers to quickly screen experimental parameters and develop a scalable process avoiding major redesigns later in process development

FBRM® and PVM® provide the ability to immediately optimize and control the particle distribution in a manufacturing process to ensure batch consistency and cycle time. By understanding the particle performance, engineers link upstream process control parameters (addition rate, mixing, or temperature) to downstream process efficiency (filtration/separations efficiency, dissolution rate) and optimized product quality (yield, bulk density):

- Batch to batch consistency
- Eliminate batch failures
- Achieve targeted product specifications
- Improve cycle time
- Optimize filtration rates
- Enhance product stability
- Scale up to production providing the desired particle size distribution, yield, and purity

# Appendix A:
## Focused Beam Reflectance Measurement (FBRM®)

**Measurement for optimization in real time – FBRM® is a highly precise and sensitive technology which tracks changes to particle dimension, particle shape, and particle count. Over a wide detection range from .5 to 3000µm, measurements are acquired in real time while particles are forming and can still be modified enabling process optimization and control. No sampling or sample preparation is required – even in highly concentrated (70% and higher) and opaque suspensions.**

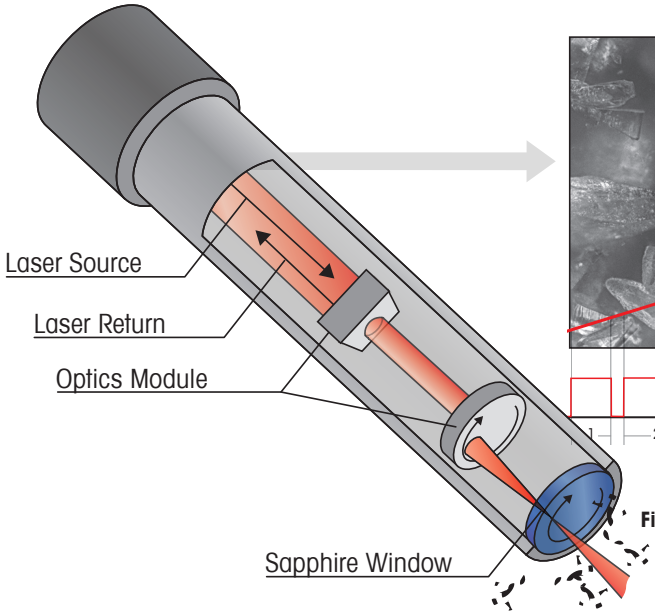



**Figure 10.**

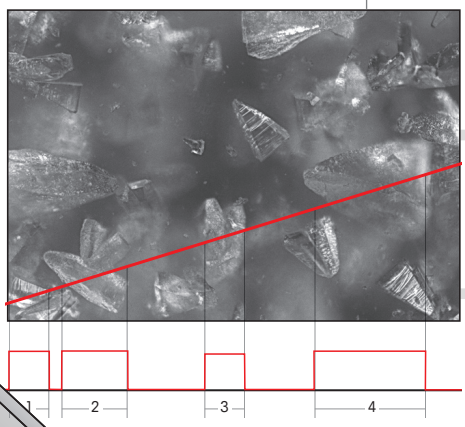



**Figure 11.**

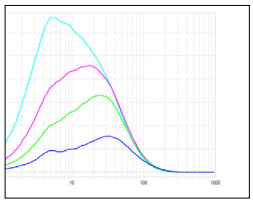


**Figure 12.** Chord Length Distributions

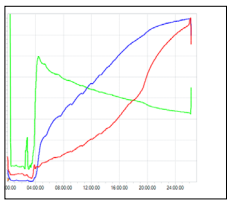


**Figure 13.** Trended Statistics

### How does FBRM® work?

The FBRM® probe is immersed into a dilute or concentrated flowing slurry, droplet emulsion, or fluidized particle system. A laser is focused to a fine spot at the sapphire window interface (Figure 10). A magnified view shows individual particle structures will backscatter the laser light back to the probe (Figure 11). These pulses of backscattered light are detected by the probe and translated into Chord Lengths based on the simple calculation of the scan speed (velocity) multiplied by the pulse width (time). A chord length (a fundamental measurement of particle dimension) is simply defined as the straight line distance from one edge of a particle or particle structure to another edge. Thousands of individual chord lengths are typically measured each second to produce the Chord Length Distribution (CLD) (Figure 12). The CLD is a "fingerprint" of the particle system, and provides statistics to detect and monitor changes in particle dimension and particle count in real time (Figure 13).

