Application Note by Anitoa Systems, LLC

DNA detection and quantification using Anitoa CFI324 Fluorescence Imaging System

Introduction

In this application note, we describe DNA detection and quantification using Anitoa CFI324 Fluorescence Imaging System and the Quant-iT DNA quantification assay. The CFI324 Fluorescence Imaging System is equipped with ULS24 CMOS molecular imager.

DNA detection and quantification is one of the most common procedures in molecular biology. It is frequently performed to accomplish several essential tasks, such as,

1. Evaluate the yields of DNA during purifications and amplification procedures such as PCR;
2. Assess efficiency in the construction of cDNA libraries and the generation primer extension products,
3. Detect residual DNA in samples such as drug and protein preparations.

The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm (A260). The major disadvantages of the absorbance method are its relative insensitivity (an A260 of 0.1 corresponds to a 5 ug/mL dsDNA solution), its inability to distinguish between signals from DNA and RNA, and its potential for interference from contaminants found in nucleic acid preparations.

PicoGreen® (Life Technologies) is a nucleic acid stain that fluoresces on binding to double-stranded DNA. The reagent has been developed as the basis of a highly sensitive assay kit. The assay has found wide acceptance within the research community for several reasons. First and foremost, PicoGreen® dye–based fluorescence assays are up to 10,000-fold more sensitive than UV absorbance measurements. They are also less affected by the presence of common UV contaminants, such as proteins, free nucleotides or very short oligonucleotides, making quantification of intact oligonucleotides and nucleic acids much more accurate in complex mixtures such as serum or whole blood. The assay utilizes a simple protocol that requires only that reagent be mixed with sample, making it ideal for automated, high-throughput measurements. It is also easily adaptable to apparatus utilizing spectro-fluorometric detection. Overall, the performance characteristics of PicoGreen®-based dsDNA quantification reagents make them very suitable for protocols involving small amounts of dsDNA such as in molecular biology applications.

Materials:

- CFI 324 “Compact Fluorescence Imager” by Anitoa Systems, LLC. Use the 525nm "green" channel.

- Objective lens diameter: 25mm. Focal length: 17mm. AOI: <10 degree.
- Emission filter: Chroma Et525/50m. OD6, center wavelength 525nm. Bandwidth: 50nm.
- LED: Blue (470nm) Rebel Start LED. 70lm at 700mA. LED light source filtered with Chroma Et470/40x band pass filter, OD6. Center wave length 470nm. Bandwidth 40nm.
- Real time PCR reaction tube by Eppendorf.
- Quant-iT PicoGreen dsDNA Assay Kit P11496 (Life Technologies)
- DNA template dsDNA concentration of 100ng/uL. Provided by Zhejiang California Nano Technology Institute (ZCNI).

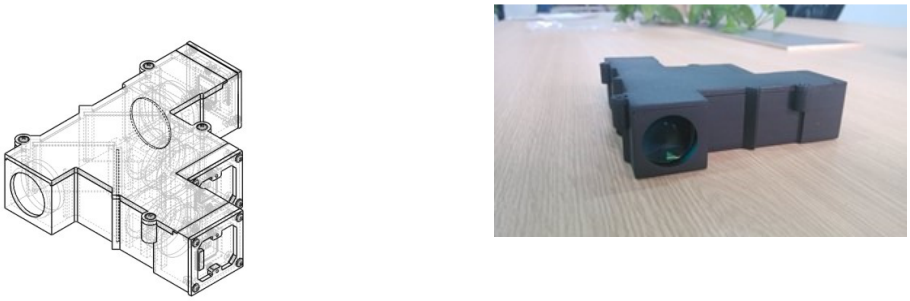

Figure 1. Compact multi-wavelengths fluorescence imager, CFI 324

Methods

Within 2 hours of the experiment, a stock of the PicoGreen dsDNA Quantitation Reagent was prepared by making a 1:200 dilution of the concentrated dye solution in Quant-iT buffer solution.

DNA template was diluted 20x with buffer to create a dilution of dsDNA for quantization at 5ng/uL.

