

# Single-use Sensors for Upstream Applications

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One of the largest factors limiting the proliferation of single-use bioreactors in cGMP and production environments is the lack of reliable and well engineered single-use sensors. Although the same process parameters are measured in both stainless steel and single-use reactors, the details of the calibration, sterilization, and introduction of the sensors into the bioreactor are substantively different. Additionally, single-use vessels have idiosyncrasies that are different from traditional vessels and must be addressed in novel ways.

In traditional stainless steel and glass vessels, the probes are often calibrated before insertion into the vessel and always calibrated before sterilization (autoclavation or SIP/CIP). This requisite order of operations leads to drift in the calibration, but has been tolerated or compensated for since the inception of “modern” bio-processing. On the other hand, single-use containers are typically sterilized with gamma radiation, and most electrochemical probes are not compatible with this method of sterilization. The ideal single-use probe would be sealed into the bioreactor container before exposure to gamma radiation so that the sterile barrier is never broken. This stipulates that the desired sensor is pre-calibrated and immune to gamma radiation or has very predictable changes after exposure so that the pre-calibration maintains its validity or can be corrected by a simple one point correction process.

Moreover, single-use bioreactors are generally constructed from films made of low-density polyethylene (LDPE) or ethylene/vinyl acetate (EVA), or a laminate of both. These films are dielectrics and as such have a bound charge associated with them. During a growth run, the agitation of the media inside the bioreactor can lead to the build-up of a significant static electric charge. There have been many reports where a sudden release of this charge has destroyed sensor equipment and has jolted or physically moved bio-processing professionals working on the reactor. Therefore, the ideal single-use sensor would also have the capability of removing electrostatic charge build-up to an earth ground.

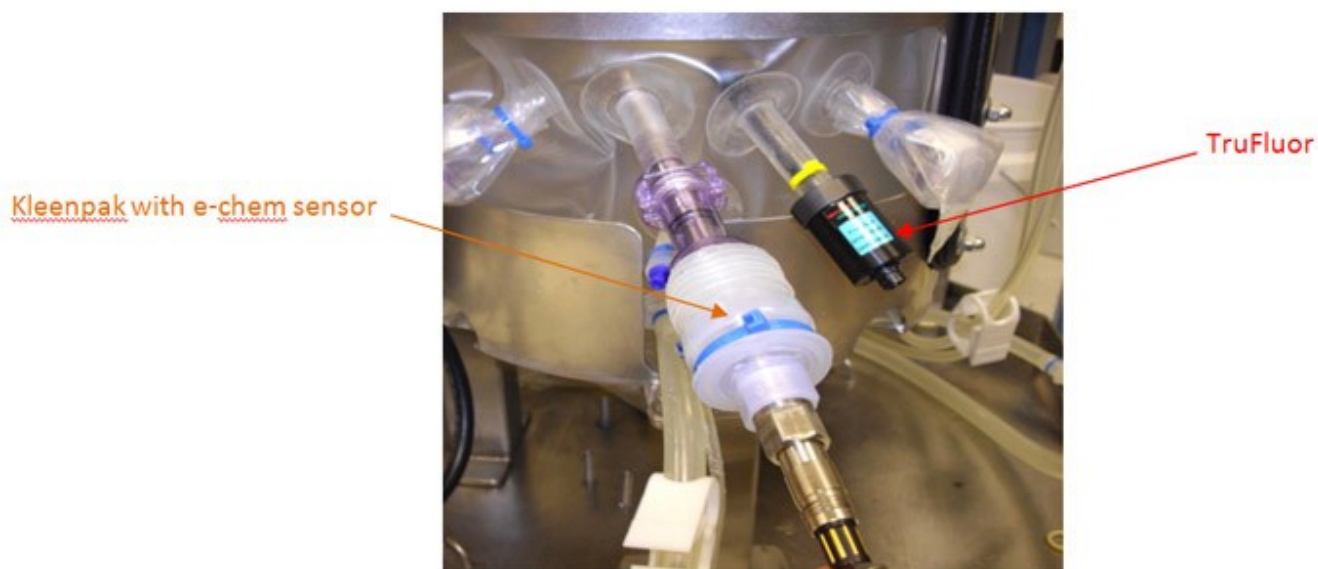
## **Finesse Single-use Sensors**

Finesse offers a suite of truly plug-and-play single-use sensors. The motivating idea is to allow engineers and operators to focus on bio-processing, rather than the concomitant sensors that are a necessary but not central focus of their work. Fundamentally, this means that the single-use sensors need to account for all of the aforementioned issues. In addition, the sensors need to be gamma sterilizable, pre-calibrated, accurate, precise, reliable, and easy to use. Finesse currently offers the following single-use sensors which meet these criteria: an optical dissolved oxygen sensor, an optical pH sensor, and a headspace pressure sensor.

