



NanoPhotometer[®] **N120/NP80/N60/N50/C40**

User Manual

Version 4.3.4

Software Version 4.3.15670





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1. NANOPHOTOMETER® AT A GLANCE

NANOPHOTOMETER® OVERVIEW

The Implen NanoPhotometer® is a mobile, simple to use UV/Visible spectrophotometer with a CCD array detector with options ranging from NanoVolume to standard cuvette. The NanoPhotometer® family comprises five different models.

There are four single sample models available:

The NP80 spectrophotometer is a combination of NanoVolume and cuvette option, the N60 and N50 are NanoVolume spectrophotometers only and the C40 is a standard cuvette solution with the option to upgrade to NanoVolume measurements.

The NanoPhotometer® N120 is a multi-sample spectrophotometer which can measure up to 12 NanoVolume samples simultaneously.

The NanoPhotometer® runs on a Linux-based operating system (NPOS) that is designed for the use of pre-programmed and custom applications with a high degree of flexibility and processing power.

Sample Compression Technology™ provides easy sample handling which is independent of surface tension. This technology squeezes the sample between two quartz surfaces allowing for unmatched precision and accuracy without the need for dilutions. Combined with our True Path Technology™ the system offers lifetime accuracy and precision without the need for maintenance or recalibration.

Note: It is recommended to use a properly calibrated pipette with high-quality tips to ensure delivery of appropriate sample volumes for NanoVolume sample applications.

Sample Control™ is a quality control technology that identifies air bubbles, sample impurities, turbidity, lint residues and potential contaminations. Sample Control™ monitors handling characteristics and sample quality in real time to ensure that the measured concentrations are reproducible and most precise.

Blank Control™ gives a warning message for blanks with high background. High background absorbance can be caused by a contaminated blank, blank buffer or by residues from previous users. Insufficient blank readings are the main cause for inaccurate measurements. Blank Control™ will protect the user from wasting time and precious sample on inaccurate readings caused by high background blanks or inappropriate cleaning. For more detailed information see also Technical Note #1 Blank Control™.

NANOPHOTOMETER® N120



Item Number	Touchscreen	Battery Pack
N120-Touch	+	-
N120-Mobile	+	+

NANOPHOTOMETER® NP80



Item Number	Touchscreen	Battery Pack
NP80	-	-
NP80-Touch	+	-
NP80-Mobile	+	+

NANOPHOTOMETER® N60

Off Switch

7" Color Touch Screen

Illuminated Sample Window

Low Vibration Vortexer

Front USB Port



Item Number	Touchscreen	Battery Pack
N60	-	-
N60-Touch	+	-
N60-Mobile	+	+

NANOPHOTOMETER® N50

Off Switch

7" Color Touch Screen

Illuminated Sample Window



Front USB Port

Item Number	Touchscreen	Battery Pack
N50	-	-
N50-Touch	+	-

NANOPHOTOMETER® C40

Off Switch

7" Color Touch Screen

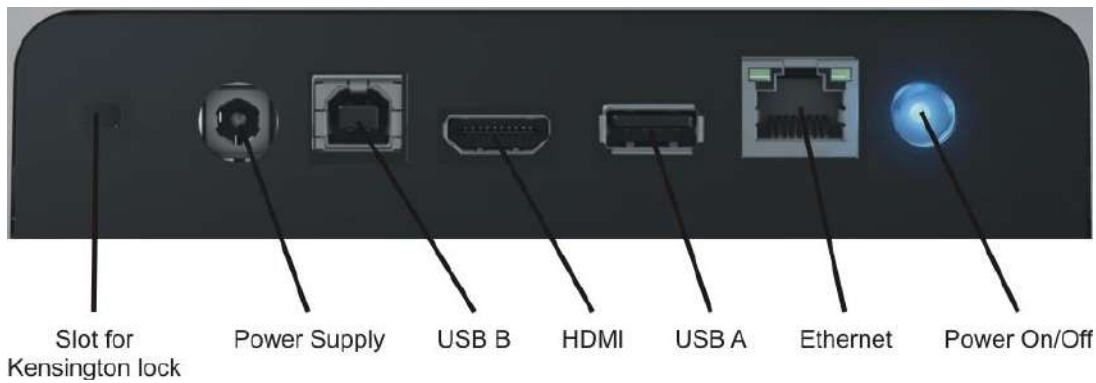
Temperature Controlled Cuvette Holder



Front USB Port

Item Number	Touchscreen	Battery Pack
C40	-	-
C40-Touch	+	-
C40-Mobile	+	+

INSTRUMENT REAR PANEL (N120/NP80/N60/N50/C40)



To boot/shut down the NanoPhotometer® push shortly (< 1 second) on the power on/off button at the back of the NanoPhotometer®.

Note: A long push (> 3 seconds) initiate a hard reset. Only activate a hard reset of the NanoPhotometer® when necessary. To avoid unnecessary hard reboots, it is recommended to power down the unit from the onboard touch screen by pushing on the power button (⏻) in the bottom left corner of the home screen.

INSTRUMENT BOTTOM VIEW (N120/ NP80/N60/N50/C40)



Model name, device serial number and FCC ID are located on the identification plate on the bottom of the instrument.

ACCESSORIES

STANDARD ACCESSORIES

- **Connecting Cable**



USB connecting cable to connect the NanoPhotometer® to a computer to control the NanoPhotometer® via computer (please see page 26 Software Installation).

- **NanoPhotometer® Power Adapter**



Power adapter for the NanoPhotometer® and for charging the optional built-in battery pack.

Note: Use only the power adapter supplied with your instrument or a replacement part from the manufacturer or your supplier.

- **Dust Cover**

OPTIONAL ACCESSORIES

- **Didymium Glass Filter (C40/NP80)**



The certified didymium glass filter can be used to verify the wavelength and photometric accuracy of the NanoPhotometer® cuvette applications.

- **Standard Solution (N120, NP80, N60, N50)**



The NanoPhotometer® is recalibration free therefore it is not necessary to review the photometric accuracy on a regular basis. If the SOPs of a laboratory require a routine control of the photometric accuracy standard solutions can be used.

Note: Specifications of the standard solution are guaranteed for at least one year. Please see expiration date. Once a vial is opened it can be used for 30 minutes.

Note: Please read the Material Safety Data Sheet carefully prior to using this product.

- **IQ/OQ documentation (N120, NP80, N60, C40)**

The NanoPhotometer® IQ/OQ package consists of a Standard Solution and/or a Didymium Glass Filter and a corresponding Excel-based software tool depending on the type of instrument. The Excel-based software produces an automated report displaying all relevant data for an audit.

Note: The IQ/OQ documentation is not available for the NanoPhotometer® N50.

- **Submicroliter Cell (C40 only)**



The submicroliter cell provides the option of upgrading the NanoPhotometer® C40 to a device that handles NanoVolume samples as well as cuvette samples. The sample volume range of the submicroliter cell ranges from a minimum of 0.3 µl to 5 µl with the path length options including 0.04 mm, 0.1 mm, 0.2 mm, 1 mm and 2 mm which correspond to virtual dilution factors of 1:250, 1:100, 1:50, 1:10, and 1:5 respectively.

- **DiluCell™ (C40/NP80 only)**



The DiluCell™ is a disposable cuvette with shortened path length for virtual dilution of cuvette-based samples. Due to the reduced path length the DiluCell™ provides an automatic dilution without the need of a physical dilution of higher concentration samples. There are two different sizes of DiluCells available DC 10 and DC 20 which allow an automatic 1/10 and 1/20 dilution of the sample respectively. Bypassing manual sample dilutions reduces dilution errors and cross-contamination making DiluCell™ ideal for GLP. Combined with small sample volume requirements and bubble free filling, the DiluCell™ allows for convenient spectrophotometric analysis from 340 - 950 nm.

▪ Field Kit

Implen aluminum roller case for safe transportation, designed to fit in most airplane overhead bins; special compartments for all necessary accessories, cleaning tools and samples.



Note: Delivered w/o NanoPhotometer® and accessories

Note: Operate the NanoPhotometer® in the roller case only when cover is open. Ensure that air circulation is possible. Always switch NanoPhotometer® off for transportation.

▪ Barcode Reader

It is possible to import sample names from 1D and 2D barcodes. Connect a compatible barcode reader to the USB port of the NanoPhotometer® and push on the sample name input window. After scanning a barcode the sample name will be shown in the input window. The imported name can then be edited or replaced completely.

