

# Cleaning Methods AccuSizer® FX Nano

## *Contamination of FX Nano sensors and recommended cleaning methods*

Many FX Nano sensors have been manufactured over the past several years, and there have been essentially no failures of hardware components (laser module and electronic circuit board) under normal operating conditions. In short, the reliability of these hardware components has been exceptional. The same can be said of the electronic counter modules and associated power supplies there have been negligible failures in all of the systems produced in this time period.

The only service/repair needs that have arisen to date have involved the optical flow cells – specifically, involving the problem of excessive "background," or "baseline," light scattering. This is indicated by an abnormally high voltage level in the high gain (HG) scattering mode, and corresponding abnormally high voltage level in the low gain (LG) scattering mode of operation. (Note: These two voltages differ by a constant factor, typically in the vicinity of 5, depending on various sensor details. It is only useful, therefore, to refer to the background scattering voltage level of the HG mode, because the LG voltage level will simply be a fixed fraction of the latter for any given sensor.)

**An abnormally high voltage level that is encountered in the HG (and LG) mode is almost always caused by one problem:** adherence, or adsorbance, of particles onto one or both of the inner glass surfaces of the flow channel in the precision optical flow cell. When the intense focused laser beam impinges on those adsorbed particles, light is scattered, often strongly, in all directions. A portion of that spurious scattered light reaches the detector, just as it would if the particles causing the scattering were passing freely through the illuminated region defined by the focused laser beam, resulting in desired signal pulses suitable for particle size analysis. Typically these surface adsorbed particles belong to a recent sample after inadequate flushing of the fluidics system. This "coating" of particles causes an increase in the background light scattering level, as indicated by an increase in the HG (and LG) background voltage. In extreme cases, especially in HG mode, the increase in scattering will be strong enough to cause "saturation" of the amplifier output, resulting in a fixed, maximum displayed voltage of 10.14 V. In this case signal pulses, due to particles flowing through the optical sensing zone, will no longer be detected or recorded for analysis.

In normal operation, with the absence of surface adsorbed particles, the normal (low) background scattering level in the HG (and LG) mode, originates from two sources. First, when the incident focused laser light beam passes through the optical flow cell, there is a certain amount of reflection and diffuse scattering of that beam at the outer surfaces of the glass cell (i.e.; the points at which the beam enters and exits the cell). The optical elements of the FX Nano sensor are designed and aligned in such a way that almost all of the diffusively scattered light that originates from those entry and exit points is excluded from reaching the light scattering detector. This alignment is optimized carefully during the final alignment of each sensor, prior to calibration. The outer surfaces of the optical glass flow cell are polished, so that the incident laser beam that impinges on the leading surface of the flow cell is reflected at the air glass interface in the backward direction. The refractive index of glass is approximately 1.5. Therefore, roughly 4% of the incident light will be reflected by the outer glass surface of the flow cell. However, that surface is not perfectly polished, diffusing a relatively small amount of scattered light in all directions. Nevertheless, only a very small fraction of this unwanted background light reaches the detector because the optical system is designed to "spatially filter" this spurious component of scattered light, so that very little of it actually reaches the detector. The same is true for the similar diffusely scattered light that originates from the back surface of the flow cell, where the focused laser beam exits the cell.

There is a second contribution to the background light scattering level in the HG (and LG) mode. That is the low level of scattered light that is produced at the two water glass interfaces of the flow channel, where the sample liquid or flushing/cleaning liquid is in contact with the inner glass surfaces of the flow cell. Unlike the situation attributed to the outer surfaces of the flow cell, all of the scattered light originating from within the flow channel (i.e.; from detectable particles flowing through the focused laser beam or from the glass surfaces defining the latter) will reach the detector. However, in contrast to the situation that obtains at the two outer surfaces of the flow cell, the scattered light intensity originating from the water glass interfaces of the flow channel (i.e.; where the focused laser beam impinges on those two

surfaces) is much lower. First, given the refractive indices of 1.33 for water and 1.5 for glass, only about 0.4% of the incident light beam will be specularly reflected at the water glass interfaces in the flow channel (i.e.; only one-tenth the value for the outer air glass interfaces). Second, the inner glass surfaces are deliberately more highly polished than the outer glass surfaces. As a result, the intensity of the diffusely scattered light originating from the inner surfaces, which contributes to the background scattered light signal in the HG (and LG) mode, is exceptionally small owing to the closer match of the refractive indices of glass and water and to the high degree of polish of those inner surfaces.

Small differences in alignment of the various optical elements, incident laser beam, flow cell, and light scattering detector, as well as small differences in the quality of the polished surfaces of the optical flow cell, inevitably will give rise to differences in the background scattered light level in the HG (and LG) mode. HG voltage levels ranging from approximately 0.5 – 2 V will typically be experienced for a given FX Nano sensor after final alignment and subsequent calibration. The seemingly large range (factor of three) of the values cited above might seem to be a reason for concern. However, any HG voltage value falling within this range should be considered good.

The key point is that signal pulses that are detected due to the passage of individual particles through the optical sensing zone are processed using the well known method of "AC coupling," in which the constant "DC" background level effectively disappears. As long as this level is low enough (certainly true for 2 V), it essentially has no effect on the measured heights of scattered light signal pulses resulting from detectable particles passing through the active sensing zone. And therefore the HG (and LG) background value has essentially no effect on the measured pulse height distribution (PHD) and resulting particle size distribution (PSD).