The wetted materials of all of the probes are constructed using fully USP Class VI or ISO 10993 approved materials that are carefully chosen to be gamma radiation resistant. We have performed statistical testing of the materials after exposure to ~50 kGy gamma radiation. The sensors are all designed to fit into a standard single-

use vessel port. Many vendors perform testing of the seal between the port and the sensor at pressures in excess of 25 psi to ensure fidelity of the seal under any foreseeable condition. Once the disposable component is sealed into the single-use container, the system is ready for gamma radiation and the sterile barrier is never broken. The entire single-use system can then be validated for sterility without complications or additional burden.

In sharp contrast, typical aseptic connection devices such as the Pall Kleenpak® are fitted with a traditional probe, autoclaved, and then inserted into the single-use vessel. Using this system, the probes are still subject to sterilization induced drift, and there is a real and measurable occurrence of contamination, despite autoclavation. It is this potential for contamination that necessitates validation to be performed on each port using a Kleenpak or similar device. A comparison between using the TruFluor sheath and a Pall Kleenpak is shown in Figure 1 below.



*Figure 1: Comparison of Pall Kleenpak® with a traditional electrochemical sensor and a Finesse TruFluor single-use sensor.*

### **Phase Fluorimetric Sensors**

The Finesse optical dissolved oxygen and pH sensors are based on the concept of phase fluorimetry. In phase fluorimetry, a fluorescent “dye” is used to sense the presence of the analyte under study. The dyes have the property that they are “quenched” or their fluorescent lifetime is reduced by the analyte. If the excitation light is sinusoidally modulated at a low enough frequency (compared to the inverse of the fluorescent lifetime), the fluorescent light is also sinusoidally modulated – but has a different phase than the excitation light. This is shown in Figure 2 below.

