BioTrak Real-Time Viable Particle Counter Algorithm Performance Assessment

Application Note CC-135 (A4)

Why is an Algorithm Performance Assessment Necessary?

An out-of-tolerance (OOT) condition identified during the as-found calibration of test equipment indicates that the results obtained since its previous calibration were inaccurate. The impact that inaccuracy may have had on product quality needs to be assessed. To do this, the effect that the OOT condition had on the measurement needs to be understood. This can be simple to determine for equipment like balances and thermometers where any drift has a direct effect on the measurement. For example, if a thermometer is found to be reading 10% too high, the assessment would need to determine what the impact would be of having the actual temperature 10% lower than what was reported.

Unlike a balance or a thermometer, biofluorescent particle counters (BFPC), like the BioTrak™ Real-Time Viable Particle Counter, feed measurements from multiple sensors into an algorithm to obtain a result. If drift is observed in these sensors during calibration, the resulting impact cannot readily be determined like in the thermometer example. Therefore, an assessment based on the combined effect from all the sensors is needed to understand what impact any drift had on the performance of the algorithm, and ultimately, the detection of microorganisms.

Assessment Method Concept

As discussed above, the algorithm performance assessment is intended to provide objective information regarding the overall performance of the instrument when various sensors have undergone drift during the calibration interval. Here’s how it works on a conceptual level.

Let’s take as a simple example of a BFPC instrument that uses two optical measurements—light scatter intensity and fluorescence intensity. During as-found calibration, each of the two optical measurement systems is individually assessed to determine the degree of signal drift that occurred during the calibration interval. Both optical systems will have a resulting error but, the individual errors are not very useful for evaluating the overall functionality of the instrument—in other words these errors do not answer the question, “How does the observed sensor drift impact the detection of microorganisms?”.

To objectively evaluate the combined impact of both sensor errors on microorganism detection, optical data from real microorganisms must be used. As conceptually depicted in Figure 1, the optical characteristics of many test particles, both viable and non-viable, are recorded during training and validation of the algorithm. This data was collected with the sensors calibrated to essentially zero error. In this simple example, the algorithm is a 2-dimensional space that delineates the optical characteristics of microorganisms. If a particle has fluorescence intensity and light scatter intensity that falls within the algorithm space then it is counted as a microorganism.

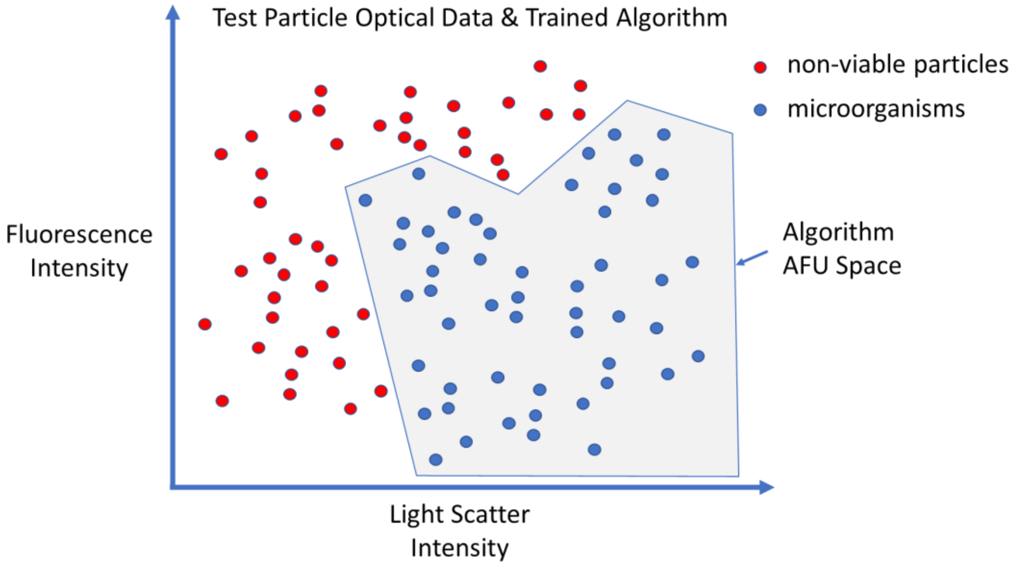

Figure 1 — Conceptual representation of test particle optical characteristics recorded during the algorithm training and validation of a hypothetical BFPC instrument. Viable (blue) and non-viable (red) test particles are indicated. In operation, particles with optical characteristics that fall within the auto-fluorescent unit (AFU) space are counted as viable particles, or AFUs.

