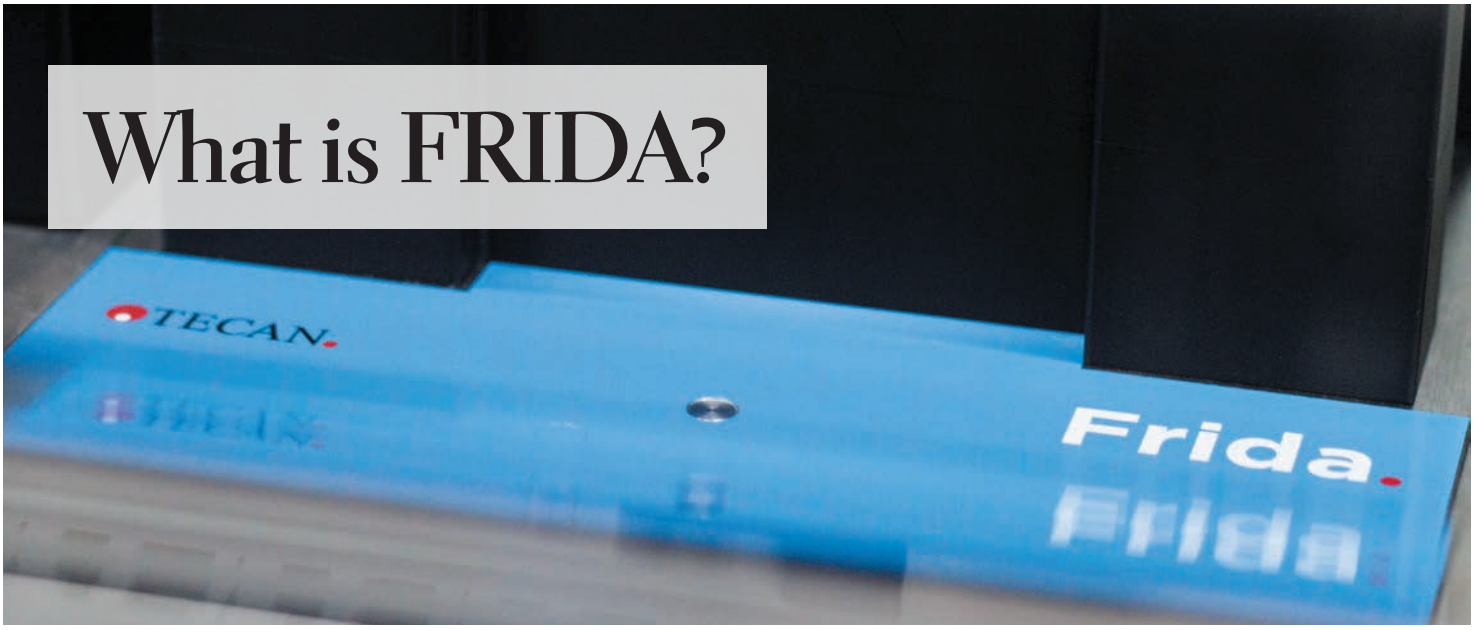
Frida Reader™

ACCURATE NUCLEIC ACID QUANTIFICATION
AND QUALITY CONTROL WITHOUT SAMPLE LOSS



What is FRIDA?

The Frida Reader is an add-on module for the Fluent® Automation Workstation that offers UV-based concentration and purity measurements of nucleic acids in a hanging drop. This unique, patented method completely avoids sample loss, as the hanging drop is aspirated back into the tip and can be used for further processing (eg. normalization). No additional preparation steps, labware or reagents are required for the measurement, which offers precision and accuracy comparable to a reference reader over a range of 2 to 1,000 ng/µl.

QUALITY WITHOUT COMPROMISE

Nucleic acid quantification by measurement of sample optical density (OD) at 260 and 320 nm is typically performed at the end of nucleic acid purification (NAP) workflows to determine the yield and allow normalization between samples for downstream processing, such as genotyping and NGS. In addition, evaluation of the A260/280 and A260/230 ratios can be used to identify the presence of protein or salt contamination in samples.

