

Semi-automated spectrophotometric DNA quantification using the ASSIST PLUS pipetting robot

Introduction

Microvolume UV/Vis spectrophotometers are commonly used for nucleic acid quantification and quality analysis. They require very small sample volumes (usually 1-2 µl) and can determine nucleic acid concentration in the 2-8000 ng/µl concentration range. The ratio of absorbance at 260 and 280 nm, and at 260 and 230 nm, is used to assess DNA purity. Implen has developed a 12 channel microvolume spectrophotometer called the NanoPhotometer® N120, which can be used to determine dsDNA, ssDNA, RNA, mRNA, miRNA, oligos, protein and antibody concentrations. It can be

operated as a stand-alone device either in the laboratory or remotely, due to an integrated battery.

Thanks to its small footprint, the NanoPhotometer N120 can be placed directly on the ASSIST PLUS pipetting robot, allowing samples to be automatically transferred to the spectrophotometer, making nucleic acid measurements even easier and less error prone.

Key benefits:

- It is possible to measure 12 samples at a time with the N120, allowing the user to analyze 96 samples up to 126 times faster, with only 33 % of the operational steps that are required for microplate readers.
- It can be challenging to keep the perfect pipetting height with a 12 channel pipette when sample transfers are performed manually. This task is easy for the ASSIST PLUS pipetting robot, providing reproducible results.
- With the VOYAGER adjustable tip spacing pipette, samples from a 96 well plate can be transferred to the NanoPhotometer N120 12 times faster than with a single channel pipette.
- The final DNA concentration values can be easily copied to the normalization worksheet of VIALAB, and a subsequent [normalization](#) can be automated on the ASSIST PLUS.

Overview: How to measure DNA concentration efficiently

In this application note, we show how to measure DNA concentration with the NanoPhotometer N120 on the ASSIST PLUS pipetting robot (**Figure 1**). The touchscreen of the N120 is removed, so that the ASSIST PLUS can freely move between the spectrophotometer and the waste bin. The N120 is operated via the NanoPhotometer software, which can be installed on a compatible PC and connected to the instrument via WiFi.



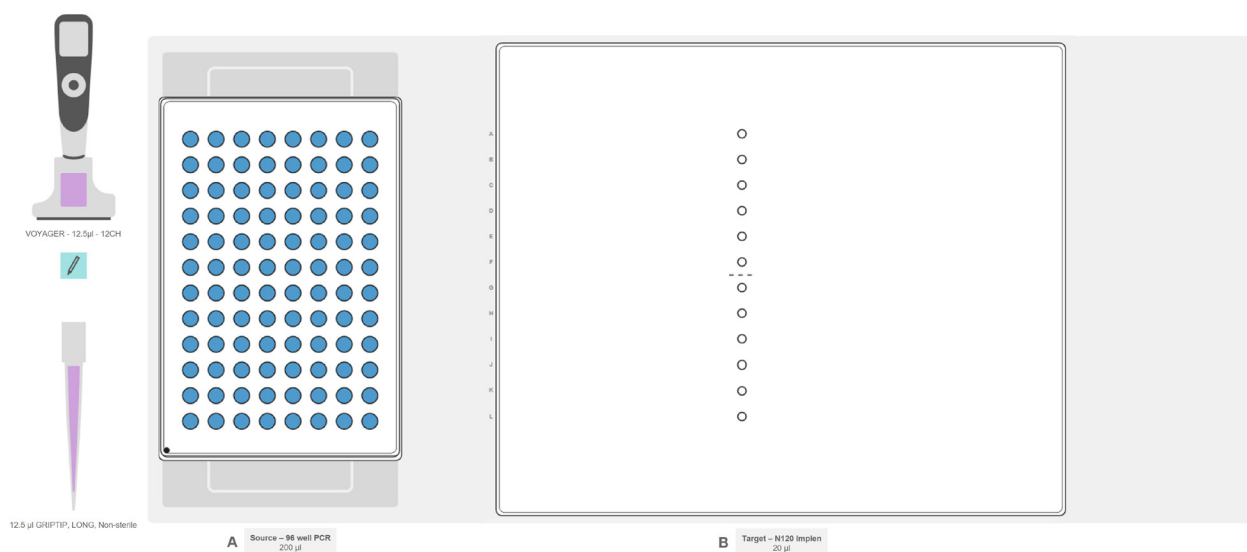


Figure 1: Deck set-up for DNA concentration measurement. Position A: Source – Sapphire 96 well PCR plate on a cooling block. Position B: Target – NanoPhotometer N120 on a black board. Position C: Empty.

