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Spectrophotometry's Next-Generation Technology

by Charles Robertson

Significant ground has been gained in spectrophotometry through the introduction of a unit that offers the ability to measure extraordinarily small samples, making quantification of DNA and RNA fast and easy.

The NanoDrop® ND-1000 spectrophotometer (**NanoDrop Technologies, Inc.,** Rockland DE) has the capability of measuring 1-µL samples in 10 sec without cuvettes or capillaries. Users pipet the sample directly onto the measurement surface (*Figure 1*); no containers or other measuring devices are needed. When a fiber-optic tip is brought down to touch the sample, it is drawn out like an hourglass using surface tension when the tip is pulled back (*Figure 2*).

It should be noted that only surface tension holds the sample in place. The full-spectrum measurement is then automatically taken through the center of the hourglass. Quantification is made based on the tightly controlled pathlength of 1 mm.

Once measurement is complete, the sample is wiped off the surfaces with an ordinary laboratory wipe. This fast and easy cleanup is completely effective for eliminating carryover or contamination and quickly readies the instrument for its next measurement.

Figure 1 With the sampling apparatus open, a droplet of sample is pipetted onto the lower measurement pedestal.

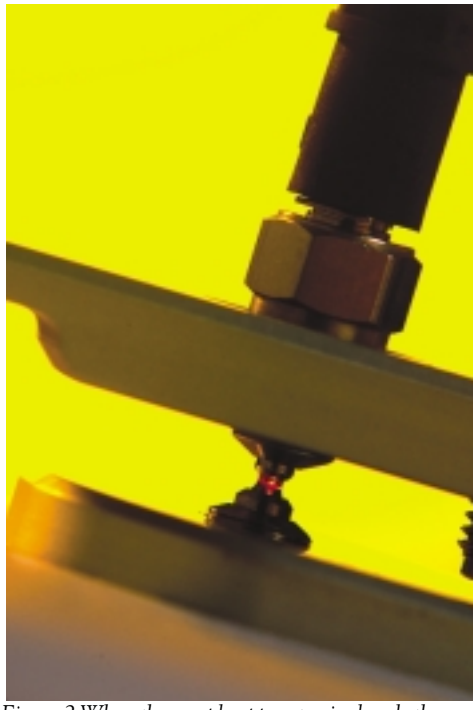

Figure 2 When the sample apparatus is closed, the upper measurement pedestal slightly compresses the droplet and then a sample column is drawn. Surface tension alone holds the sample in place.

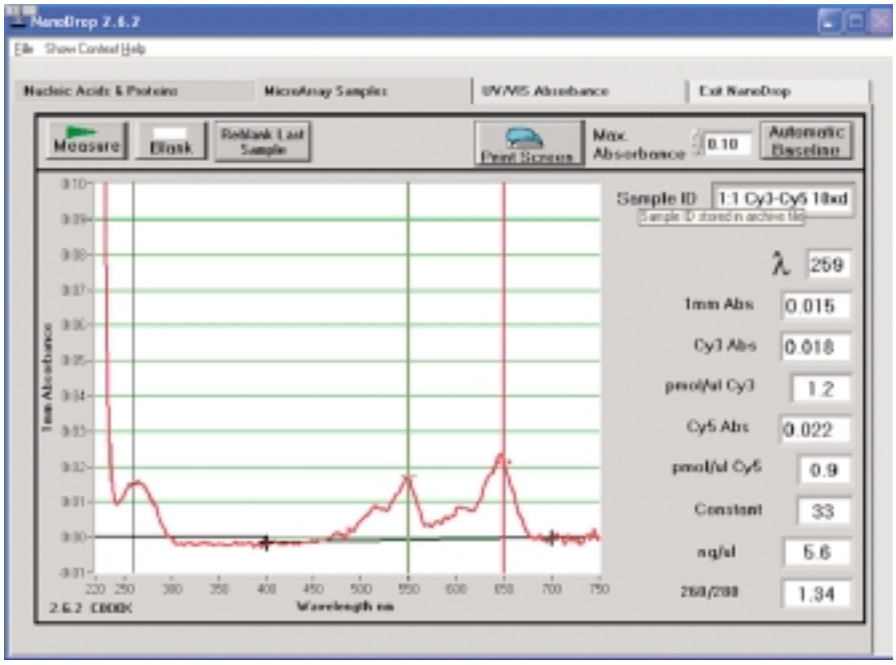

Figure 3 *On the screen, the green vertical line represents the peak wavelength position for Cy3, and the red vertical line represents the peak wavelength position for Cy5. There is a general wavelength/absorbance cursor to aid in reading values from the graph.*

Because it measures both visible and ultraviolet light, the spectrophotometer can be used to:

- Determine microarray Cy-Dye label concentrations. The unit can detect Cy3 and Cy5 at concentrations as low as 0.2 pmol/µL. The software will automatically calculate a baseline between 400 and 700 nm (*Figure 3*).