However, many laboratories working with rare or low volume samples do not quantify and normalize DNA at the end of their NAP workflow, as quantification consumes a portion of their precious sample. This can lead to poor quality results or downstream failures if insufficient genetic material is available or samples are contaminated, wasting time and causing unnecessary consumption of expensive reagents.

The Frida Reader has been developed specifically to address this issue, offering labs a completely sample loss-free method for nucleic acid quantification and normalization. By ensuring a sufficient quality and quantity of genetic material is available before beginning downstream processes, this approach will lead to better quality results and lower processing costs. Crucially, it has been designed to virtually eliminate the risk of hanging drops falling during measurement – over 4,600 drop were generated during instrument development without losing a single drop.

SEAMLESS INTEGRATION

The compact design of the Frida Reader – which occupies a single SLAS-format position on the Fluent's Dynamic Deck™ – allows easy integration alongside up- and downstream processes on the Fluent Automation Workstation. Designed to work in combination with the platform's Air Flexible Channel Arm™ (Air FCA) using 50 µl filtered disposable tips, it is compatible with all Fluent platform sizes, in both benchtop and cabinet-based configurations, offering flexible integration for labs looking to eliminate sample loss for quantification and purity check from their workflow.

How it works.

MEASUREMENT PRINCIPLES

The Frida Reader measures the UV absorbance of a liquid through a hanging drop at the end of a pipette tip. As the amount of light absorbed depends on the optical path length through the drop, it is vital that drops are consistently sized and accurately positioned within the optical path.

Hanging drop assessment

The reader uses the optical set-up shown in Figure 1 (yellow lines) to accurately determine the position and diameter of sample drops. Prior to nucleic acid measurement, each drop is illuminated by two orthogonally-positioned LEDs, and two mirrors (M1 and M2) are used in combination with a beam splitter (BS) and objective to generate two separate images using the built-in CMOS camera. Automated comparison of these images allows drop size and position to be rapidly assessed and adjusted via closed loop feedback to the Fluent workstation's Air FCA.

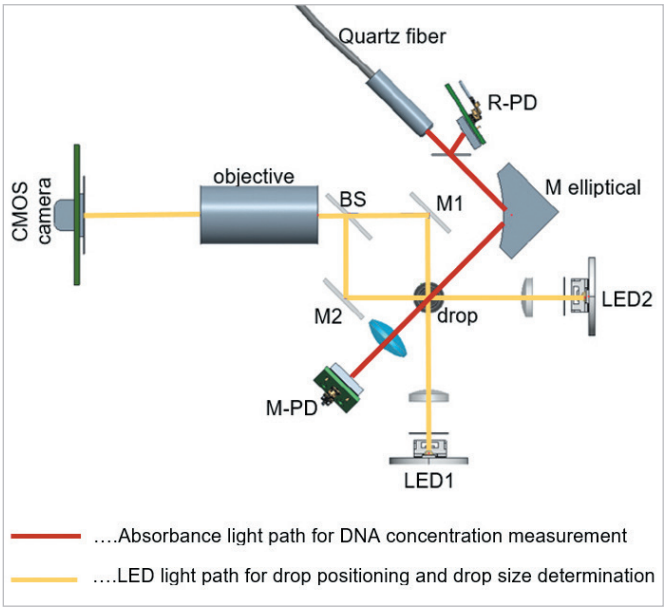


Figure 1: Frida Reader's optical set-up for both hanging drop size and position assessment (yellow lines) and nucleic acid quantification/ purity assessment (red lines).

Sample measurement

Following hanging drop assessment and positioning, UV light from a monochromator equipped with a xenon flash lamp is used to measure the OD of the sample (red lines in Figure 1). The UV light is guided with a quartz fiber and focused on the drop with an elliptical mirror (M elliptical). After passing through the drop, the light is focused with a lens and detected with a photodiode (M-PD). In addition, a small part of the light beam is split directly after the fiber output, and used to provide a reference with another photodiode (R-PD).

