**SpectraMax Plus or SpectraMax 190 and SoftMax Pro Software automatically:**
- Make $A_{260}$ and $A_{280}$ measurements
- Measure the optical pathlength of each sample with the PathCheck sensor
- Normalize the absorbance values to a 1 cm pathlength
- Calculate concentrations
- Make specialized calculations (e.g., aliquot size needed for sequencing)
- Report final results in your own customized format

**Introduction**
Ultraviolet (UV) measurements in microplates became possible only 5 years ago when Molecular Devices introduced the first UV-capable microplate spectrophotometer. Since then, many users have moved to the higher-throughput microplate format for making DNA, RNA, or protein absorbance measurements. Accurate and reproducible $A_{260}$ measurements can be made easily in SpectraMax Microplate Spectrophotometers with awareness of the optical properties of microplate materials and attention to technique. Using the PathCheck® Sensor, results can be automatically normalized to a 1 cm pathlength so that the values are equivalent to those obtained in a standard cuvette. SoftMax® Pro Software can then automate specialized calculations and report formats.

**Materials**
- SpectraMax Plus or SpectraMax 190 Microplate Spectrophotometer (Molecular Devices, Inc.)
- UV-transparent microplates; *e.g.* UV-Plate™ (Corning-Costar Cat. # 3635), UV-Star® (Greiner Cat. # 655801, E & K Scientific Products), UVMax™ (Polyfiltronics, from Whatman LabSales), Quartz Plate (Hellma Cells, Inc.)

**UV-transparent microplates**
While quartz microplates are commercially available, they are expensive (> $1,000). Standard polystyrene microplates cannot be used for UV measurements because they do not transmit light below approximately 300 nm. In addition to quartz, at least three UV-transparent plastic microplates that can be used below 300 nm are available (Figure 1). The Corning Costar UV and Greiner UV microplates transmit down to approximately 215 nm. The Polyfiltronics UV Plate is usable down to 240 nm, though it has a small absorbance peak at 280 nm.

**Recommendations for best quality results**
- Use clean microplates and particle-free solutions.
- Note absorbance values of buffer blanks. If they do not fall into the expected range, the microplate is most likely dirty, defective, or has particles in the wells. OD$_{260}$ values for a water-filled quartz microplate should be 0.03–0.04 with a standard deviation (SD) < 0.002. Costar UV and Greiner UV microplates vary slightly between lots, but should have mean OD$_{260}$ values of 0.045–0.06 with SDs < 0.002. Polyfiltronics UV microplates have a higher background, but good results can be obtained by pre-reading the plates and subtracting the background on a well-by-well basis (automatically performed by SoftMax Pro Software).
- If your procedure calls for a small sample (1–20 µL) plus large volume of a diluent, pipet the small volume first, followed by the larger volume. Automix in the SpectraMax instrument for 10–20 sec to complete the mixing.
- Be aware that increasing ionic strength decreases absorptivity. DNA has approximately 30% higher absorbance when dissolved in water rather than buffered saline.
- For low-absorbing samples, use the maximum possible volume (250–300 µL) to get the maximum optical pathlength. Include duplicates or triplicates if possible.
- Use PathCheck Sensor to normalize $A_{260}$ values to a 1 cm pathlength.

**Example of DNA measurements**
Figure 2 shows DNA $A_{260}$ results. The limit of detection (amount producing an absorbance value higher than 3 positive SDs of the blank values) was ~25 ng/well and the limit of quantitation (10 positive SDs of the reagent blank) was ~75 ng/well. The microplate results agree with published limits of detection of DNA using $A_{260}$ measurements in spectrophotometers. Quantitation of lower DNA concentrations require alternative techniques, such as reaction with a fluorescent dye.
DNA (or RNA) estimation using absorptivity

DNA (or RNA) concentration is commonly estimated by dividing the $A_{260}$ value by the 1-cm absorptivity value (or multiplying by its reciprocal). Using the PathCheck Sensor, SpectraMax Instruments automatically normalize sample absorbance values to a 1-cm pathlength and calculate the concentrations. The 1/absorptivity value for double stranded DNA is commonly assumed to be 50 µg/mL for a 1-cm pathlength. However, this value is correct only when the solution has a relatively high salt concentration (Figure 3). DNA has 30% higher absorptivity in water compared to buffered saline.

Calculating DNA (or RNA) concentration with SoftMax Pro Software

DNA (or RNA) assay protocols often call for $A_{260}$ measurements of small (2–10 µL) samples to which a diluent is added. SoftMax Pro Software can automatically normalize $A_{260}$ values from SpectraMax Instruments, determine the concentrations of the original samples and calculate the volumes required for subsequent assays. SoftMax Pro Software can also accommodate liquid handling systems that can be set in 0.5 µL increments. Figure 4 shows such an example in which 5 µL was used for the $A_{260}$ measurements and the target amount for use in a sequencer was 0.4 µg. The nominal aliquot volume was calculated for each sample and rounded off to the nearest 0.5 µL. These results are easily exported to other data handling systems.

Summary

Accurate and reproducible DNA (or RNA) measurements can be made easily in microplates. The lower limits of quantitation are comparable to those obtained in conventional UV-VIS spectrophotometers. The PathCheck Sensor automatically normalizes the results to a 1 cm pathlength. SoftMax Pro Software automatically calculates concentrations, as well as the volumes needed for transfer to a subsequent step.

1 McGown, E.L. 1999. MAXline Application Note # 32
2 McGown, E.L. 1999. MAXline Application Note # 33