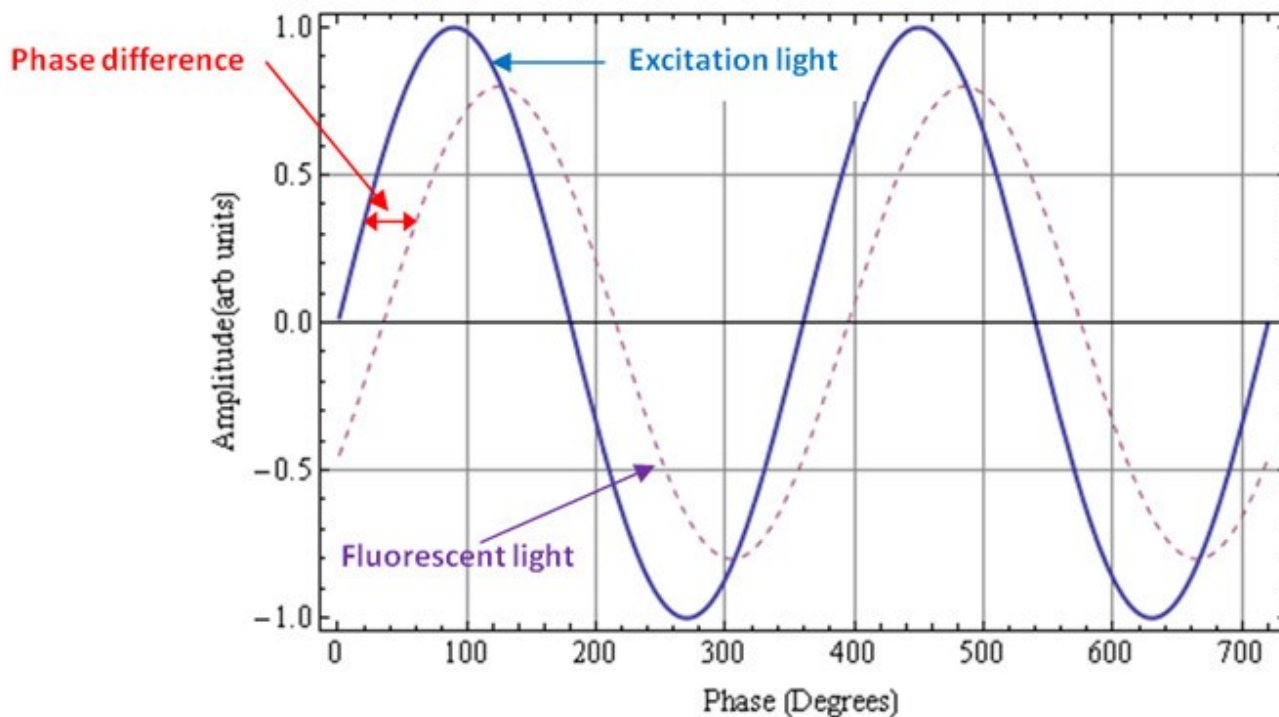


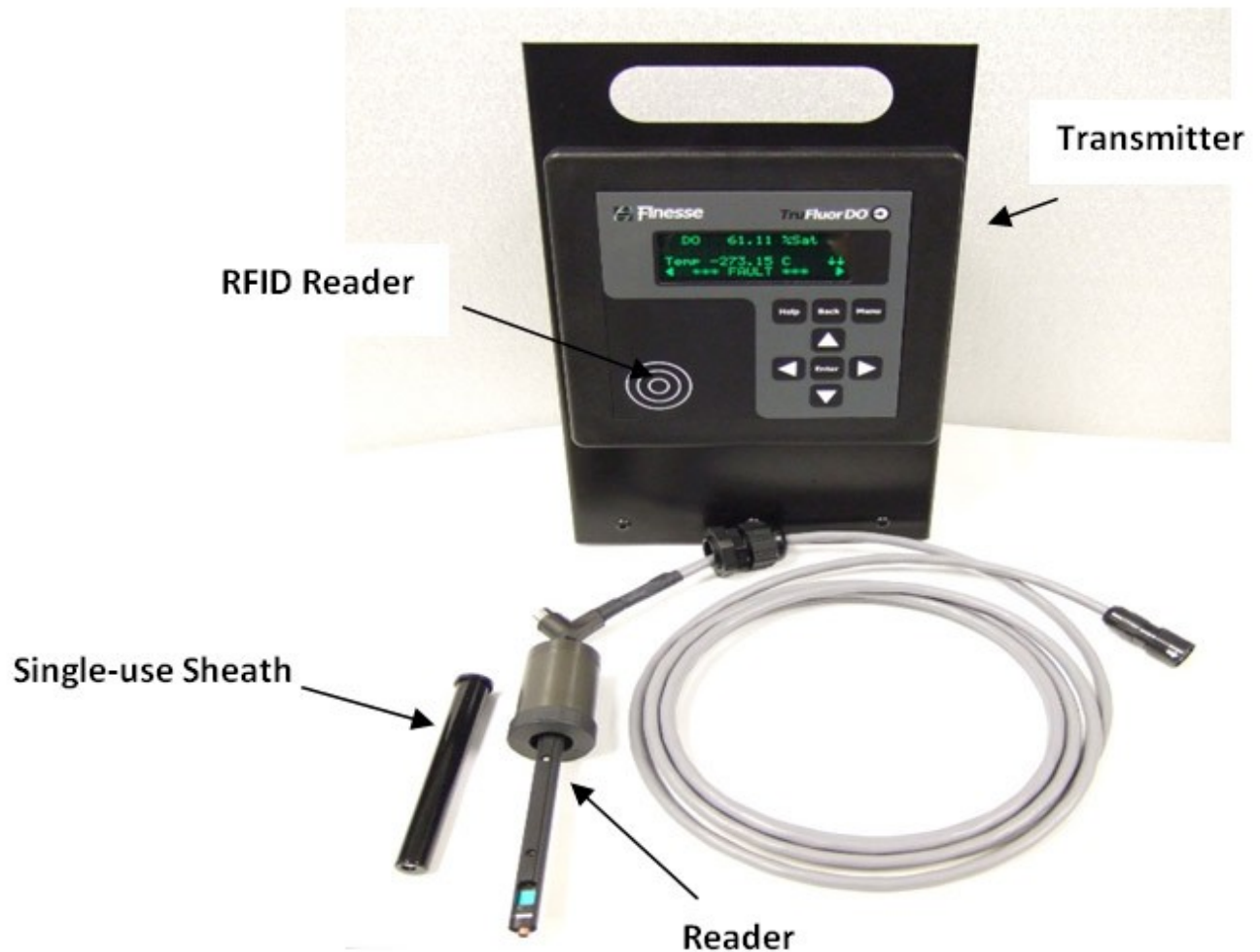
Figure 2: Phase fluorimetry

This phase relationship changes as a function of the concentration of the analyte under study. We utilize this relationship between phase and analyte concentration and create a mathematical model of the process. This is the basic transduction method underlying our TruFluor product family.

The dyes used in all phase fluorimetric sensors to date are subject to photo-degradation. Specifically, the dyes' fundamental properties change when exposed to a combination of light and the analyte that quenches their fluorescence; this photo-degradation manifests itself as drift in the calibration. While there are ways to chemically stabilize the dyes, these additives are often complicated to construct and deploy, and can be toxic to cells. We have addressed the photo-degradation issue in the TruFluor product family by using a patented free space optical design, instead of fiber optics. Namely, a large area detector does not have the light collection limits imposed by fiber optics, and our design can capture a much larger amount of the emitted fluorescent light – thereby allowing us to use far less excitation light (approximately 20 times less than an optimized fiber based system). Using simple mathematical and fundamental physical arguments, we can show that the decrease in excitation light is linearly proportional to a decrease in the photo-degradation rate. We have shown that with a very stringent test (10 days at 37.5 °C in 21% oxygen gas, sampling every 2 seconds), there is less than a 0.5% change in the TruFluor DO reading. Therefore, a 30 day run should have the same drift when sampling every 5 seconds; a sampling rate that is more than adequate to capture any change occurring in the growth run. This is especially true since the sensor dye responds in approximately 30-40 seconds in liquid – and such a response is faster than the comparable electrochemical probe. Comparative life-test studies are underway to unequivocally demonstrate the advantage of our design over optical fiber-based systems.

### TruFluor Product Family

The TruFluor product itself is shown below in Figure 3.



*Figure 3: TruFluor product – transmitter, optical reader, cable, and single-use sheath*