The dsDNA samples were quantified with CFI-324 fluorescence imager utilizing the PicoGreen® dye reagent and detecting the fluorescent signal in the Eppendorf PCR tube.

Before taking measurement, the dsDNA sample is mixed with Quant-iT Reagent to create a 200uL sample mix. Thus with 1uL of dsDNA template and 199uL of Quant-it Reagent, we have a sample mix with 5ng of dsDNA, with 2uL of dsDNA template and 198uL of Quant-it Reagent, we have a sample mix with 10ng of dsDNA, and so on. We made sure that the sample in the tube appeared homogenous and that air-bubbles were absent. The sample mixes are allowed to incubate at room temperature for 5 minutes before analysis. As per the manufacturer's instructions, control samples without any dsDNA were used to create "a blank point" in the fluorescence detection system. And the most concentrated sample in the series was used to calibrate the gain of the fluorescence detection system. Because of this calibration scheme, we decided to generate standards curves, for dsDNA concentrations covering the

range roughly from 0-60 ng/mL. Sensitivity adjustment is achieved primarily through the selection of integration time and gain mode. We chose 200mS integration in high gain mode for this series of measurement. We did so in order to demonstrate how the fluorescence detection system can be adjusted to obtain the optimal signal for the dsDNA being studied.

Multiple readings of the same sample mix were conducted in order to confirm the precision of the reading. The assay had good precision; the CV between readings on the same sample was less than 6 units in digital readout. A single set of fluorescent readings were used to generate standard curves.

Results

We created a series of concentration samples containing 0ng, 5ng, 10ng, 15ng, 20ng and 30ng of dsDNA. Fluorescent readouts were recorded for each sample and repeated several times. We "blanked the system using 0ng sample to create a dark image. We chose a fixed integration time to establish the signal gain of the system, and found that 200ms is adequate.

The captured image is processed by simply averaging the pixel digital readout that defines the light spot.

Below is a series of images of the light spot.