Quantification and purity assessment

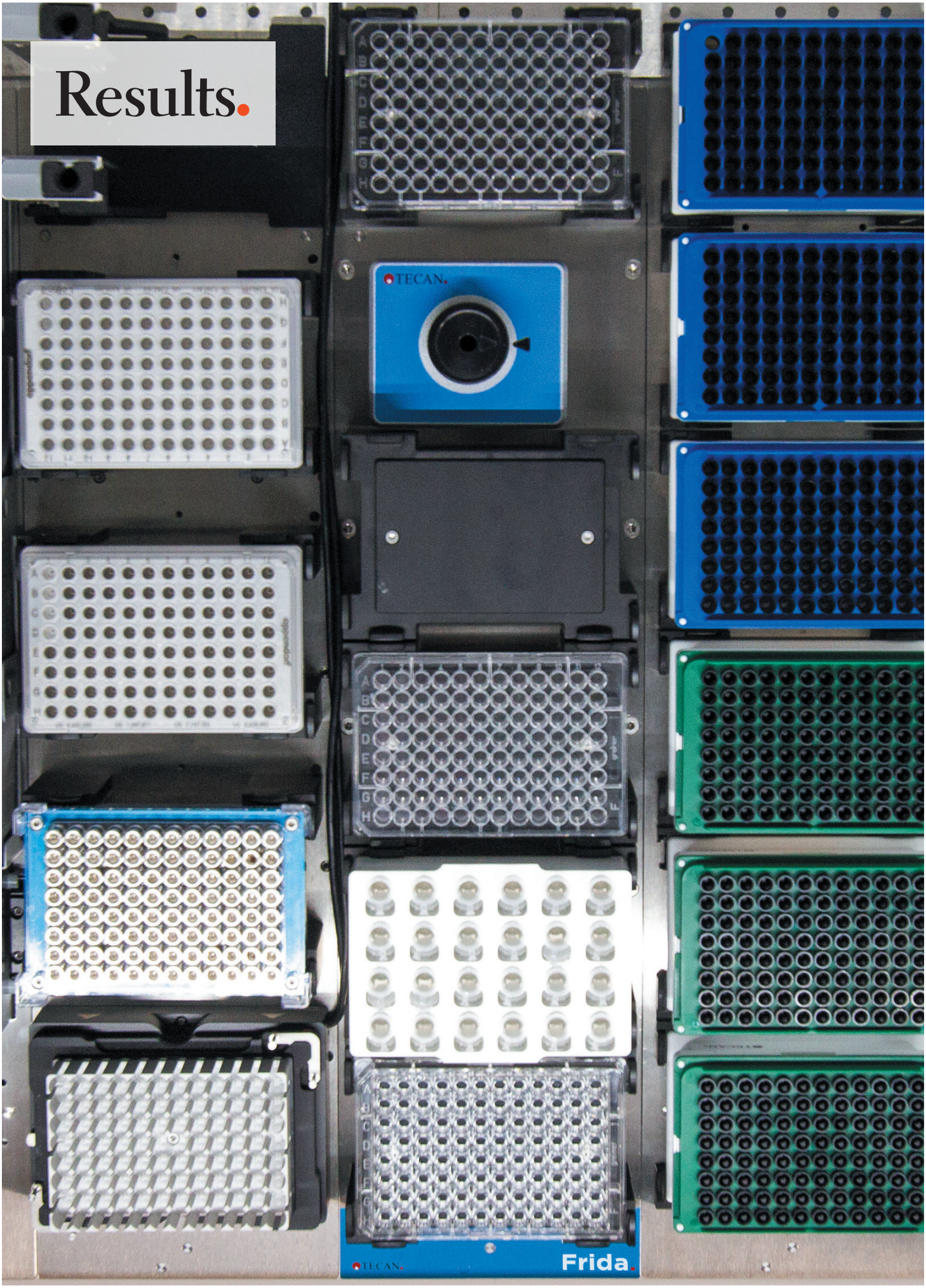
The OD values at 260 and 320 nm are compared to a DNA-free control (blank) drop to determine the DNA concentration (C) in the sample. The DNA concentration can be determined using the known extinction coefficient (ε) of nucleic acids, and the diameter of the drop (d):