- Measure concentration and quality of nucleic acid samples up to 3700 ng/µL without dilution. The nucleic acid concentration and 260/280 ratio are calculated by the software and can be read directly on the PC screen (*Figure 4*). For those with special needs, any of several analysis constants can be selected, or the constant set to any value between 20 and 55. For low nucleic acid concentrations, measurement precision is approx. 1.0 ng/µL under normal circumstances.

- Measure protein concentration.

- Measure cell density.

The instrument's 1-µL capability saves precious laboratory samples, and is ideal for laboratories that need to conserve reagents in general. Because of its shorter pathlengths, the instrument has an expanded dynamic range that allows measurement of samples that are up to 50 times more concentrated than those used for standard spectrophotometers. Along with eliminating the need to fill a certain volume in a cuvette, this means that laboratories can eliminate the time-consuming sample dilutions that were needed with past-generation instruments.

For busy laboratories the time and effort saved by eliminating cumbersome dilutions and washing cuvettes is substantial. The spectrophotometer is so simple to set up that users can start using it within minutes. The spectrophotometer includes everything required for immediate incorporation into a laboratory's work flow: power supply cord, USB cable, user's manual, and proprietary software on CD.

The instrument uses a PC with at least a 233-MHz processor that runs Windows 98, ME, XP, or 2000 (**Microsoft Corp.,** Redmond, WA) and has an open USB port, a CD-ROM drive, 32 MB of RAM, and 40 MB of free hard drive. The manufacturer offers a **Dell**

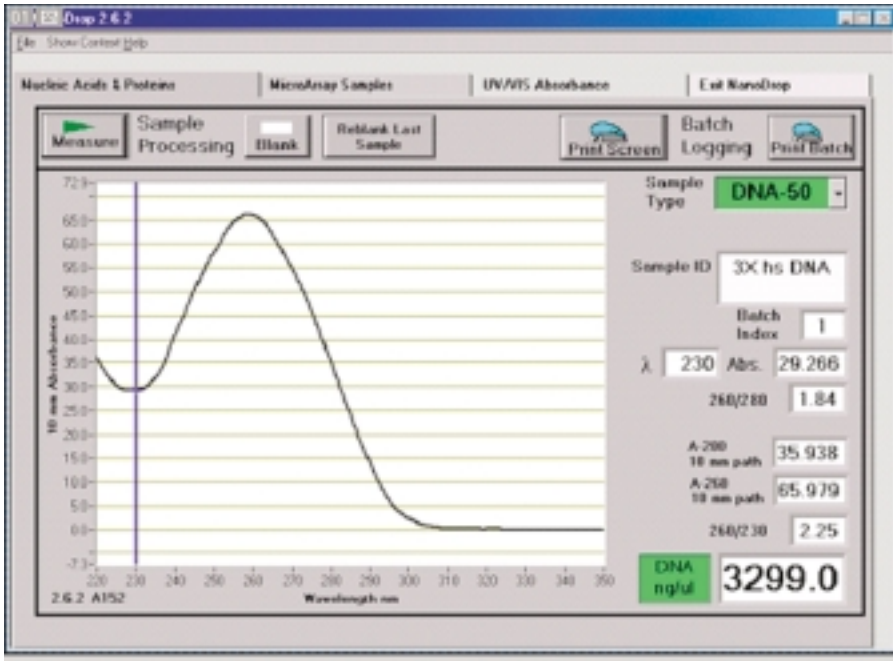

Figure 4 *The nucleic acid concentration and 260/280 ratio are calculated by the software and can be read directly on the PC screen. For those with special needs, any of several analysis constants can be selected, or the constant can be set to any value between 20 and 55.*

(Round Rock, TX) notebook for purchase by those who need a computer to use with the instrument.

For organization and convenience, the DYMO LabelWriter 330 label printer (**NanoDrop Technologies, Inc.**), which prints 2.3 × 4 in. self-adhesive labels, is available. The labels can be placed directly into laboratory notebooks with data from a single sample printed individually or batches of samples logged and printed together.

The spectrophotometer features a small footprint (20 × 15 × 12 cm). This, combined with the fact that a semiskilled individual can easily do four samples a minute, makes the unit a wise choice for today's busy spectrophotometry laboratories.

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