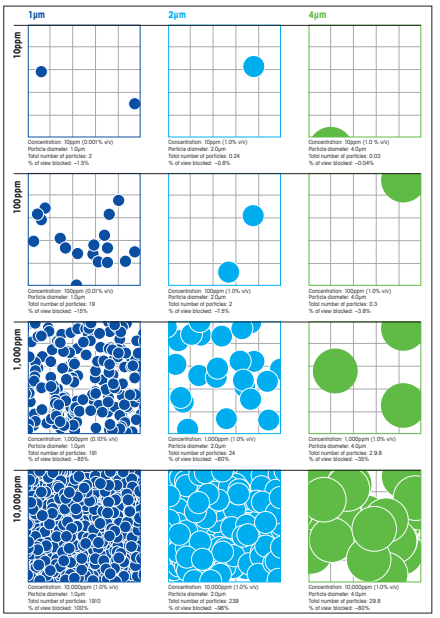
Unlike other particle analysis techniques, with FBRM® measurement there is no assumption of particle shape. This allows the fundamental measurement to be used to directly track changes in the particle dimension, shape, and count.

## Importance of a Fixed Focal Spot

In Focused Beam Reflectance Measurement of concentrated suspensions, the user should be aware that having a fixed focal spot at the probe window has been shown to provide optimum results.[9]

Technologies that use a varying focal depth will have inconsistent and often unpredictable results due to the inability of light to penetrate even small distances (< 1mm) at relatively low concentrations (1000ppm) as shown in Figure 5. The result of an oscillating focal position is a measurement zone that shows extreme variation with concentration (Figure 14). Particles that are close to the probe window effectively interfere with portions of the measurement where the focal position is located away from the window. In extreme cases, this can actually cause a reduction in particle count with increasing concentration. At higher concentrations, the loss of resolution will be even further magnified.

**Figure 5.** See page 5



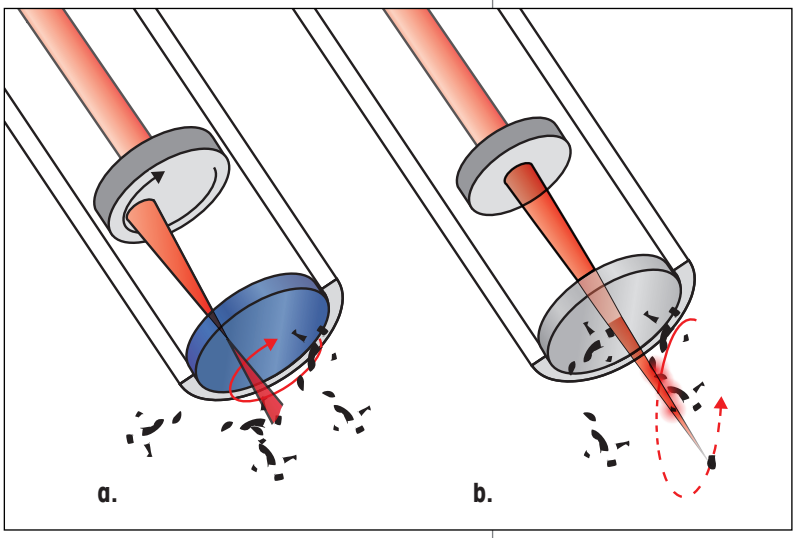




**Figure 14. a.** Ideal FBRM® measurements are made with a focal position that scans at the window surface to minimize the affect of concentration on the measurement

**b.** A variable or oscillating focal position loses measurement sensitivity as concentration increases, the same way as transmittance turbidity saturates at relatively low concentration

[9] Ruf, A., Worlitschek, J. and Mazzoti, M., Modeling and Experimental Analysis of PSD Measurements through FBRM®

# Appendix B:
## Turbidity Overview

**Turbidity measurements provide data on the concentration of undissolved, suspended particles present in a liquid phase. The determined particle concentration is used for process monitoring and optimization e.g. to control biomass growth, crystallization and filtration processes or concentration measurement of solid matters in wastewater.**

Turbidity measurement is used to refer to a number of measurements that are designed to measure the presence and/or concentration of suspended solids in a fluid. This can include low-level detection of contaminants to high concentration monitoring of various industrial process streams. However it should be understood that the measurements used in these situations can employ very different technology, and it is important to select the right turbidity measurement to provide the sensitivity and accuracy needed for a given process stream.