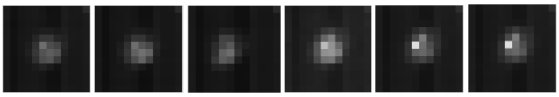

Figure 2: Fluorescent light spot

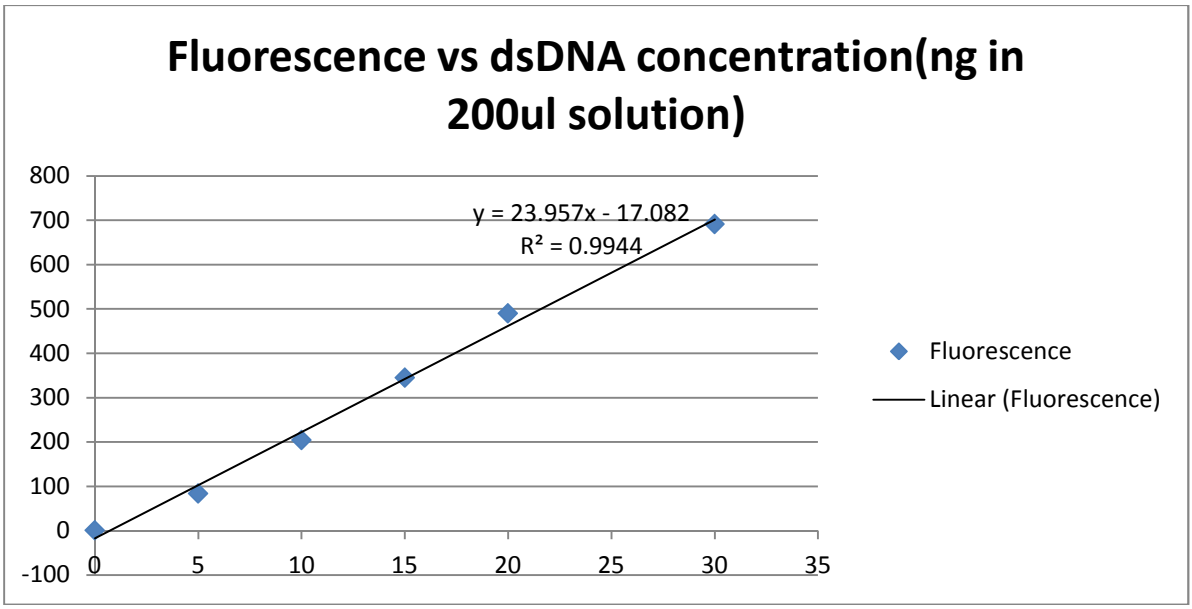

Figure 3. Fluorescence read out vs. dsDNA concentration curve

The results of analyzing samples are shown below in Figures 3 and 4. The series yielded a linear dose response curve intercepting at a point very close to zero.

The line describing the concentration dilution series had a correlation coefficient r=.9944, Closer examination of the curve demonstrates that the linear detection range of the PicoGreen(r) assay using the CFI 324 extends over three orders of magnitude.

Using a SnR ~10dB or 3-fold over background noise (standard deviation of measurement results) we determined that the assay sensitivity for the gain setting of 200mS integration was 1.0 ng. This represents >500 fold improvement in the sensitivity of this method over the standard A260 method shown in the literature.

Integration time projection

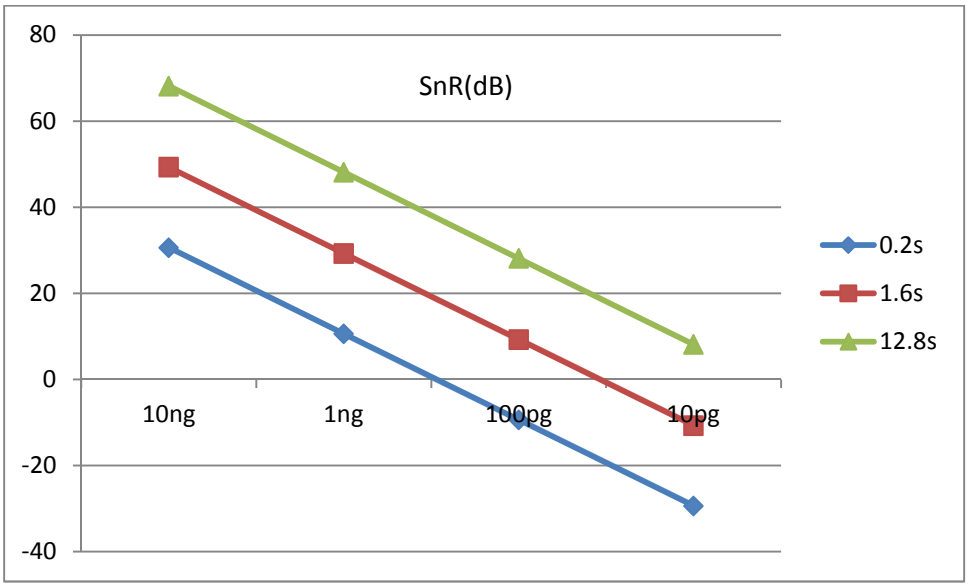

Figure 3. Signal-to-noise ratio in dB vs. dsDNA concentration curves, with different integration time

By prolonging the integration time, CFI 324 is capable of much better low light sensitivity with minimum increase of readout noise. We did linear projection of the performance data and predicted that CFI 324 can detect much lower concentration of dsDNA with longer integration time. For example, according to the curves in Figure, at 1.6s integration time, CFI 324 has a detection threshold of 120 pico-gram of dsDNA, albeit under ideal experimental circumstances[1].

Conclusion

CFI 324 is a convenient fluorescence measurement tool capable of dsDNA detection and quantification using the PicoGreen(R) assay reagent with high level of sensitivity and precision.

[1] In real experiment, at extremely low concentration of analyte, the sources of variations will mostly come from the precision in preparing and pipetting the samples. 0.5ng detection threshold has been reported using calibrated precision pipettors and the best microplate reader on the market.