Barcode readers that have been tested and verified compatible:

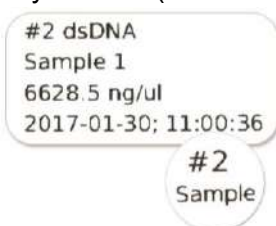
- 1D: Honeywell Voyager 95X0 Single-Line Laser Scanner
Datalogic Touch65
- 1D & 2D: AGPtEK SC36
Honeywell Xenon 1900g

▪ DYMO Label Printer

It is possible to connect a DYMO Label printer to the NanoPhotometer® for direct printing on standard or cryo labels. Recommended and tested printers are the DYMO LabelWriter 4XL (label size 10.3 x 15.8 cm) and the DYMO LabelWriter 450 (5.4 x 10 cm). Cryo labels can be printed with both DYMO printers using the following label format: 26 x 12.7 mm and 9.5 mm circle (landscape).

Note: After starting the NanoPhotometer®, plug in the DYMO printer. The home screen will be shown. Wait at least for 30 seconds for driver installation.

Cryo labels (26 x 12.7 mm and 9.5 mm circle):



Note: Orientation of cryo labels on carrier foil needs to be landscape.

Implen NanoPhotometer®	
Instrument	NP80
Version	NPOS 1.1 build 11107
Serial Number	M80700
Self-test passed	yes; 2015-11-27; 14:22
Parameter	
Method	dsDNA
Mode	NanoVolume
Volume (ul)	1-2
Units	ng/ul
Nucleic Acid Factor	50.00
Background Correction	On
Manual Dilution factor	1.000
# 1	
Date and Time	Blank 1
	2015-11-27; 15-51-48
Concentration	0.0000
A230 (10 mm)	0.000
A260 (10 mm)	0.000
A280 (10 mm)	0.000
A320 (10 mm)	0.000
A260/A280	0.000
A260/A230	0.000
# 2	
Date and Time	Sample 1
	2015-11-27; 15-52-18
Dilution	15.0
Concentration	533.55
A230 (10 mm)	4.726
A260 (10 mm)	10.72
A280 (10 mm)	7.615
A320 (10 mm)	0.049
A260/A280	1.410
A260/A230	2.282
Result 1 / 2	2015-11-27 15:53:28

Printout for DYMO LabelWriter 4XL

Implen NanoPhotometer®	
Instrument	NP80
Version	NPOS 1.1 build 11107
Serial Number	M80700
Self-test passed	yes; 2015-11-27; 14:22
Parameter	
Method	dsDNA
Mode	NanoVolume
Volume (ul)	1-2
Units	ng/ul
Nucleic Acid Factor	50.00
Background Correction	On
Manual Dilution factor	1.000
# 1	
Date and Time	Blank 1
	2015-11-27; 15-51-48
Concentration	0.0000
A230 (10 mm)	0.000
A260 (10 mm)	0.000
A280 (10 mm)	0.000
A320 (10 mm)	0.000
A260/A280	0.000
A260/A230	0.000
# 2	
Date and Time	Sample 1
	2015-11-27; 15-52-18
Dilution	15.0
Concentration	533.55
A230 (10 mm)	4.726
A260 (10 mm)	10.72
A280 (10 mm)	7.615
A320 (10 mm)	0.049
A260/A280	1.410
A260/A230	2.282
Result 1 / 2	2015-11-27 15:53:28

Printout for DYMO LabelWriter 450

Note: The printouts are optimized for the label size of the DYMO LabelWriter 4XL. All other DYMO printers can be used. However, the font size will be zoomed to utilized paper size.

▪ HP Printer

Printing from the NanoPhotometer® is possible via USB (HP printers) and through network connection. Network printing should be possible with AirPrint / IPP compatible printers supporting PDF format. For network printer settings see page 132 Network Printer.

Note: IPP version 2.2 is required and some printer configuration settings might need to be changed in order to allow communication with the NanoPhotometer®

The following HP printers have been tested and deemed compatible to print via USB connection:

- HP LaserJet 3030
- HP LaserJet m1522nf MFP
- HP LaserJet 400 color M451nw
- HP DeskJet 2543
- HP DeskJet 1110

Further HP printers are available upon request.

Note: After starting the NanoPhotometer®, plug in the HP printer. The home screen will be shown. Wait at least for 30 seconds for driver installation.

CONNECTIVITY

▪ USB A

There is a USB A port on the front as well as the rear panel of the NanoPhotometer® which is compatible with standard portable USB 2.0 storage devices (back) and USB 3.0 (front) for direct data transfer in a variety of formats including Excel. It is also possible to connect a mouse, keyboard, barcode reader, DYMO printer or HP printer directly to the NanoPhotometer®.

Note: We recommend using FAT/FAT32 formatted 2.0 USB flash drives. The USB flash drive size is due to the standard formatting currently limited to 32 GB. Encrypted USB flash drives are not compatible with the NanoPhotometer®.

Note: Cordless Bluetooth mice are not supported. Use wired mice only.

Note: Connect mouse and keyboard before starting the NanoPhotometer®.

▪ USB B

There is a USB B port located on the rear panel of the instrument which is compatible with the USB cable provided to connect the NanoPhotometer® to a computer. This USB connection can be used to control the NanoPhotometer® via computer.

▪ LAN

There is an Ethernet (LAN) connection port on the rear panel of the instrument which enables the NanoPhotometer® to connect with the local network. This Ethernet connection can be used for data transfer from the NanoPhotometer® to a local network, to control the NanoPhotometer® via a control device and network printing.

Data transfer is possible by saving on a defined network folder (see page 131 Network Folder) or on the NanoPhotometer® file server (see page 56 Data Transfer via File Server).

Note: Plug in the LAN cable before starting the NanoPhotometer®.

Note: The maximum LAN cable length is 10 meters. Bit rate is 1 Gbit/s

▪ WiFi

The NanoPhotometer® is equipped with WiFi, which can be used as a WiFi network or as a WiFi Hotspot. The WiFi network allows same functionality as the Ethernet connection including direct printing via AirPrint / IPP compatible printers supporting PDF format.

Note: IPP version 2.2 is required and some printer configuration settings might need to be changed in order to allow communication with the NanoPhotometer®

The WiFi Hotspot provides the option to control the NanoPhotometer® by other WiFi devices like computer, tablets or smartphones (tablets and smartphones not for N120).

WiFi Hotspot connection details:

SSID: NanoPhotometer® serial number

Password: Implenuser

Note: Due to the limitations of some handheld devices saving to a wireless device is limited to 40 measurements per dataset. Larger datasets can be saved on the NanoPhotometer® itself.

- **HDMI**

There is an HDMI port located on the rear panel of the NanoPhotometer® which is compatible with HDMI 1.4 cables (or better) to connect the NanoPhotometer® to HDMI compatible monitors.

Note: The maximum HDMI cable length is 5 meters.

NANOPHOTOMETER® SPECIFICATIONS

NanoVolume Performance

Detection Range dsDNA	N60, NP80: 1 - 16,500 ng/μl N50: 5 - 7,500 ng/μl N120: 2 - 8,000 ng/μl
Detection Range BSA	N60, NP80: 0.03 - 478 mg/ml N50: 0.15 - 217 mg/ml N120: 0.06 - 230 mg/ml
Sample Volume	N50, N60, NP80: 0.3 - 2 μl N120: 2 - 3.5 μl
Photometric Range (10 mm equivalent)	N60, NP80: 0.02 - 330 A N50: 0.1 - 150 A N120: 0.04 - 160 A
Path length	N50, N60, NP80: 0.67 and 0.07 mm N120: 1 and 0.125 mm
Dilution Factor	N50, N60, NP80: 15 and 140 N120: 10 and 80
Vortex	N60, NP80: 2,800 rpm; tube size up to 2.0 ml

Cuvette Performance – NP80 & C40

Detection Range dsDNA	0.1 - 130 ng/μl
Detection Range BSA	0.003 - 3.7 mg/ml
Photometric Range	0 - 2.6 A
Center Height (Z-Height)	8.5 mm
Cell Types	outside dimension 12.5 x 12.5 mm
Heating	37°C ± 0.5°C

Optical Specifications

Wavelength Scan Range	C40, N60, NP80, N120: 200 – 900 nm N50: 200 - 650 nm
Measure Time For Full Scan Range	C40, N50, N60, NP80: 2.5 – 4.0 seconds N120: 1.7 – 2.5 seconds per sample
Wavelength Reproducibility	C40, N60, NP80, N120: ± 0.2 nm N50: ± 1nm
Wavelength Accuracy	C40, N60, NP80, N120: ± 0.75 nm N50: ± 1.5 nm
Bandwidth	C40, N60, NP80: 1.8 nm N50: 5 nm N120: < 2.5 nm
Absorbance Reproducibility	C40, NP80 (cuvette): < 0.002 A @ 0 - 0.3 A @ 280 nm CV < 1% @ 0.3 – 2.0 A @ 280 nm N60, NP80 (Lid 15): < 0.002 A @ 0 – 0.3 A @ 280 nm CV < 1% @ 0.3 – 1.7 A @ 280 nm