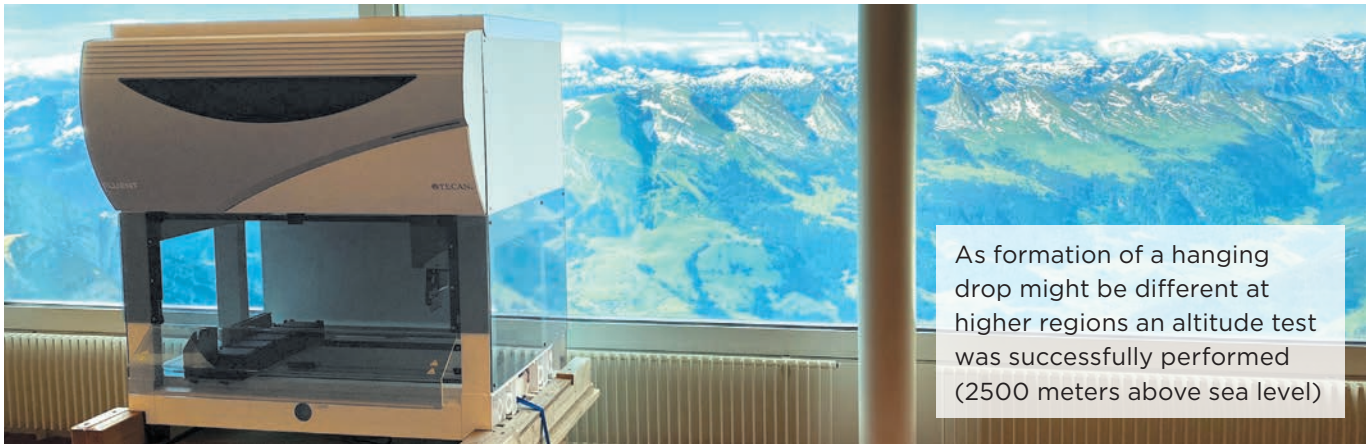
$$C = \frac{OD_{260,corr}}{d \cdot \varepsilon}$$

$$OD_{260,corr} = (OD_{260,sample} - OD_{260,blank}) - (OD_{320,sample} - OD_{320,blank})$$

In addition, OD values at 230 and 280 nm are used to provide an idea of sample purity. This fully automated process, including droplet assessment and nucleic acid quantification and purity assessment, takes around 10 seconds for a single sample (without blank measurement).

Results.





As formation of a hanging drop might be different at higher regions an altitude test was successfully performed (2500 meters above sea level)

PERFORMANCE

Example CV determination

A 96-well plate was filled with aliquots of herring sperm dsDNA (Promega, #D1815) dissolved in 1x TE buffer (10 mMol Tris, 1 mMol EDTA) to a nominal concentration of ~5 ng/µl. The Frida Reader quantification protocol

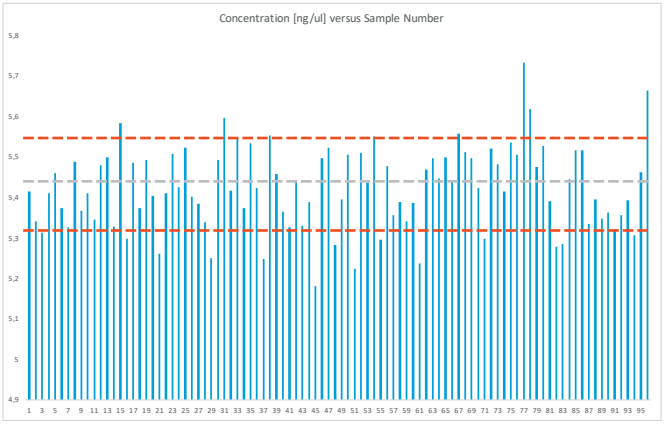


Figure 2: Quantification performance of Frida Reader for 5 ng/µl herring sperm dsDNA. 96 separate aliquots were measured, giving a mean concentration of 5.42 ng/µl (dotted black line), with standard deviation of ±0.10 ng/µl (dotted red lines), and a CV of 1.87 %.

was used to determine the average concentration and CV values across all 96 samples. The results (Figure 2) showed a mean concentration of 5.42 ng/µl (standard deviation = ±0.10 ng/µl), with a CV of just 1.87 % – well below the target CV of 10 %.
The %CV was also determined across multiple runs (n=24) using two Frida Readers integrated in separate Fluent platforms under dfferent environmental conditions. Various concentrations of herring sperm dsDNA were tested, using the same experimental set-up as previously, demonstrating instrument performance well in excess of the target CVs at all concentrations (Table 1).