Turbidity is defined as the cloudiness or haziness of a fluid caused by suspended particles or droplets. As light passes through a fluid sample containing a quantity of suspended particles, a fraction of the light will be attenuated. A portion of the light may be absorbed by the fluid itself – which would be related to the fluids optical density (OD), while additional light may be absorbed and/or scattered by the suspended particles.

Across a fixed distance, as the concentration of suspended particles increases, the quantity of attenuated light will also increase. In many practical situations, the total attenuation is a direct function of concentration according to the Beer-Lambert Law, which allows the use of light transmittance as a measurement of solids concentration at relatively low concentrations. Figure 15 provides an example of an instrument designed for low level detection of solids concentration and detection of process disturbances such as filter breakthrough.

At higher concentrations (a rule of thumb is concentration of suspended solids greater than 1000 ppm), the amount of

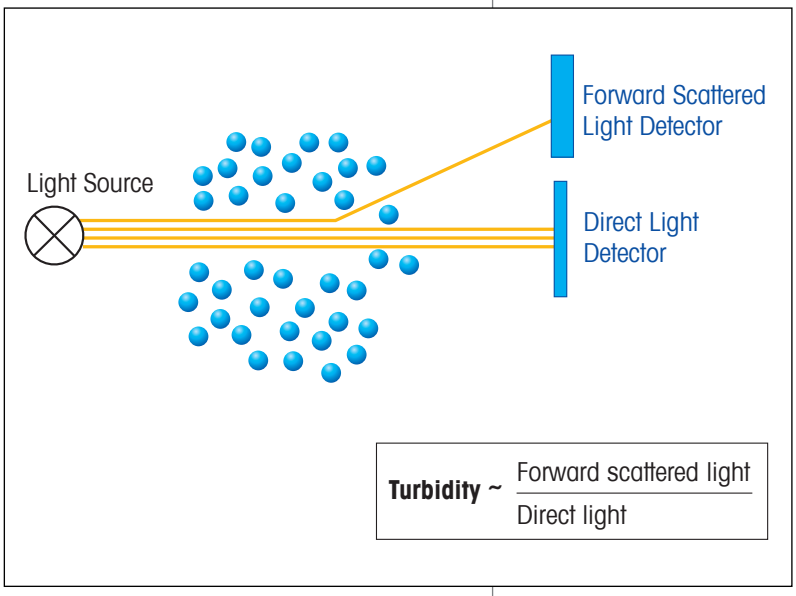



Figure 15.

15a. Schematic of turbidity measurement based on forward scattering for monitoring at low solids concentration

16b. Forward scattering turbidity cell for low concentration solid particulates (METTLER TOLEDO InPro 8400)

transmitted light becomes insufficient for sensitive detection of concentration changes, and it becomes more effective to use backscattering or nephelometry as a measurement of the solids concentration (Figure 16). The quantity of backscattered light continues to increase with increasing concentration – well beyond the limits of transmission based turbidity or optical density measurements, permitting calibration that will report particle concentrations well beyond the limits of traditional turbidity. Figure 17 provides an example of a backscatter sensor that is used in applications such as monitoring the progress of cell growth in a bioreactor.

Sensors based on turbidity and backscattering technologies continue to advance, with many probes now offering sensors to detect multi-angle scattering, and using multiple light sources (white light, red, green, blue, near infrared, etc) to provide increased sensitivity to changes in both the fluid and the particulate properties. (Figure 18).

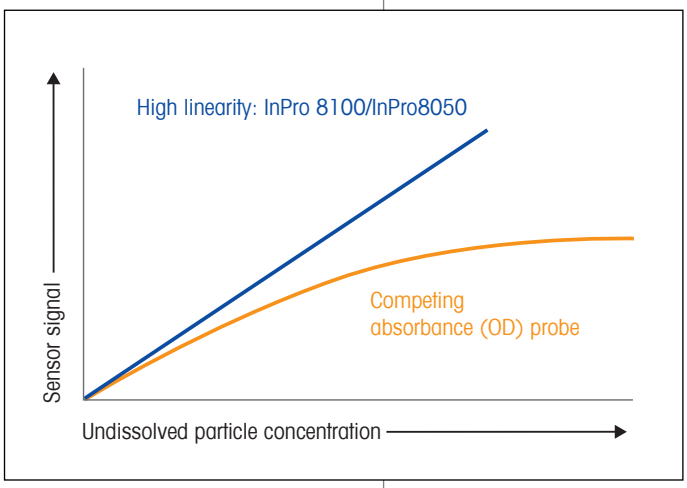



**Figure 17.** Backscattering technology provides a larger dynamic measurement range than transmission/attenuation measurements.