	N50 (Lid 15):	< 0.004 A (0.67 mm path) @ 280 nm CV < 1% @ 0.3 – 1.5 A @ 280 nm
	N120 (Lid 10):	< 0.004 A @ 0 – 0.3 A @ 280 nm CV < 0.4% @ 0.8 A @ 280 nm
Absorbance Accuracy		< 1.75 % @ 0.7 A @ 280 nm of the reading
Stray Light		N60, NP80, C40: < 0.5% @ 240 nm using NaI N50: < 2% @ 240 nm using NaI N120: < 1% @ 240 nm using NaI
Optical Arrangement		1 x 3648 CCD Array
Lamp		Xenon flash lamp
Lifetime		10 ⁹ flashes, up to 10 years

Processing Power and Compatibility

Operating System	Linux based OS
Onboard Processor	Intel Celeron dual core 2.4 GHz
Internal Data Storage	C40, N50, N60, NP80: 32 GB N120: 128 GB
Software Compatibility	Windows 8, 10 (32 & 64 bit), OS X (Intel x86 und Apple M1), iOS and Android OS

General Specifications

Main Body Size	200 mm x 200 mm x 120 mm
Weight	3.8 – 5.2 kg depending on configuration
Operating Voltage	90 - 250 V ± 10%, 50/60 Hz, 90 W, 18/19 VDC
Display	1024 x 600 pixels; touchscreen glove compatible
Built-in Battery Pack	Optional rechargeable lithium ion battery: C40, N60, NP80: 95 Wh, 6.6 Ah; 8 h N120: 47.5 Wh, 3.3 Ah, 3 h Min. charging cycles: 800
Certifications	CE, IEC 61010-1:2012 and EN 61326-1:2013
Battery Certification	IEC 62133 and UN38.3 transport test
In- and Output Ports	2x USB A, USB B, HDMI, Ethernet, WiFi
Security	Slot for Kensington lock

Features and specifications are subject to change without notice.

US Patents 20080204755 and 20080106742

Windows is a trademark of Microsoft. Mac OS & iOS are trademarks of Apple, Inc. Android OS is a trademark of Google. Linux is a trademark of Linus Torvalds.

2. GETTING STARTED

SPECTROPHOTOMETER INSTALLATION

Note: Translations of the Safety Information and Unpacking and Positioning are available in Appendix 1 (Czech), Appendix 2 (Dutch), Appendix 3 (French), Appendix 4 (German), Appendix 5 (Italian), Appendix 6 (Polish), Appendix 7 (Spanish), Appendix 8 (Swedish) and Appendix 9 (Portuguese)

SAFETY INFORMATION

Before commencing installation, please take time to familiarize yourself with warning labels and symbols on your instrument and their meaning. These are to inform you where potential danger exists or particular caution is required. Improper use may cause personal injuries or damage to the instrument. The instrument must only be operated by appropriately trained and experienced personnel. Please read the complete user manual prior to use.

— direct current

Overvoltage category: Class II

Maximum operating altitude: < 2000 m

Protection degree: 2

Do not open the instrument as this can expose the operator to electrical power, UV light, and delicate fiber optics or damage the instrument.

If the submicroliter cell is used with the NanoPhotometer® C40 make sure that the lid is placed on the measurement head prior to the measurement.

WARNING: UV exposure when measuring without lid.

Do not use damaged power cords, accessories, and other peripherals with your NanoPhotometer®. Use only the delivered and specified power supply/charger.

Do not expose the NanoPhotometer® to strong magnetic, electrical fields, water, chemicals or any type of liquid as heavy rain or moisture.

Do not put the instrument into fire, as it may swell or explode (battery). Do not store at or use near any type of heat source, especially temperatures above 60°C or in an explosive atmosphere.

Do not leave your NanoPhotometer® on your lap or near any part of your body to prevent discomfort or injury from heat exposure.


Do not place objects on top of the NanoPhotometer®.

The NanoPhotometer® with battery pack (mobile version) has to be switched off during transportation. The on/off button has to be protected during transportation from turning on itself caused by shock or vibration.

Biological samples may contain or have the potential to transmit infectious diseases. Be aware of the health hazard presented by such samples and wear appropriate protective equipment. Handle such samples with the greatest of care and according to applicable

regulatory and organization requirements before working with such potential infectious materials.

Note: Do not spill any biological samples on instrument components. If spill occurs, disinfect the instrument immediately following your laboratory protocols and the cleaning instruction of the instrument (see page 144 Maintenance).

The symbol  on the product, or on the documents accompanying the product, indicates that this appliance may not be treated as household waste. Instead it shall be handed over to the applicable collection point for the recycling of electrical and electronic equipment. Disposal must be carried out in accordance with local environmental regulations for waste disposal.

UNPACKING AND POSITIONING

Note: Translations of the Safety Information and Unpacking and Positioning are available in Appendix 1 (Czech), Appendix 2 (Dutch), Appendix 3 (French), Appendix 4 (German), Appendix 5 (Italian), Appendix 6 (Polish), Appendix 7 (Spanish), Appendix 8 (Swedish) and Appendix 9 (Portuguese)

Check the contents of the package against the delivery note. If any shortages are discovered, inform your supplier immediately.

Inspect the instrument for any signs of damage caused in transit. If any damage is discovered, inform your supplier immediately.

Ensure your proposed installation site conforms to the environmental conditions for safe operation: indoor use or dry environment.

Note: Do not expose your NanoPhotometer® near liquids, chemicals, rain, moisture or dusty environments.

Working temperature range 10 - 40°C (NP80, N60, N50 and C40) and 10 – 32°C (N120); If the cuvette heating is used the range is 10 - 27°C.

Storage temperature range is 0 - 40°C. Do not store the instrument below or above this temperature.

If the instrument is subjected to extreme temperature changes, it may be necessary to allow the instrument to equilibrate. Turn the instrument off and then on again once thermal equilibrium has been established (~2 - 3 hours).

Maximum relative humidity (non-condensing) of 80% up to 31°C decreasing linearly to 50% at 40°C.

The instrument must be placed on a stable, level surface that can support 4-5 kg. Ensure that air can circulate freely around the instrument. Confirm while powered on that no materials reduce air circulation. Avoid direct sunlight as it may bleach parts of the instrument and can cause damage to plastic parts.

The equipment should be positioned such that in the event of an emergency the main plug can be easily located and removed.

Always carry the instrument by holding the main corpus of the instrument and not e.g. on the optional attached display, pipetting support or NanoVolume pedestal.

The equipment must be connected to power with the 90W power supply/cord supplied by Implen. The power outlet must have a protective conductor (earth/ground). It can be used on 90-250 V \pm 10%, 50-60 Hz power supply system.

For mobile models please charge the battery pack at least for 3 hours prior to the first use. To avoid deep discharging of the battery pack charge the battery pack at least once a month even if the NanoPhotometer® is not in use. Recharging of deep discharged batteries is not possible they need to be replaced by new ones.

Please read the complete user manual before first use.

Turn the instrument on using the power button on the rear panel after it has been plugged in. The instrument will perform a series of self-diagnostic checks.

Please contact original supplier immediately if technical or sample handling difficulties are experienced.

Note: If this equipment is used in a manner not specified or in environmental condition not suitable for safe operation, the protection provided by the equipment may be impaired and the instrument warranty voided.

SOFTWARE INSTALLATION

NPOS OVERVIEW

NPOS is a Linux-based operating system designed for the NanoPhotometer®.

NPOS can store data either to a common directory or be configured to save to independent directories according to file format and/or instrument.

NPOS can save data in an Implen .IDS format, PDF or as an Excel format file.

Note: PDF and Excel files cannot be opened on the NanoPhotometer®. Files need to be transferred to a computer or device where Excel or a PDF reader is available.

Note: Please do not connect the instrument to a computer until the NanoPhotometer® NPOS software is installed on the computer.

REQUIREMENTS AND COMPATIBILITY

The NPOS user interface is designed that all features can be operated by using a touchscreen. If the software is installed on a computer without touchscreen, the user interface can be operated by using keyboard and mouse. Before starting the installation process, ensure that the software of the control device is compatible.