Step by step procedure:

The DNA concentration measurement protocol consists of two steps:

1. Blank measurement
2. DNA concentration measurement

Experimental set-up

Deck position A: 96 well plate with the DNA samples on a cooling block

Deck position B: NanoPhotometer N120

Deck position C: Empty

1. Blank measurement

STEP: The blank measurement is done with an elution buffer.

Select the method on the software by tapping on the nucleic acids icon, which will open the method immediately. In the parameter setting, select the multi-sample application, dsDNA, the unit ng/µl and horizontal sample loading. The sample map will then appear. This set-up allows the user to measure 12 samples at a time. A blank measurement of elution buffer (or water) is required before sample measurements can be taken. To do this, manually pipette 2 µl of elution buffer into the sample windows on the pedestal (**Figure 2**), close the lid and select blank on the software to initiate the reading.



Figure 2: Manual sample loading onto the NanoPhotometer N120 with the help of the positioning guide.

After the reading is complete, open the lid, clean the sample windows on the pedestal and mirrors in the lid arm with a lint-free laboratory wipe. The instrument is now ready to measure the concentration of the DNA samples.

2. DNA concentration measurement

STEP: The transfer and measurement of 96 DNA samples.

HOW TO: If the lid is open and the sample windows and mirrors are clean, start the VIALAB program 'Nucleic acid concentration Implen'. The ASSIST PLUS pipetting robot will start to transfer the first 12 samples (2 µl each) from the 96 well PCR plate (A1-A12) to the sample windows in a single repeat dispense step (**Figure 3**). When the transfer is complete, the pipette informs the user to measure the concentration. Close the lid and press the sample button on the software to initiate the DNA concentration measurement or select AutoSample to start the measurement automatically each time the lid is closed. After the measurement is complete, lift the lid arm and clean the windows and mirrors with a lint-free tissue. The ASSIST PLUS transfers the next samples (up to 96 samples in total) in the same way. To avoid any bubble formation by blow-out, repeat dispense steps were programmed in VIALAB. For precise transfer of low volume samples, 0.5 µl pre- and post-dispense, tip touch and slow dispense speed (set to five) should be used.



Figure 3: Automated sample loading onto the NanoPhotometer N120 with the ASSIST PLUS pipetting robot.

At the end of the measurement, click on the 'Save as type' option on the software. With this option, the data can be saved as a .csv file, so that it is possible to copy the data to a VIALAB worklist for concentration [normalization](#).

Results

We analyzed the DNA concentrations of three replicates of 96 different samples both automatically and manually, to highlight how much easier the process is using the ASSIST PLUS. The DNA concentrations decrease column 1 to column 12 of the 96 well plate (concentration values can

be found in the Appendix). We saw lower standard deviation (SD) values for the automatically transferred samples compared to the manually transferred ones (**Figure 4**). The ASSIST PLUS pipetting robot also required half of the time that was needed for manual sample transfers.