Example accuracy determination

Various concentrations of DNA from different suppliers were measured in a reference spectrophotometer, two Tecan multimode microplate readers and three different Frida Readers to compare measurement accuracy.*
The results shown in Table 2 and Figure 3 show a good correlation between the Frida Readers, Tecan multimode readers and reference spectrophotometer, demonstrating the suitability of the Frida Reader approach for nucleic acid quantification.

* Published typical performance values represent the average observed factory tested values, and do not represent specified accuracy performance.

Typical values for accuracy performance FRIDA

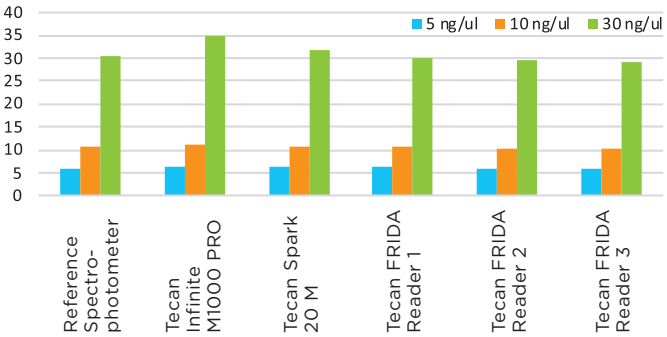


Figure 3: Comparison of Frida Reader measurement accuracy against a reference spectrophotometer and Tecan multimode microplate readers.

Target Conc. [ng/µl]	Target CV [%]	CV [%]
5	10	2.45
10	6	1.58
30	2	0.81
1,000	2	0.95

Table 1: Frida Reader quantification performance at varying concentrations of herring sperm dsDNA.

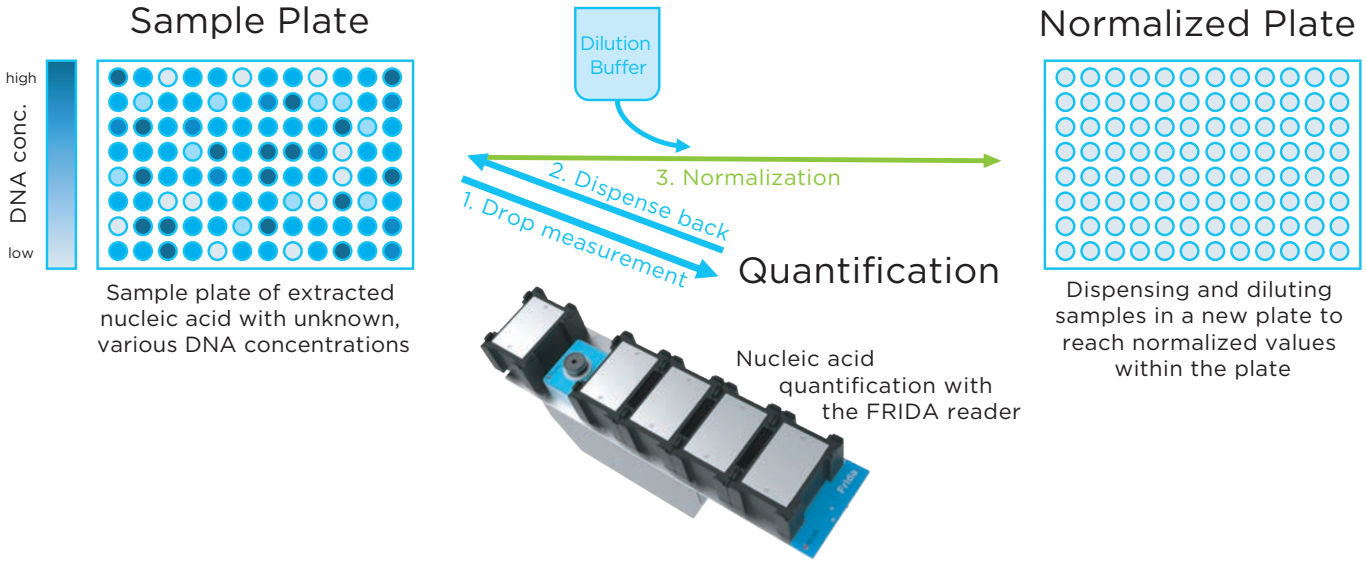
Nominal target DNA concentration from supplier (ng/µl)	Reference spectro-photometer (ng/µl)	Tecan Infinite® M1000 PRO (ng/µl)	Tecan Spark® 20 M (ng/µl)	Frida Reader #1 (ng/µl)	Frida Reader #2 (ng/µl)	Frida Reader #3 (ng/µl)	Average difference between Frida Reader and reference spectrophotometer (%)
5	5.7	6.2	6.2	6.1	5.7	5.8	2.8
10	10.5	11.2	10.7	10.6	10.3	10.2	2.1
30	30.4	34.9	31.7	30.1	29.5	29.3	2.9
300	302.7	N/A	N/A	299.5	301	301.1	0.7
800	790.1	N/A	N/A	772.6	773.1	766.3	2.5