Compatible Control Devices

Computer:

PC: Windows 8 / Windows 10 (32 & 64 bit)

Mac: macOS Catalina / Big Sur (Intel x86 und Apple M1)

Tablets (minimum requirements):

iPad: iOS 13

Android (quad core 1.2 GHz with 1 GB RAM): Android version 10

Smartphones (minimum requirements):

iPhone: iOS 13

Android (quad core 1.2 GHz with 1 GB RAM): Android version 10

Windows is a trademark of Microsoft. Mac OS & iOS are trademarks of Apple. Android OS is a trademark of Google. Linux is a trademark of Linus Torvalds.

Note: There are two user interfaces of the software available; one for built-in touchscreen, computer and tablets and one for smartphones (not available for NanoPhotometer® N120).

INSTALLING SOFTWARE ON COMPUTER

The NanoPhotometer® software can be installed on compatible Windows and Mac computer systems. Various operating systems and computer hardware may cause the set-up procedure to differ from that described here. This process is given as guidance only; it may need adaptation for other systems.

Note: Do not connect the NanoPhotometer® to the PC/Mac before NPOS installation.

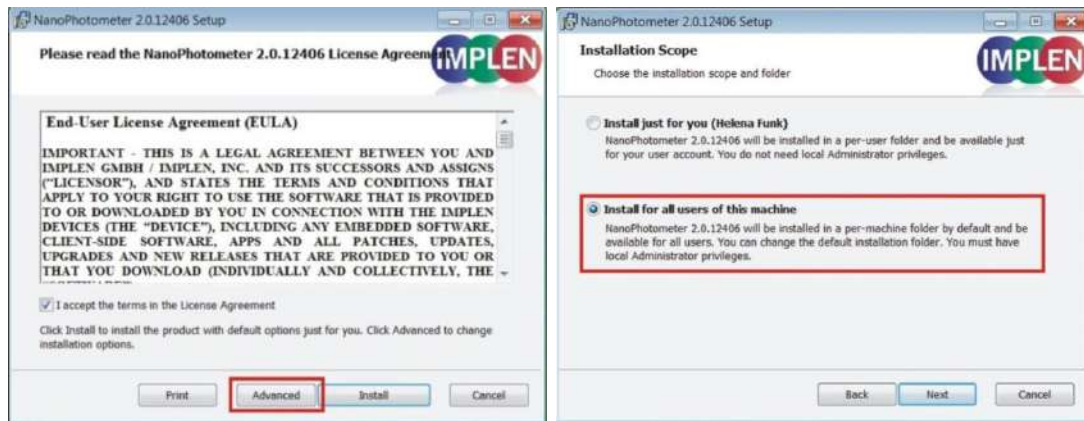
Note: If a previous version of the NPOS software is already installed on the computer, remove the USB cable and uninstall the NPOS software before installing the new software version.

Note: The Windows and Mac installation files are located on the Implen USB flash drive which is included with the NanoPhotometer® at time of delivery. The files are available for free download at any time in the download area of the Implen website (www.implen.de/downloads).

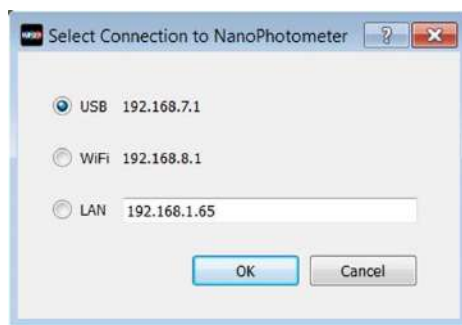
- **NPOS installation for single/multi user on Windows computer**
 1. Check the installed version of the NanoPhotometer® firmware (Preferences/About) and update it to the latest version if necessary, before starting the installation/update of the NPOS software on your computer.
 2. Start the NPOS installation file and follow the installation routine for **single user installation**.

Installation file can be found on the Implen USB flash drive which is included with the NanoPhotometer® at time of delivery or can be downloaded from the Implen webpage: www.implen.de/downloads.

Full administration rights are required for the installation. If you have insufficient privileges, installation may fail. If in doubt consult your computer administrator.
 3. For **multi user installation** (only necessary for Windows installation) choose on the License Agreement dialog the option “Advanced” and on the following dialog “Install for all users of this machine”



4. Start the NPOS software and select the desired connection. For a connection via USB cable, connect the NanoPhotometer® to the PC using the USB cable supplied. For a connection via WiFi hotspot, ensure a stable WiFi connection between the PC and NanoPhotometer® WiFi HotSpot (**SSID**: serial number, **password**: Implenuser). For a network connection, connect the NanoPhotometer® to the local network via an Ethernet cable or WiFi network (see page 128 Network).

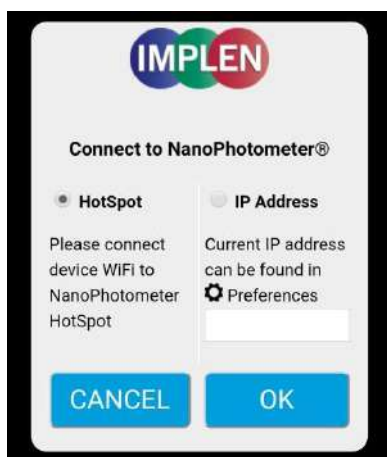


Note: If there is Avira installed on your computer, it is recommended to switch off the browser safety. This may interfere with the NPOS running on your computer.

INSTALLING NANOPHOTOMETER® APP ON TABLET OR SMARTPHONE

The NanoPhotometer® App can be installed as an application on tablets and smartphones with compatible Android and iOS operating systems (see page 26 Requirements and Compatibility). The NanoPhotometer® App is available for free download in the app store (Apple Store and Google Play Store).

1. Download and install the NanoPhotometer® App from the app store
2. Connect the tablet or smartphone via WiFi HotSpot (**SSID**: Serial number, **password**: Implenuser) or WLAN network to the NanoPhotometer®.
3. Open the NanoPhotometer® App and choose the connection type:



4. When connected the NanoPhotometer® will recognize the tablet/smartphone as a remote control device and measurements can be initiated from the tablet or smartphone.
5. Results will be shown on the tablet or smartphone once measurements have been taken.

Note: In order to install the NanoPhotometer® App on a tablet or smartphone, the device must have an established internet connection to access the app store for app download.

Note: The version of the app and the software of the NanoPhotometer® should be the same. Different versions may have not full functionality.

FIRST STEPS AND CONFIGURATION WIZARD

When starting the Implen NPOS the first time an Implen configuration wizard is shown. Please accept the End User License Agreement (EULA) and select the country in which the NanoPhotometer® is used and confirm.

PRINTER INSTALLATION

For printers connected via USB connection:

1. Switch NanoPhotometer® on / home screen
2. Connect DYMO/HP printer via USB cable
3. DYMO/HP printer is ready to use after 30 seconds

Note: Make sure that the home screen is shown when connecting a printer. If the USB connection between the printer and the NanoPhotometer® is established while a method is open, the printer function may fail.

Always return to the home screen before connecting a printer.

Note: Check printer compatibility (page 19 HP Printer)

For network printers:

1. Assure either LAN or WiFi network connection
2. Set printer IP in preferences (page 132 Network Printer)
3. Printer is available in methods for printing

Note: Check printer compatibility (page 19 HP Printer)