Many of the aforementioned shortcomings of traditional probes and other optical probes are addressed by this product. The single-use element, the sheath, is constructed entirely from ISO10993 rated material and 316L electro-polished stainless steel. The sensor dyes used have undergone full USP Class VI testing including heavy metals, cytotoxicity, and implantation testing. The readers are all normalized to read the same value within a very small uncertainty so that they are interchangeable. The disposable sheaths are pre-calibrated in a computer controller station. The calibration is entered on a gamma radiation resistant RFID tag along with the material lot, dye lot, and fabrication date information. This RFID tag is simply held to the transmitter and the system is calibrated. Both one-point standardizations and full two-point calibrations are possible using the transmitter, if desired by the user.

Because the energy levels in the sensor dyes are temperature sensitive, TruFluor has a built-in temperature sensor (accurate to better than 0.25 °C over the entire range of operation) for automatic temperature compensation. The temperature is measured through a small 316L electro-polished thermal “window” in the sheath. This temperature measurement is also available from the transmitter as a process variable (4-20 mA output), along with the dissolved oxygen level. The 316L stainless steel plate and temperature sensor are also connected through the reader and transmitter to earth ground and serves to bleed charge out of the bioreactor,

in order to avoid dangerous build-up of static charge.

### TruFluor DO

The specifications for TruFluor DO have been tested in detail. TruFluor DO performance compared to two traditional electrochemical probes is shown below in Figure 4a on the left, where the TruFluor DO reading is plotted on the x-axis and the two electrochemical probe DO readings are plotted on the y-axis. As expected, the lines are almost precisely at 45 degrees, indicating that the electrochemical probes and TruFluor DO yield similar readings in identical conditions. Figure 4b on the right shows the reproducibility of the non-disposable optical reader. We have taken 48 different readers and tested the consistency of their readings in units of % Sat with one disposable sheath.