Table 2: Comparison of Frida Reader dsDNA concentration accuracy against a reference spectrophotometer and Tecan multimode microplate readers.

Workflow.

INTEGRATED QUANTIFICATION AND NORMALIZATION

The combination of the Frida Reader and Fluent Automation Workstation allows walkaway quantification and normalization of 96 samples (including blank and blank buffer measurement) in around 40 minutes. The illustration below describes the workflow:



The protocol begins with measurement of the reader background and buffer blank, which are then valid for all samples. Samples are then processed column-by-column, ie. samples 1 to 8 are quantified and normalized, then the process is repeated for each column of 8 samples until all 96 samples are complete. The processing steps are:

1. Sample drop measurement

The Air FCA picks up 50 µl filtered disposable tips for all 8 channels, then aspirates 10 µl of DNA sample from the first well in the column. The sample is measured in the Frida Reader, then dispensed back into the original source well before the next sample is aspirated.

2. Normalization calculation

FluentControl™ software uses the quantification data to calculate the correct volumes of the samples and diluents that need to be dispensed to achieve the target concentration and target volume.

3. Individual volume transfers

The same disposable tips are then used to transfer the calculated volumes from the Sample Plate to the Normalization Plate, before discarding the used tips.

4. Diluent transfer

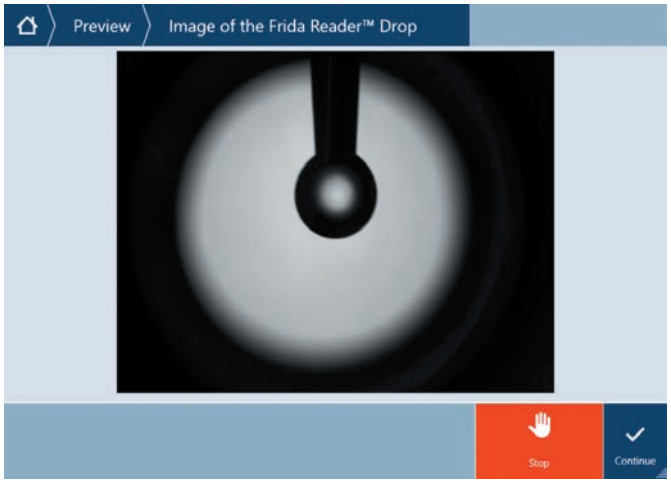
The Air FCA picks up new 200 µl filtered disposable tips with all 8 channels, and transfers the calculated volumes of diluent to the Normalization Plate from a 100 ml trough. The same tips are used to mix the samples and diluent, then discarded.