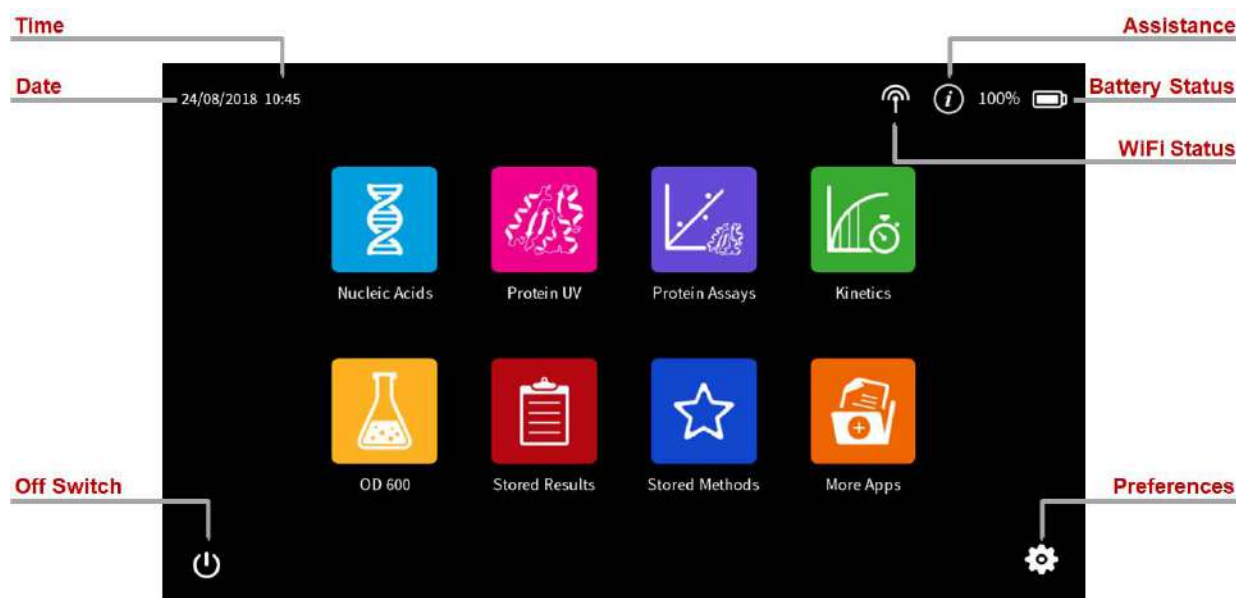
3. NANOPHOTOMETER® BASICS

The NanoPhotometer® product line offers a complete solution for NanoVolume (N120/NP80/N60/N50/C40) and standard cuvette (C40/NP80) applications. The NanoPhotometer® N120 is a multi-sample model that can measure up to 12 samples per run. The multi-channel option is available in Nucleic Acid, Protein UV, Protein Assay and Wavescan method.

NanoVolume applications start with a minimum sample volume of 0.3 µl (NP80/N60/N50) and 2 µl for the NanoPhotometer® N120. Standard cuvette applications can be performed with 10 mm, 5 mm, 2 mm, 1 mm and 0.5 mm path length quartz, glass, or plastic cuvettes with a center height of 8.5 mm.

APPLICATIONS OVERVIEW

The NanoPhotometer® comes with pre-programmed applications as well as the ability to create custom applications. To select a method, tap the corresponding icon and the method opens immediately.



Method Icons

Description



Nucleic Acids

Concentration, purity, and dye incorporation for DNA, RNA, Oligo, and other nucleic acids




Protein UV

Protein UV determination at 280 nm (or in a range of 200 - 330 nm), purity and dye incorporation































Kinetics

Time vs. Absorbance readings

	Protein Assays	BCA (562 nm), Bradford (595 nm), Lowry (750 nm), and Biuret (546 nm; cuvette only) Assays
	OD600	Measures cell density at 600 nm (or in a range of 200 – 900 nm)
	Stored Results	Archive of stored results
	Stored Methods	Collection of stored custom methods
	More Apps	Additional applications found on a secondary method screen
	Wavelength	Define one or multiple wavelength between 200 – 900 nm (N50: 200 – 650 nm) for absorbance measurements
	Wavescan	Define desired full scan range anywhere between 200 - 900 nm (N50: 200 - 650 nm)
	Concentration	Define extinction coefficient for automatic concentration calculations
	Absorbance/ Ratio	Define two wavelengths absorbance/ratio calculation
	Standard Curve	Create a standard curve at a defined wavelength
	Custom Apps	Optional custom applications for personalized methods tailored to individual spectroscopy needs

ICONS

Icon	Name	Action
	WiFi Network	WiFi network active; status of WiFi connection 
	WiFi Hotspot	WiFi hotspot active
	Assistance	Opens the assistance page
	Battery Status	Shows the actual battery status (only shown with optional battery pack)
	Preferences	Opens the preferences page
	Home Screen	Returns to home screen with application icons for method selection.
	Store Method	Opens a dialog pop up with the possibility to store the actual method parameter to a custom method
	Save Data	Opens a save dialog pop up
	Leave Method	Returns to the previous application selection
	Back	Returns to the previous page (smartphone only)
	Next/Confirm	Confirms parameter and opens next screen (smartphone only)
	Print Data	Opens a print dialog pop up (only shown when a printer is available)
	Delete Data	Opens a delete dialog pop up
	Sample ID	Opens sample ID pop up to define sample IDs (N120 only)
	Graph	Opens graph overlay for multi-channel mode (N120 only)
	Parameter	Opens parameter window
	Results	Opens result window
	Graph	Opens graph result window
	Table	Open/shows results in table format
	Sample Map	Opens sample map window for computer versions (N120 only)
	Standard Map	Opens standard map window in Protein Assay method to measure standard/replicates for defined standard curve (N120 only)
	Standard Curve	Opens standard curve window in Protein Assay method (N120 only)
	Add Folder	Adds a new folder to the directory
	Manage Data	Opens a dialog pop up with several action options including delete, rename or import folders/files/data as well as copying or move folders/files/data to defined directories
	Delete	Deletes added functions in parameter; empties input windows
	Full Scale	Restores graph to original size without zoom
	Cancel	Returns to the previous screen/closes window without implementing any changes

BUTTONS



When opening a method and starting a sample measurement, a blank measurement is required. For the blank measurement, either water or sample buffer can be used to give the NanoPhotometer® a reference of what zero should be. It is recommended to re-apply the blank solution and measure it as a sample to ensure the graph of the blank spectra is a flat line.



To initiate the spectral scan of the sample push the sample button. The data will be temporarily stored until the method is exited; at this time the user needs to define if the samples should be saved.



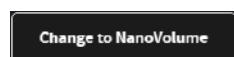
The auto sample button is disabled by default. When switched on sample measurements are started automatically as soon as the lid arm is closed. Auto sample function is only available for sample and not for blank measurements.

Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.



The Change to Cuvette button (NP80) is located at the bottom of the parameter window. The change to Cuvette/NanoVolume button toggles between NanoVolume and cuvette applications. Selecting the Change to Cuvette button activates the cuvette compartment (opens the sliding cover and switches the red arrow LED on). Only cuvette measurements are possible when this mode is selected. Selecting the Change to NanoVolume button while the cuvette port is activated will deactivate the cuvette compartment (closes the sliding cover and switches the LED off) and only NanoVolume measurements are possible.

Note: Please make sure that the lid arm is closed during cuvette measurements.



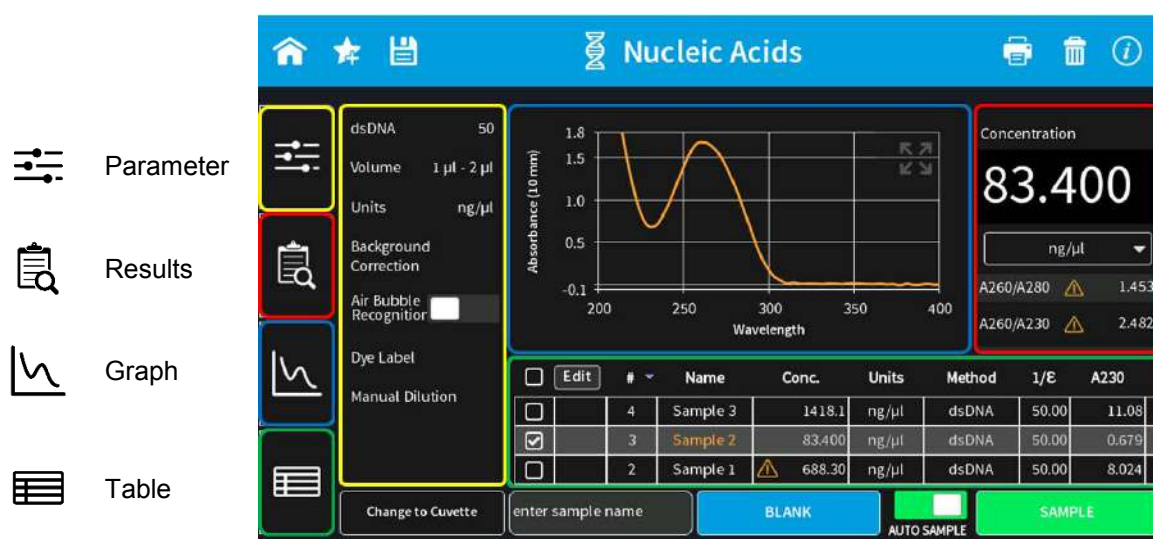
The Change to NanoVolume button (C40) is only shown when submicroliter cell applications are enabled in the preferences. The Change to NanoVolume/Cuvette button toggles between cuvette and submicroliter cell applications. With the NanoVolume submicroliter cell application the parameter of each method shows a list for lid selection (Lid 5, 10, 50, 100 and 250). Choose the correct lid and the results are automatically calculated considering the virtual dilution.

MEASUREMENT SCREENS

SINGLE SAMPLE METHODS

Side Tab Bar

On the left side of the measurement screen there is a vertical tab bar that contains four tabs including: parameters, results, graph, and table. The different tabs allow the user to organize the measurement screen. It is possible to show or hide the different areas on the screen. Default screen for computer shows all areas, for the built-in screen and the tablet version the table is hidden.



