NanoDrop Spectrophotometers and Fluorospectrometers

Micro-Volume Spectroscopy

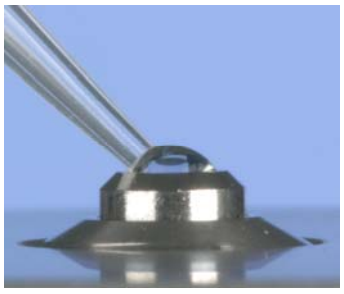
Absorbance and fluorescence measurements with minimal consumption of sample have become paramount as methods evolve using ever smaller amounts of material for analysis. Conventional methods are impractical given the limited sample volumes produced by such techniques as laser-capture microdissection. By using fiber optic technology and the inherent surface tension properties of liquid samples, NanoDrop Technologies' micro-volume instrumentation can accurately quantitate a wide range of biomolecules in volumes as small as 1 microliter. Here we describe the novel micro-volume spectrophotometric and fluorometric systems and how measurements are made using the Thermo Scientific NanoDrop® ND-1000 Spectrophotometer and the Thermo Scientific NanoDrop® ND-3300 Fluorospectrometer respectively.

Sample Retention Technology

The sample retention system enables absorbance and fluorescent measurements to be performed without traditional containment devices such as cuvettes or capillaries. The system uses inherent surface tension to hold a one microliter (1ul) sample in place between two optical surfaces during the measurement cycle.

Micro-Volume Absorbance Measurements

In order to make a measurement, 1ul of sample is pipetted directly onto the lower optical (measurement) surface (Figure 1a). An upper optical surface automatically engages the sample, using surface tension to form a liquid column of mechanically-controlled path length (Figure 1b).



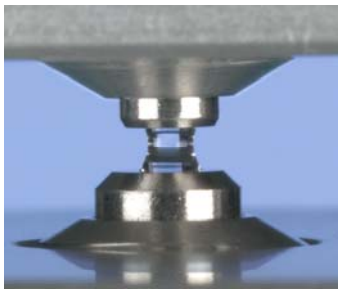


Figure 1a: Loading 1ul sample. **Figure 1b: Retention system.**

Once the measurement is complete, the user simply cleans both optical surfaces with a standard laboratory wipe to prepare for the next sample.

The UV-Vis wavelength range of 220nm to 750nm provides a versatile platform for absorbance analysis of various chromophores, including nucleic acids and proteins. The variety of modules within the NanoDrop 1000 operating software represents the diversity of possible measurements and the ease of use (Figure 2).

Figure 2: Main menu of the NanoDrop 1000 operating software

The Microarray module is a good example of how the software is designed to quickly provide investigators critical information. After the ten-second measurement cycle is complete, the module displays a full UV-Vis absorbance spectrum as well as the calculated concentrations of both the nucleic acid and the fluorescent label. Investigators can readily determine in-

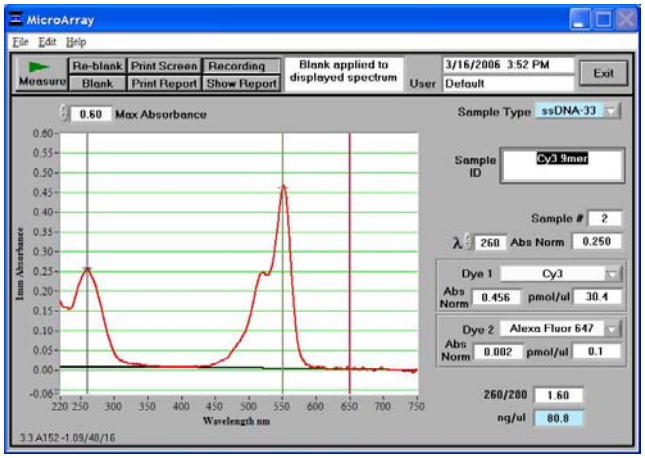


Figure 3: 1ul absorbance measurement of a Cy3 labeled

By design, the NanoDrop-1000 eliminates fixed containment devices such as cuvettes and capillaries, enabling an automatic path length change from 1mm to 0.2mm during the same measurement cycle of a given sample. This allows for an unprecedented range of sample concentration to be assessed through absorbance spectroscopy (2 ng/ul to 3700 ng/ul for dsDNA), essentially eliminating the need to perform dilutions.

Micro-Volume Fluorescence Measurements

Using the same sample retention technology, the NanoDrop 3300 performs full spectrum fluorescent analysis of one microliter samples without the use of cuvettes or capillaries. The lever arm of the NanoDrop 3300 is opened and 1-2ul of sample is pipetted onto the optical surface. The lever arm then engages the upper surface of the sample to capture and hold the sample in place during the measurement cycle. Once complete, the sample is wiped away using an ordinary laboratory wipe. Fluorophore excitation occurs from one of three solid-state light emitting diodes (LEDs): UV, Blue, or White. The broad excitation range allows for a wide range of common fluorophores to be measured without the need for filter changes (Figures 4).