Seamless Integration.

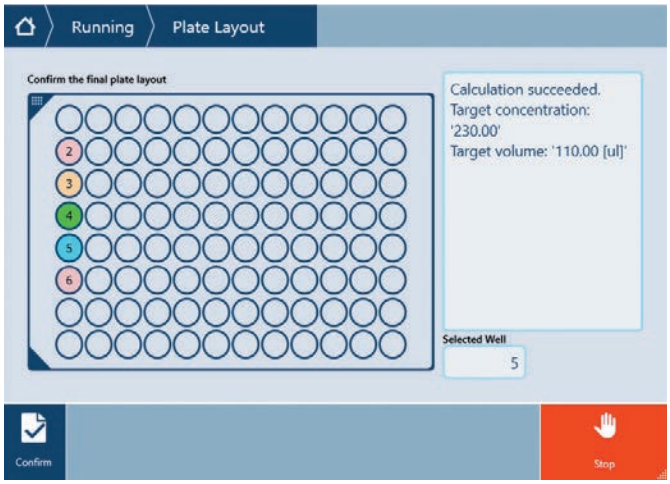
EFFORTLESS CONTROL

The Frida Reader is completely integrated into FluentControl, allowing all measurement and normalization parameters to be adjusted directly in the Fluent workstation's operating software. The reader is supplied with an example method. Please make a copy of this method in order to customize it to accommodate and validate your workflow according to your desired intended use and laboratory protocols.

For complete peace of mind, FluentControl provides real-time imaging of the hanging drop within the measurement chamber during measurements:



The system also automatically displays the progress of the protocol on a verification map after a column has been processed, making it easy to visualize quantification results during processing:



None: No sample liquid detected
Blue: Selected well
Green: Final concentration and volume corresponds exactly with targets
Yellow: Target concentration and volume can be reached within tolerances
Red: Target concentration and volume cannot be reached

"We had the unique chance to beta test the new Frida Reader for Tecan prior to the launch of the product. The seamless integration into FluentControl was simple and the nice layout was easy to follow. Overall we were very happy and impressed by this new, innovative product and the new possibilities it provides as regards the quantification of nucleic acids without sample loss."

Dr. Marc Brehme, CTO, Ribbon Biolabs GmbH

ribbon BIOLABS

www.ribbonbiolabs.com

Specifications+ for absorbance measurement

Light source	Dedicated xenon flash lamp
Operating wavelengths	230, 260, 280 and 320 nm
OD range	6 mOD to 3.2 OD
Corresponding to a concentration range	2 to 1,000 ng/µl for dsDNA, and 2 to 800 ng/µl for RNA
Concentration range (nucleic acids)	2 to 1,000 ng/µl
Wavelength accuracy	≤0.8 nm
OD accuracy (260 nm) @ 1 OD	≤2.5 %
OD precision (260 nm) @ 1 OD	≤2.0 %

Reproducibility values in single hanging drop (measured)

dsDNA concentration	Equivalent RNA concentration	CV limit
5-10 ng/µl	4-8 ng/µl	≤10 %
>10-30 ng/µl	>8-24 ng/µl	≤6 %
>30-1,000 ng/µl	>24-800 ng/µl	≤2 %

The CV value reflects the reproducibility of the measurement:

- Determined in absorbance measurements
- On fluid drop samples with 1.5 to 1.6 mm diameter
- With Tecan 50 µl filtered disposable tips
- Nucleic acid dissolved either in TE buffer or water
- Assuming a 2-sigma confidence interval

Nominal reproducibility compared to a 10 mm cuvette

Measurement reproducibility within a 1.5 to 1.6 mm drop size, calculated to an OD 10 mm (ie. path length = 10 mm) value:

- ≤100 ng/µl dsDNA (80 ng/µl RNA) measurement range: ±2 ng/µl
- >100 ng/µl dsDNA (80 ng/µl RNA) measurement range: ±1.5 ng/µl

+ Specifications are subject to change.

The Frida Reader is intended for use on the Fluent Automation Workstation, which is intended for general laboratory use.

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