Note: There is no tab bar available for smartphone versions. The parameter, results and graph screens are shown full screen. Parameters need to be confirmed (>) to get to the measurement screen. It is possible to toggle between the results and graph area by swiping left and right. There is no table area available for smartphones.

Parameter area

In the parameter area it is possible to define all necessary parameters for a measurement as well as turn on cuvette mode and initiate cuvette heating. The standard measurement screen shows the parameter area open by default. The parameter area is automatically hidden when starting either a blank or sample measurement by pushing on the Blank or Sample button. It is also possible to hide the parameter area by tapping the parameter tab in the vertical side tab bar.

Results area

The results area shows the method specific results of the grey highlighted measurement in the table including concentration, absorbances, and relevant ratios. It is also possible to change the units of the calculated concentrations in the results area with a dropdown selection menu.

Table area

The table area collects the results of all samples in an active method. The first table column shows a tick box. Selecting the samples with the tick box the graphs are overlaid in the

graph area. With the header tick box it is possible to select/unselect all samples (maximum number of sample selection is 30). The second column of the table indicates whether the measurement is saved (☑) or not saved (blank field).

With the edit button the sample name of a single sample can be edited.

1. Select one sample in the table (selected sample will be highlighted in grey)
2. Push on Edit button
3. Change sample name
4. Confirm with the “Confirm” button


Note: It is not possible to edit sample names of opened IDS files.

Graph area

The graph area shows a chart with the graph of the actual measurement or the selected line(s) in the table (tick box selection). There is an overlay toggle switch on the left bottom of the graph area. If the overlay option is enabled the graphs of the measurements will be automatically overlaid. To change the overlaid graphs use the tick boxes in the table.

Note: It is only possible to overlay up to 30 graphs in a chart. If more than 30 data are selected a message will appear that says “More than 30 samples have been selected. Only 30 will be shown in graph.”

Note: The overlay button is not available on the NanoPhotometer® touch screen and smartphones, only on tablets and computer versions.

It is possible to zoom in and out any area of the diagram (x- and y-axis). Undo the zoom by pushing the full scale icon ().

Note: Maximum zoom is 20 nm for the x-axis and 0.01A for the y-axis.

As legend option the sample name in the table is colored in the same color as the graph in the chart.

Pushing on the graph opens a pop up which shows the sample name, wavelength and absorbance of the selected wavelength. It is possible to display the results of other graphs by changing the sample name with the dropdown option.



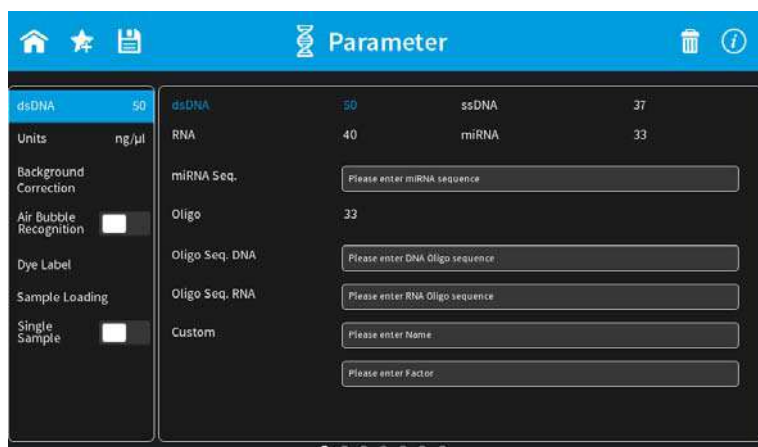
The screenshot shows a dark-themed pop-up window with a close button (X) in the top right corner. It contains three rows of information:

Sample Name	L10
Wavelength	260
Absorbance	0.207

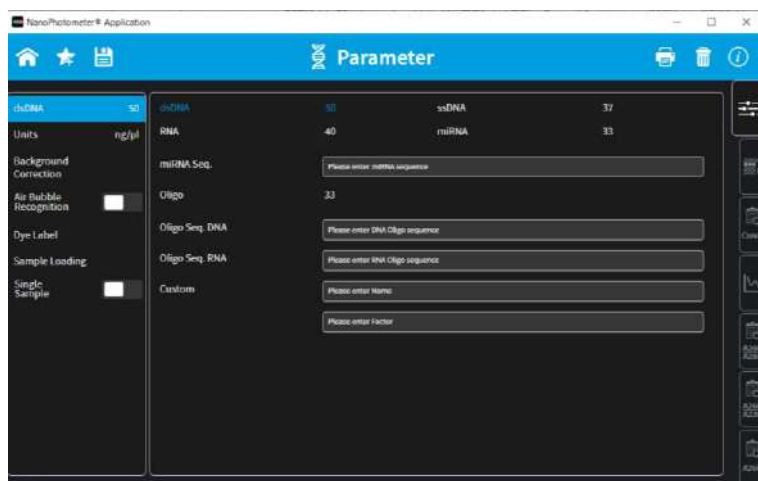
MULTI SAMPLE METHODS

- **Change Screens**

Opening a multi sample method (Nucleic Acid, Protein UV, Protein Assay or Wavescan) shows the parameter screen first. To confirm the parameter swipe the screen to the left and the sample map is shown. It is possible to change between the parameter, sample map and result screens by swiping left or right at any time. At the bottom of the screen is a screen carousel that shows the active and available screens.



For computer versions without touchscreen a vertical tab bar is shown to change between the different screens (parameter, sample map, results and graph; for Protein Assay additionally standard map and standard curve)



- **Sample Map / Definition of Sample IDs**

The sample map screen shows the sample IDs as defined or names it automatically if nothing is defined. For measurements it is not necessary to select any cell or define sample IDs. If no cell is selected/Sample ID defined, all 12 (horizontally) or 8 (vertically) samples are measured. Sample IDs are named automatically in this case.

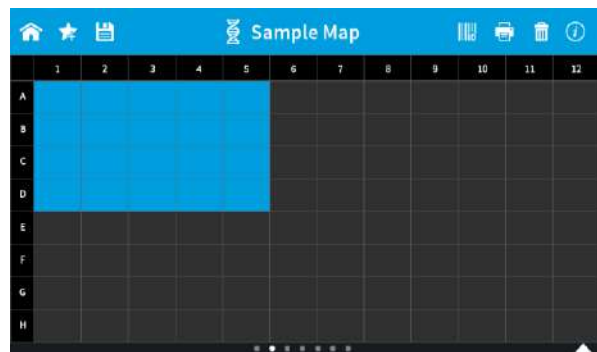
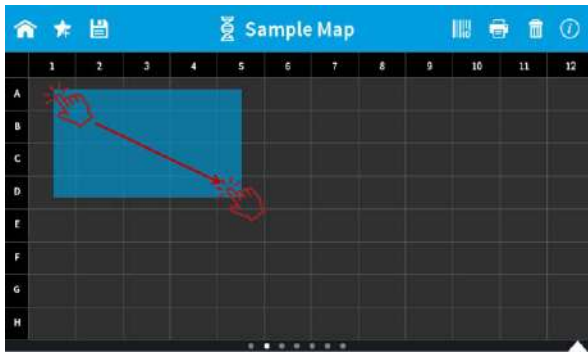
Selection Options

Selection of cells is only possible in the sample map screen. Cell A01 must always be selected.