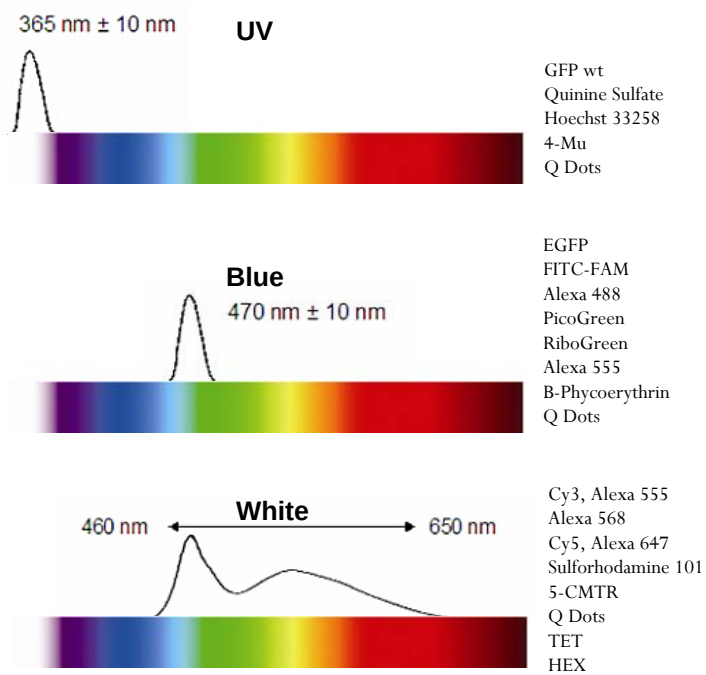


Figure 4: LED excitation spectra and comon fluorophores

The uniquely clean optics of the retention system and proprietary signal processing enable the unconventional use of the broad-spectrum, white LED. The broad excitation of the white LED can be used to excite several fluorophores, allowing for a multiple emission profile from a single sample (Figure 5).

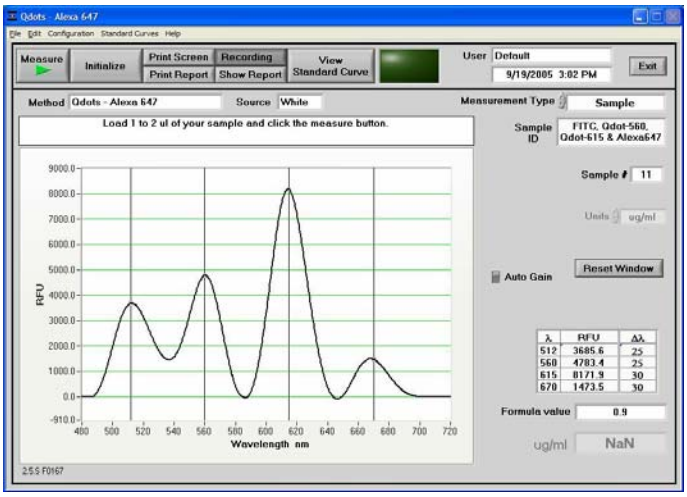


Figure 5: Multiple fluorophore emission output.

Ultra-Low Sample Mass Detection Limit

The small sample volume requirement of the NanoDrop 3300 allows significantly scaled-down fluorescent reaction volumes (2-10ul) and therefore utilizes only a fraction of the sample consumed by cuvette- or plate-based fluorescent measurements. Even though the NanoDrop 3300 does not measure ultra low *concentrations*, it does lower the fluorescent detection limit of sample *mass* by more than an order of magnitude. For example, using the PicoGreen® dye, the NanoDrop 3300 can detect as little as 2 pg dsDNA, while a cuvette or microplate PicoGreen® assay would need at least 25-50 pg dsDNA for detection (Table A)

Table A: PicoGreen® assay detection limits

	Nano-Drop 3300	Microplate reader	Cuvette-based Fluorometer
Detection limit by sample mass	**2 pg (in 2 ul)**	**50 pg (in 200 ul)**	**25 pg in 1 ml**
Detection limit by concentration	**1 pg/ul**	**0.25 pg/ul**	**0.025 pg/ul**

For those working with limited sample quantities, the NanoDrop 300 enables measurements that are simply not possible using cuvette or plate-based fluorescent spectroscopy.

In Summary

Our novel retention system not only enables quality absorbance and fluorescence measurements with only a minute fraction of sample material needed to perform similar analyses on more traditional systems, but its ease of use provides a practical alternative for all spectroscopy measurements.