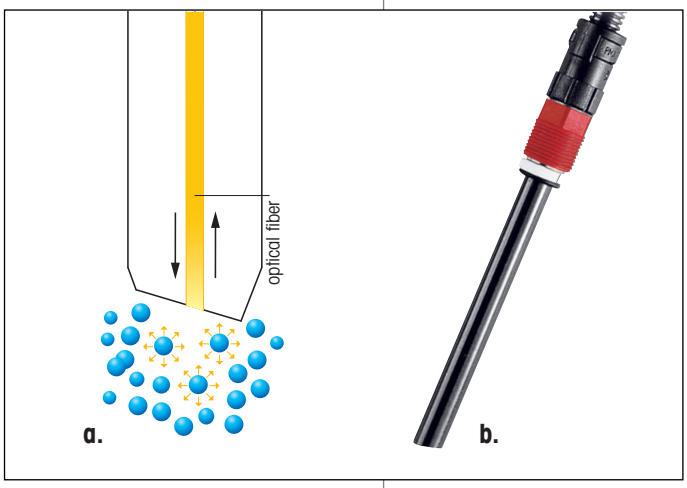



**Figure 18. a.** Schematic of measurement principle for detection of backscattering (nephelometry). **b.** Backscattering turbidity probe for direct installation into a concentrated suspension (METTLER TOLEDO InPro 8050)

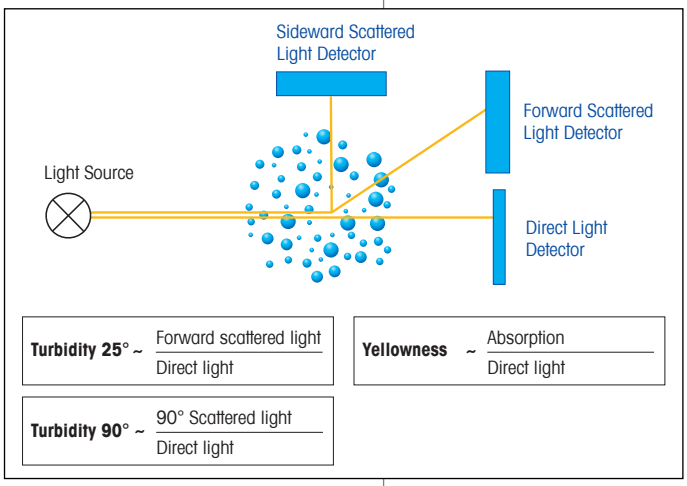
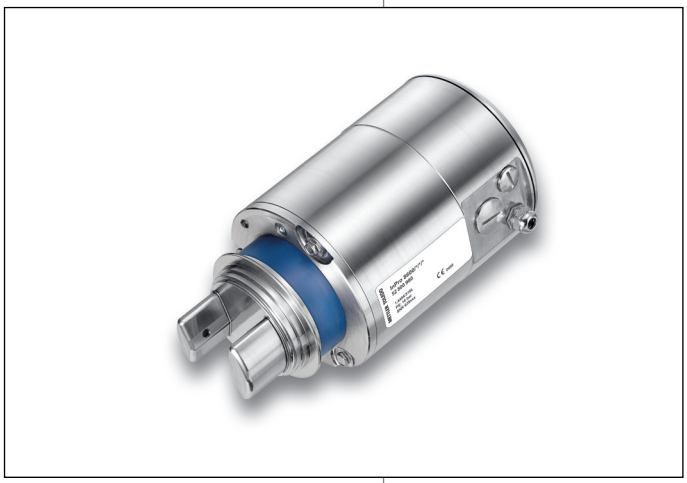



**Figure 19.**

19a. Schematic of color detection and turbidity detection at multiple scattering angles



19b. An example of a sensor using both turbidity and scattering detection. (METTLER TOLEDO Inpro 8600)

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