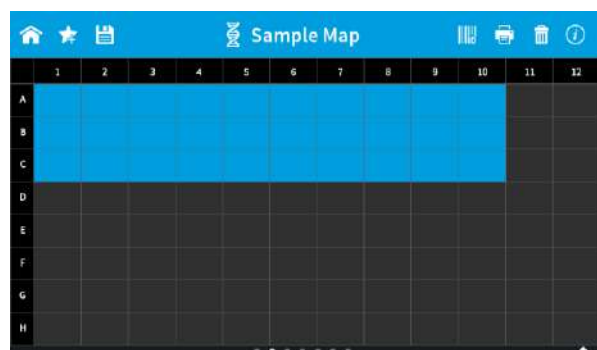
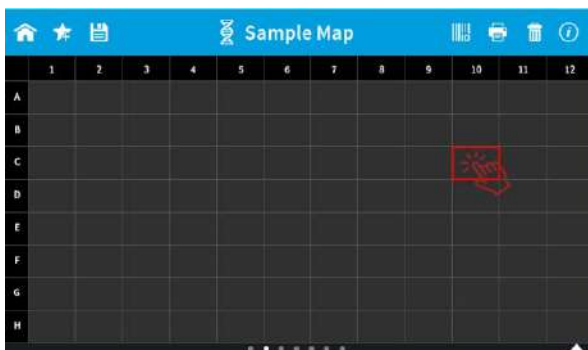
A push in the top left corner selects/deselects all 96 cells at once:



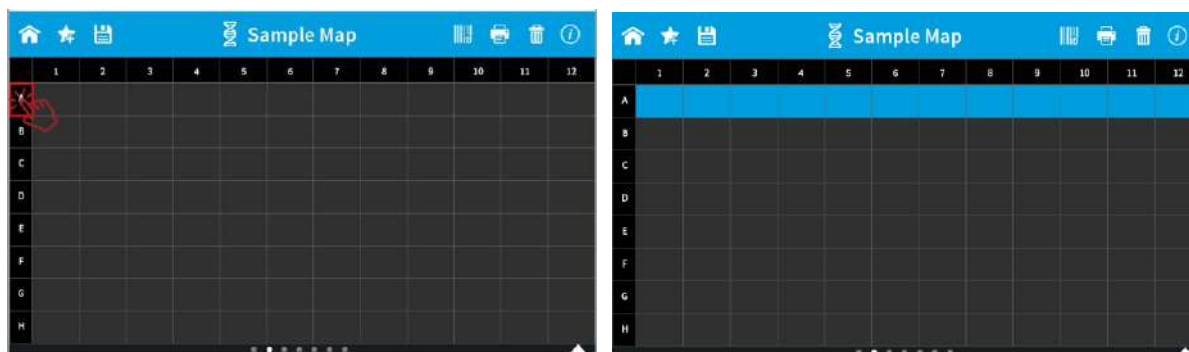
Manual selection is possible by selecting any area starting in cell A01.



or by pushing in any cell:



A push to the row/column header selects/deselects the whole row/column:

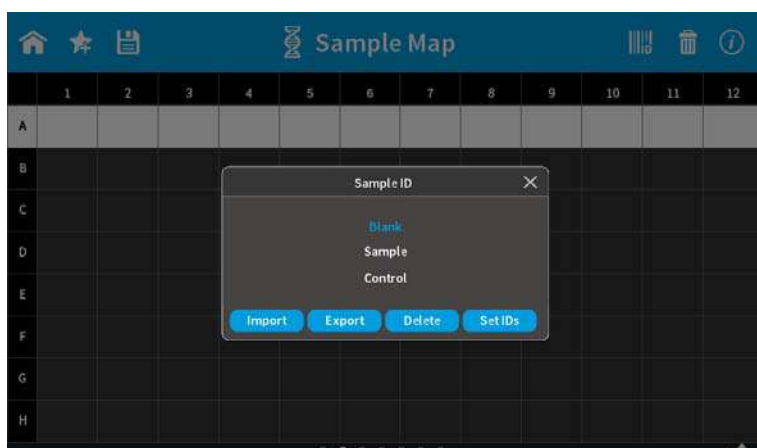


Note: As a blank is necessary for each measurement it is not possible to select more cells as in the previous row/column.

Sample ID Definition

There are multiple options to define Sample IDs. If no Sample IDs are defined the Sample IDs are named automatically.

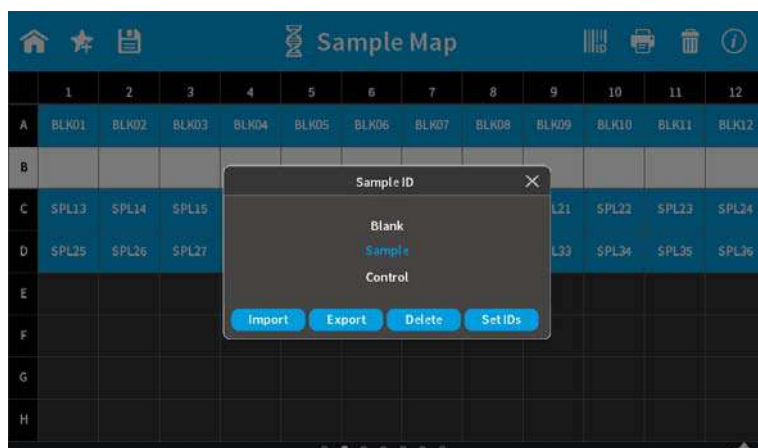
To define sample IDs open the Sample ID pop up (☰) on the Sample Map screen. The Sample ID pop up offers options to set sample IDs, delete sample IDs, import or export sample IDs from a csv file.



To set sample IDs, select Blank (BLK), Sample (SPL) or Control (CRL) and push on “Set IDs” button. Sample IDs are numbered automatically.



To delete sample IDs, close the pop up, select the row/column for which the sample IDs should be deleted and reopen the sample ID pop up and push the Delete button.



Individual Sample IDs can be changed by double-clicking on the Sample ID. To change several Sample IDs simultaneously, open the table view after a measurement and select Edit.

Sample IDs can be imported with the Import function. For the import of Sample IDs, the sample IDs must first be defined in the following format and saved as a csv file.

- for horizontal Sample Map:

	A	B	C	D	E	F	G	H	I	J	K
1	Position	Content	Sample ID		Comments						
2	A01	B	BLK01								
3	A02	B	BLK02		Content field needs to be defined with B, C or S						
4	A03	B	BLK03		B = Blank measurement						
5	A04	B	BLK04		C = Control measurement						
6	A05	B	BLK05		S = Sample measurement						
7	A06	B	BLK06		It is not possible to mix Blank and Control/Sample measurements in one row.						
8	A07	B	BLK07		Control and Sample contents can be combined in one row.						
9	A08	B	BLK08								
10	A09	B	BLK09		Blank and Control have pre-defined sample IDs						
11	A10	B	BLK10		B = BLK01, BLK02, etc.						
12	A11	B	BLK11		C = CTRL01, CTRL02, etc.						
13	A12	B	BLK12		If anything else than the pre-defined sample IDs is entered for Blank and Control, the samp						
14	B01	C	CTRL01		Sample IDs for samples can be user-defined, if no sample IDs are defined for content (S) th						
15	B02	C	CTRL02		S = SPL01, SPL02, etc.						
16	B03	C	CTRL03								
17	B04	C	CTRL04		It is not necessary to set samples 1 - 12, any number > 1 is possible						
18	B05	C	CTRL05								
19	B06	C	CTRL06								

Note: Draft files are available for download on the Implen homepage (www.implen.de/downloads).

- for vertical Sample Map:

	A	B	C	D	E	F	G	H	I	J	K
1	Position	Content	Sample ID		Comments						
2	A01	B	BLK01								
3	B01	B	BLK02		Content field needs to be defined with B, C or S						
4	C01	B	BLK03		B = Blank measurement						
5	D01	B	BLK04		C = Control measurement						
6	E01	B	BLK05		S = Sample measurement						
7	F01	B	BLK06		It is not possible to mix Blank and Control/Sample measurements in one column						
8	G01	B	BLK07		Control and Sample contents can be combined in one column						
9	H01	B	BLK08								
10	A02	C	CTRL01		Blank and Control have pre-defined sample IDs						
11	B02	C	CTRL02		B = BLK01, BLK02, etc.						
12	C02	C	CTRL03		C = CTRL01, CTRL02, etc.						
13	D02	C	CTRL04		If anything else than the pre-defined sample IDs is entered for Blank and Control, the samp						
14	E02	C	CTRL05		Sample IDs for samples can be user-defined, if no sample IDs are defined for content (S) th						
15	F02	C	CTRL06		S = SPL01, SPL02, etc.						
16	G02	C	CTRL07								
17	H02	C	CTRL08		It is not necessary to set samples 1 - 08, any number > 1 is possible						
18	A03	S									
19	B03	S									
20	C03	S									

Note: Draft files are available for download on the Implen homepage (www.implen.de/downloads).

Note: It is not possible to edit imported Sample IDs.

To import sample IDs open the sample ID pop up and push the import button. A file directory is shown. Select the .csv file and push on import button. Sample IDs will be imported.

For exporting sample IDs push the export button in the sample ID pop up. Enter a file name, select a folder and confirm with export.

▪ Result Screens

There are multiple result screens available depending on parameter setting and method.