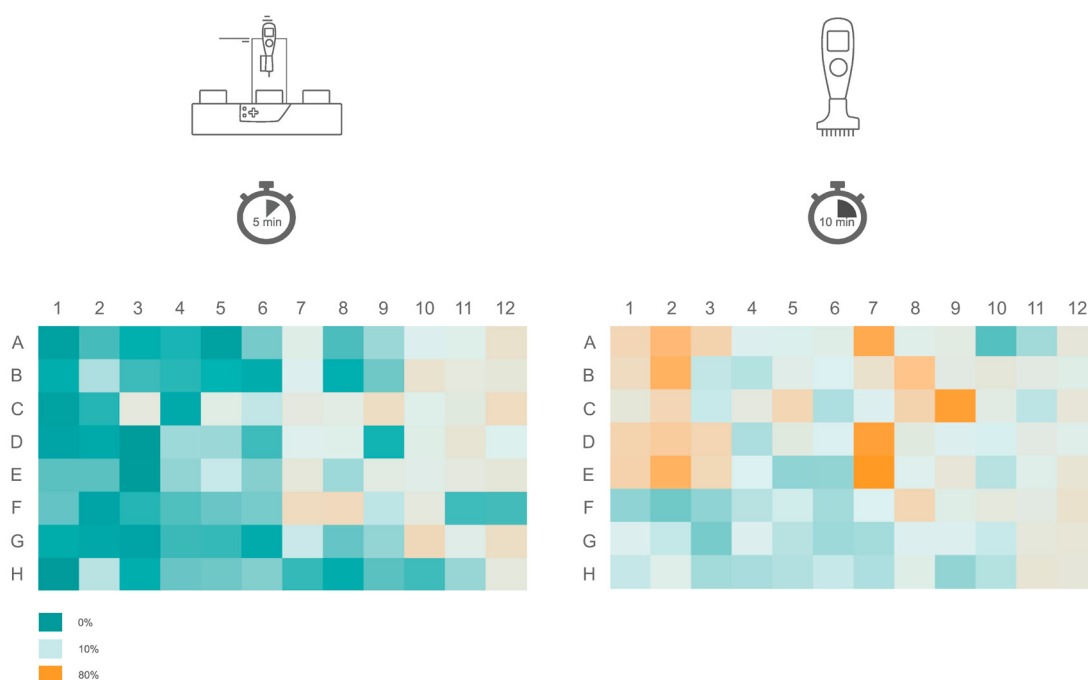


Figure 4: Heatmap analysis of the standard deviation (in %) of three parallel measurements of a 96 well plate with DNA samples. The samples were transferred automatically (left) and manually (right).

Remarks

- **VIALAB software:** VIALAB programs can be easily adapted to the user's specific labware and protocols.
- **Normalization:** the ASSIST PLUS can be equipped with a D-ONE single channel pipetting module for samples that need to be normalized after DNA concentration measurements, allowing full walk-away normalization on the same pipetting robot.

Conclusion

- The combination of the ASSIST PLUS pipetting robot, VOYAGER automatic tip spacing electronic pipette and NanoPhotometer N120 provides an easy and precise solution for nucleic acid quantification.
- Implen's NanoPhotometer product line offers a complete solution for nanovolume applications. The NanoPhotometer N120 is a multi-sample model that can measure up to 12 samples at a time.
- Fast, efficient, error free and reproducible sample transfers are easily accomplished using the ASSIST PLUS pipetting robot.
- The DNA concentration measurements for 96 samples can be performed 126 times faster than with a microplate reader, thanks to the 12 channel VOYAGER and N120.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4731	VOYAGER 12 channel 12.5 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager
INTEGRA Biosciences	6475	12.5 µl Sterile, Filter, SHORT GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6405	12.5 µl Sterile, Filter, LONG GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6250	PCR 96 well cooling block	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
Implen	N120	NanoPhotometer N120	https://www.implen.de/product-page/implen-nanophotometer-n120-12-channel-nanovolume-spectrophotometer/
Greiner Bio-One International	652270	Sapphire microplate, 96 well, PP	https://shop.gbo.com/en/switzerland/products/bioscience/molecular-biology/pcr-microplates/652270.html

Appendix

Automated sample transfer												
ng/µl	1	2	3	4	5	6	7	8	9	10	11	12
A	478.4	619.2	489.7	111.9	109.8	113.3	80.7	69.4	74.5	4.2	4.4	5.1
B	453.0	568.2	462.7	110.2	108.3	109.3	74.1	64.4	70.5	4.8	4.2	5.4
C	505.9	690.3	565.5	134.2	142.3	123.9	66.0	69.4	63.9	4.0	4.0	4.6
D	496.7	614.7	495.1	118.0	116.4	112.9	70.2	72.2	66.5	4.3	5.0	5.6
E	515.9	592.1	486.6	122.6	129.7	125.2	76.1	69.5	73.3	4.1	4.0	3.5
F	530.1	640.4	505.2	120.1	116.4	115.1	82.6	73.5	67.6	5.7	4.7	6.2
G	508.5	634.9	486.8	123.2	119.2	119.8	60.6	60.6	61.3	5.6	4.1	5.3
H	481.2	534.0	450.1	117.6	118.2	122.9	58.9	57.3	58.9	4.7	4.9	6.2

Manual sample transfer												
ng/µl	1	2	3	4	5	6	7	8	9	10	11	12
A	375.3	344.4	381.6	92.2	92.6	91.4	43.7	62.0	64.5	3.5	3.6	3.6
B	380.2	376.5	437.7	98.2	96.4	100.8	50.6	47.7	61.4	4.4	3.6	4.0
C	439.9	593.6	484.6	110.2	124.6	104.3	66.2	55.5	85.9	3.5	3.4	3.9
D	371.9	284.0	408.5	98.3	93.4	98.6	41.5	53.3	60.6	3.6	3.6	3.7
E	323.8	334.2	399.0	110.6	107.2	107.1	39.1	62.2	66.8	3.2	3.3	3.6
F	498.0	612.0	465.1	103.6	105.9	103.4	59.2	67.5	60.8	3.4	3.3	3.7
G	481.5	600.6	465.4	112.2	110.3	114.3	58.1	56.5	57.2	3.2	3.1	3.3
H	455.0	519.1	422.2	97.8	98.0	96.7	50.5	50.8	49.7	3.6	3.3	3.7

Table 1: Measured DNA concentration values.

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