When an optical sensor has drifted, the light intensities it records are different than what they would have been had the sensor not drifted. Let's examine the hypothetical case where both sensors (light scatter and fluorescence intensity) are reading 10% low. In this case, the apparent optical characteristics of all particles will have been shifted by 10% on both axes, resulting in altered algorithm performance. To evaluate the impact of this sensor drift, the optical characteristic of the test particles can be similarly shifted and the effect on algorithm performance quantitated.

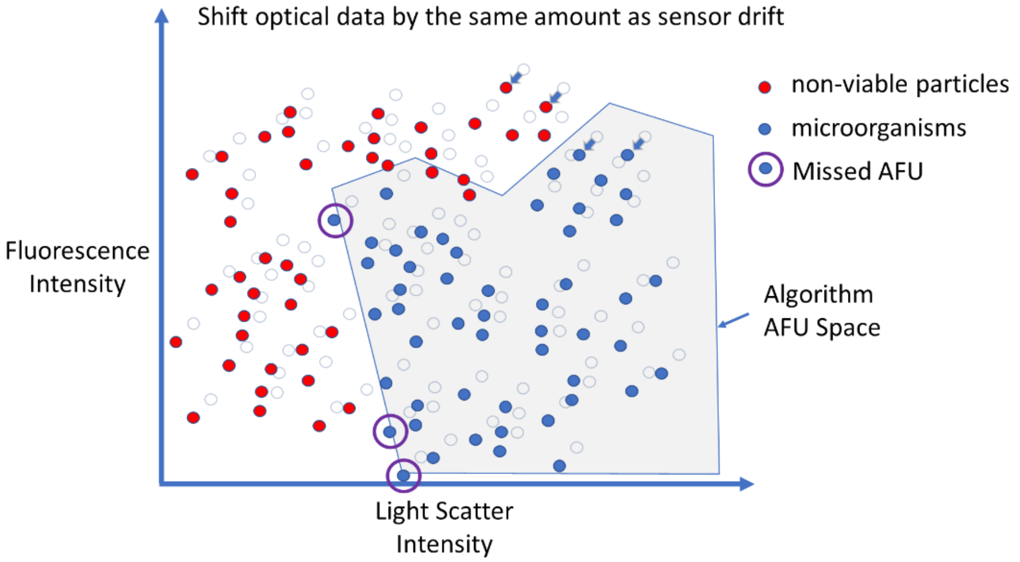

Figure 2 — Conceptual representation of test particle optical characteristics that have been shifted lower on both axes (open circles shifted to filled circles as indicated by representative arrows). The amount of shift is equal to the observed sensor drift during as-found calibration (in this example 10%). Viable (blue) and non-viable (red) test particles are indicated. Certain microorganism test particles (indicated by purple circles) now fall outside the algorithm AFU space resulting in altered BFPC performance.

Figure 2 shows the optical intensities of all of the test particles shown in Figure 1 however, in this instance, the values are shifted lower by 10%. The result of this shift is that several microorganism test particles (circled in purple) are now being incorrectly categorized as non-viable particles. The fraction of miscategorized microorganisms represents the relative algorithm performance given the observed sensor errors. In the ideal state (Figure 1), all of the microorganisms are correctly categorized (100% performance). In the state that simulates the observed sensor drift (Figure 2), 3 of the 51 microorganisms are now incorrectly categorized as non-viable particles (94% performance). In other words, this analysis objectively and quantitatively answers the question, “How does the observed sensor drift impact the detection of microorganisms?”.

Implementation for the BioTrak Real-Time Viable Particle Counter

To adequately determine the impact of sensor drift on the BioTrak™ Real-Time Viable Particle Counter, the simplified example needs to be expanded to cover the multiple measurements used in the viability algorithm. Therefore, the evaluation includes the size error (i.e. light scatter error) and two separate fluorescence intensities measured during as-found calibration. Additionally, this evaluation needs to be applied to a variety of microorganism types as sensor drift will have a different impact on the detection of each.

The data set used for the evaluation is the data collected during the execution of the primary validation. This includes data collected from 4 instruments of 11 different microorganism species, including vegetative bacteria, bacterial spores, mold spores, and yeast. The data set was compiled over 251 unique runs and is comprised of over 17,000 data points. All results are averaged to give a comprehensive analysis of the impact of sensor drift to AFU detection performance.

Conclusions

The BioTrak™ Real-Time Viable Particle Counter, a BFPC, relies on measurements from multiple sensors to detect a viable particle. The drift, or error, of each sensor is determined during as-found calibration. The impact of these individual errors cannot easily be related to the overall performance of the instrument. Instead, the combined affect that all the sensors have on the performance of the algorithm to accurately detect a viable particle needs to be determined. This assessment of the algorithm performance provides the information necessary to quantitatively evaluate the impact from an OOT condition.