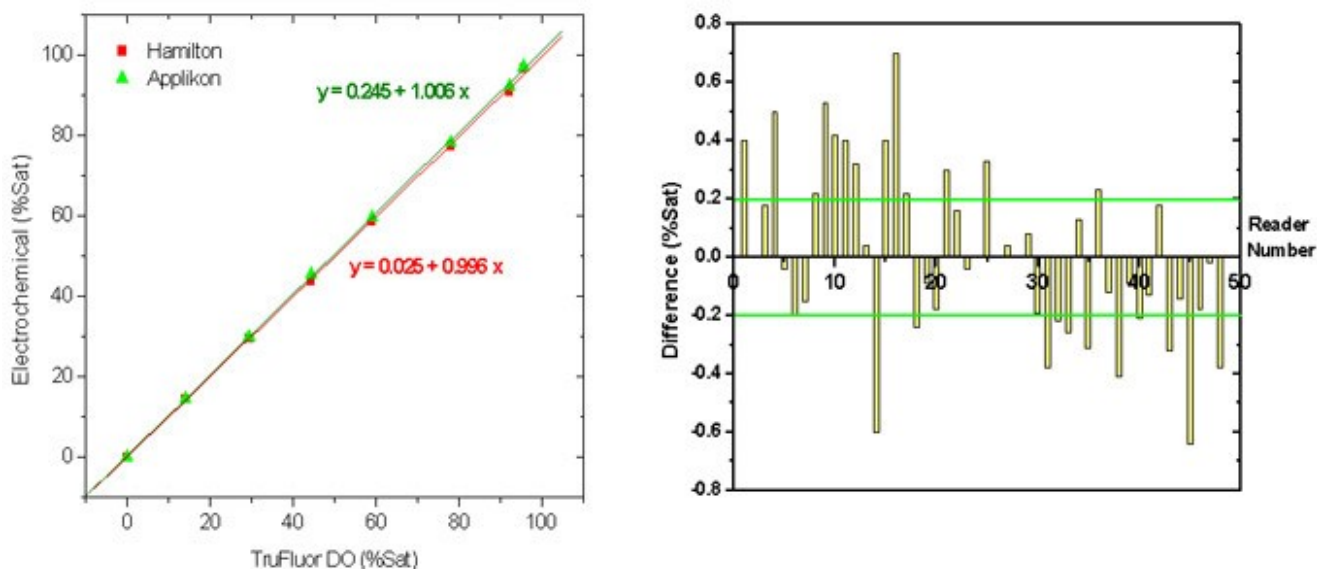


Figure 4 (left) Comparison of TruFluor DO and polarographic probe, and (right) uniformity test over 48 TruFluor DO readers in a single sheath.

In order to obtain the uniformity in readings shown above in 4b, manufacturing of the readers is performed in a repeatable and precise manner. Each component is individually inspected and tested before the systems are assembled, and each unit is built in an identical fashion. This process has led to very consistent statistical performance of the readers. Therefore, our reader to reader phase repeatability ensures if or when a reader is changed out on a given sheath, there is a very small and often unnoticeable change in the measured values.

Finally, the TruFluor DO dye is coated with an oxygen permeable, light blocking layer which makes it resilient to ambient light. This coating, combined with the sheath design which seats the spot on a rim having a smaller diameter than the physical spot makes the sensor far less sensitive to effects from exterior light sources than fiber based systems.

### Summary

Finesse single-use sensors are designed and manufactured to help fulfill the promise of single-use upstream production. The designs of these sensors resolve the limitations of their predecessors in practical operation, namely light sensitivity, drift, and unwanted build-up of electrostatic charge. In addition, calibration, gamma

radiation resistance, and USP Class VI issues are addressed for each sensor.

The current sensor product family includes DO, pH, temperature, and pressure, as these are the bare minimum required to monitor and control the single-use process. Future sensors are planned to further enable the bio-process professional and disposable manufacturing.