As mentioned earlier in Section 2, the background scattered light voltage in the LG mode is typically about one-fifth (or 20%) of the corresponding voltage in the HG mode. Therefore, the typical range of scattered light voltage in the LG mode is approximately 0.1 – 0.4 V. As in the HG mode, the lower the voltage, the "cleaner" the flow cell and associated optical system. As before, even the highest value in this range is acceptably low, resulting in no effect on the measured pulse heights in the LG mode of operation.

Finally, it is useful to review briefly the role of the extinction voltage in the FX Nano sensor. After passing through the optical flow cell, the incident laser beam impinges on a light-extinction (LE) detector, completely separate from the detector that is used in the HG and LG light scattering modes of operation. The gain of the LE amplifier is adjusted to yield a LE voltage of approximately 9.3 V (i.e.; well short of the saturation value of 10.14 V) as for the HG and LG modes. In practice this value from one sensor to the next will range from about 9.1 – 9.5 V. It tracks the power of the laser beam and should be nearly constant over time, given the fact that the temperature of the laser module is held constant by a feedback regulator. The LE signal produced by this detector is used for focused light extinction (FX) analysis, in the occasional instance in which this mode of operation is used. Otherwise, in the more common case in which only the HG and LG light scattering modes are used, the LE detector remains useful as a monitor of laser "health" (i.e.; output power level). It will be observed that immediately after the laser is turned on, the LE voltage level will quickly decrease from a starting value of roughly 9.8 – 10 V to its final resting value of about 9.3 V. This behavior occurs because the laser diode requires a short time (30 – 45 seconds) to rise in temperature and reach equilibrium with its regulated exterior, thereby reducing the beam intensity by several percentages in the process.

Absorbance of sample particles or other contaminants on the inner surfaces of the flow cell will generally have a negligible influence on the LE voltage level, unlike the strong effect they can have on the HG (and LG) background voltage level, as discussed earlier. In contrast, significant turbidity of the sample suspension can be expected to reduce to some degree the LE voltage level. However, in practice one rarely encounters this effect given the fact that such turbidity would likely cause an unacceptably large increase, likely leading to saturation, of the HG (and LG) background voltage in any case high enough to cause significant distortion in the final PSD results and therefore such significant turbidity could not be tolerated for this reason alone, apart from the fact that it would result in a decrease in the LE voltage.

In summary, the expected, acceptable ranges of the HG, LG and LE background voltages are approximately the following:

|                           |             |
|---------------------------|-------------|
| <b>HG scattering mode</b> | 0.5 – 2.0 V |
| <b>LG scattering mode</b> | 0.1 – 0.4 V |
| <b>LE extinction mode</b> | 9.1 – 9.5 V |

In the event that the background scattering voltage in HG (and LG) mode rises significantly above its "normal" value (at the time of calibration, shown in "properties" in the calibration file), including the extreme case in which it reaches the amplifier "saturation" value of 10.14 V, a cleaning procedure must be employed in order to remove the particles that have become absorbed onto the inner glass surfaces of the flow cell. Again, this is the only source of the abnormally high background scattering voltage. To date, three different cleaning procedures have been found to be effective in accomplishing this task, as outlined below.

First, the easiest cleaning procedure consists simply of flowing water through the sensor for an extended period of time (i.e.; using the autodilution (AD) module operating in continuous flushing mode, drain valve (V11) on and flow pump on high speed, preferably). Water passing through the narrow flow channel at significant velocity (e.g.; 62 cm/sec at a flow rate of 15 mL/min and >100 cm/sec at high speed) will often be able to dislodge particles if they are only weakly adsorbed onto the inner surfaces of the flow channel.

Second, in cases where the adsorbed particles consist of polymers (e.g.; PSL standard particles) or biopolymers (e.g.; proteins or other macromolecules), acetone usually succeeds as a cleaning agent. It effectively dissolves the adsorbed polymer or biopolymer particles that are the source of strong background scattering. The recommended procedure is, to use a glass syringe

to manually push a quantity of alcohol (e.g.; ethanol, methanol or propanol) through the sensor cell first, and then to push a quantity of acetone through the cell. A back-and-forth push/pull action will often enhance the cleaning effectiveness of the acetone, because of the "scrubbing-bubble" action that results from pushing and pulling acetone and air through the flow channel. At the same time this action will reduce the total volume of acetone needed. This procedure should then be followed by alcohol again before water is finally pumped through the sensor for final flushing of the flow cell.

Third, in other cases where the adsorbed particles consist of inorganic materials, including various oxides, such as silica or alumina (e.g.; CMP slurries), the use of a high-pH surfactant based cleaning solution is recommended – specifically, Contrad 70. A quantity of this agent (prediluted 1:1 with filtered water) can be manually introduced into the sensor cell using a disposable syringe.

**⚠ WARNING: Prolonged exposure of the inner glass surfaces of the flow cell to this caustic solution may cause "micro-etching" of those surfaces, which will result in a permanently elevated background scattering voltage in HG (and LG) mode, requiring the flow cell to be replaced. Therefore, this cleaning agent should be left in the flow cell for only 1 minute. The cell must then be flushed with filtered water for several minutes in order to remove all traces of the cleaning agent.**

In many (but not all) cases this action will result in a cleaner cell, and in some cases bringing the background voltage down to a value below the original value at the time of calibration. If necessary, this procedure can be repeated for a second or third time if good progress (lower background voltage) is observed after the first attempt.

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