Nucleic Acids:

- Concentration
- Graph
- Ratio A260/A280
- Ratio A260/A230
- A260

Protein UV:

- Concentration
- Graph
- Ratio A260/A280
- A280

If a dye is added three additional screens are shown:

- Dye Concentration
- Dye Absorbance
- FOI / DOL

Protein Assay:

- Standard Map
- Standard Curve
- Concentration
- Graph
- Absorbance

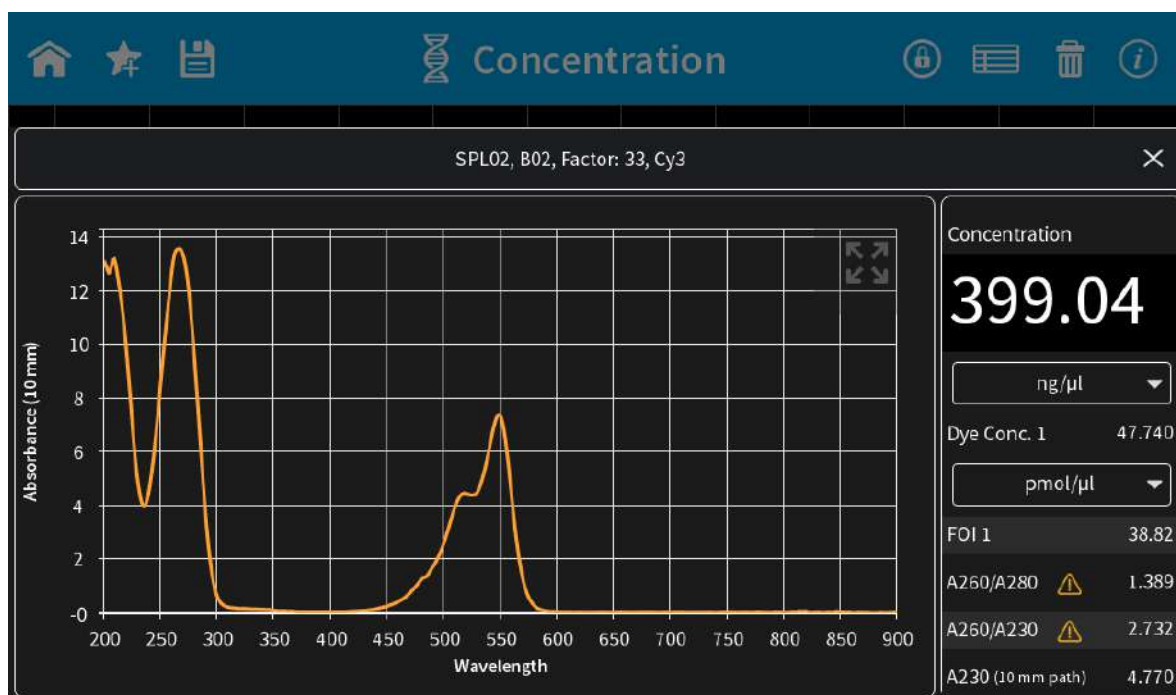
Wavescan:

- Graph

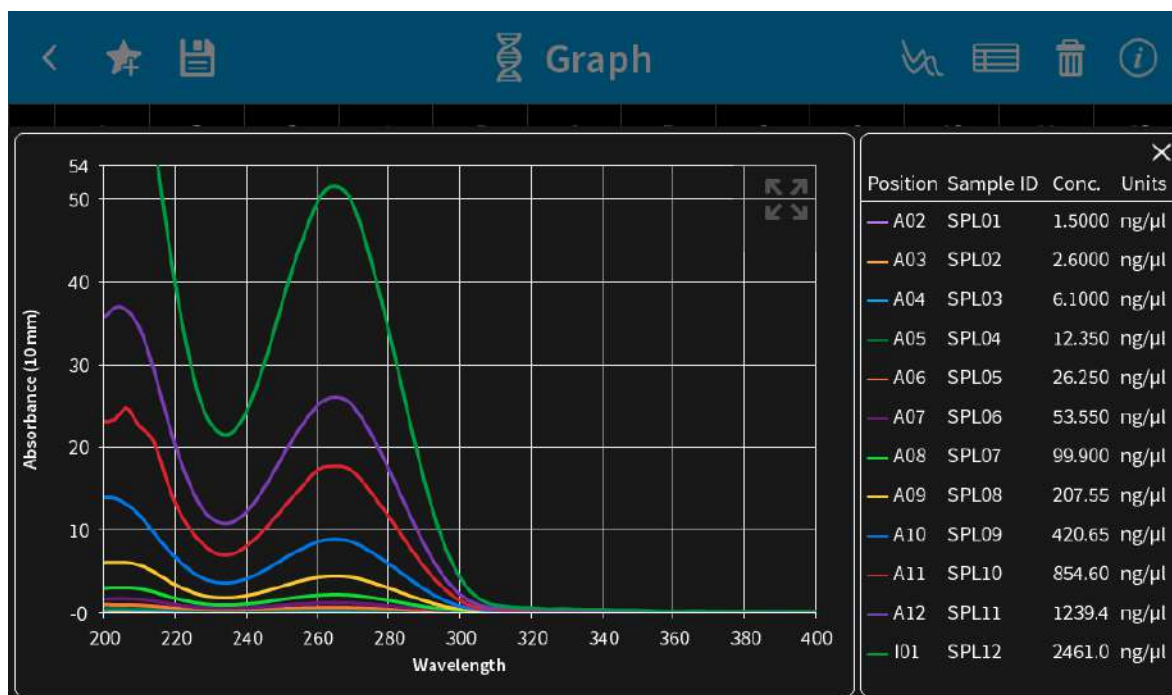
Defined sample IDs are shown on all result screens as long as the sample is not measured. Once the sample is measured the result is shown. A white frame marks the sample IDs which will be measured next.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B	SPL01	SPL02	SPL03	SPL04	SPL05	SPL06	SPL07	SPL08	SPL09	SPL10	SPL11	SPL12
C	SPL13	SPL14	SPL15	SPL16	SPL17	SPL18	SPL19	SPL20	SPL21	SPL22	SPL23	SPL24
D	SPL25	SPL26	SPL27	SPL28	SPL29	SPL30	SPL31	SPL32	SPL33	SPL34	SPL35	SPL36
E												
F												
G												
H												

A push on a result cell opens a single result overview:



A double click to a result cell shows tick boxes for all measured samples. The tick boxes can be used in the graph screen to overlay up to 12 samples. Up to 12 ticked samples will be overlaid by pushing on the graph icon (📊) in the navigation bar.



A table view (☰) is available on all result screens. The table lists all measured samples.

<input type="checkbox"/>	Edit	Position	Sample ID	Content	Conc.	Units	Method	1/ε	A230	A260	A280
<input type="checkbox"/>		A01	BLK01	B	0.0000	ng/μl	dsDNA	50.00	0.000	0.000	0.00
<input type="checkbox"/>		A02	BLK02	B	0.0000	ng/μl	dsDNA	50.00	0.000	0.000	0.00
<input type="checkbox"/>		A03	BLK03	B	0.0000	ng/μl	dsDNA	50.00	0.000	0.000	0.00
<input type="checkbox"/>		B01	SPL01	S	0.3000	ng/μl	dsDNA	50.00	0.010	0.005	-0.01
<input type="checkbox"/>		B02	SPL02	S	0.8000	ng/μl	dsDNA	50.00	0.028	0.018	0.00
<input type="checkbox"/>		B03	SPL03	S	---	ng/μl	dsDNA	50.00	-0.014	-0.009	-0.00

With the edit button selected Sample IDs can be changed.

DATA PROCESSING DIALOGS

PRINT

Selecting the print icon (🖨️) opens a full screen dialog window with various print options. The print icon is only shown if a printer is available.

The print command is sent primarily to DYMO or HP printers if they are directly connected to

the NanoPhotometer® via USB cable. If no USB printer is available, the print command is sent to the defined network printer, if configured. Network printers can be configured in the preferences by entering the printer IP (see page 132 Network Printer). All ticked samples are printed.

Note: If a printer is directly connected to the NanoPhotometer® via USB, this printer will have the highest priority and will be used by default when selecting Print on NanoPhotometer®. In order to print utilizing a printer on the network, please disconnect the connected USB printer.



Note: The print icon is only shown, if a printer is available.

Note: It is only possible to operate one printer at a time. Do not connect more than one printer to the NanoPhotometer®.

Note: The print option is not available in smartphone apps.

▪ Auto Print

If the auto print function is enabled, each measurement will be printed directly after the measurement. Auto print function is available for DYMO printer, HP printer connected via USB cable and network printer.

Note: Default setting for auto print is off. If enabled in one method it is set default on for all methods and needs to be switched off if not required.

Note: The auto print function is not available for printing via control device (local computer printer).

▪ Cryo Label Print


To print on cryo labels connect a DYMO printer to the NanoPhotometer® and insert the specified cryo label paper (26 x 12.7 mm and 9.5 mm circle / landscape mode).

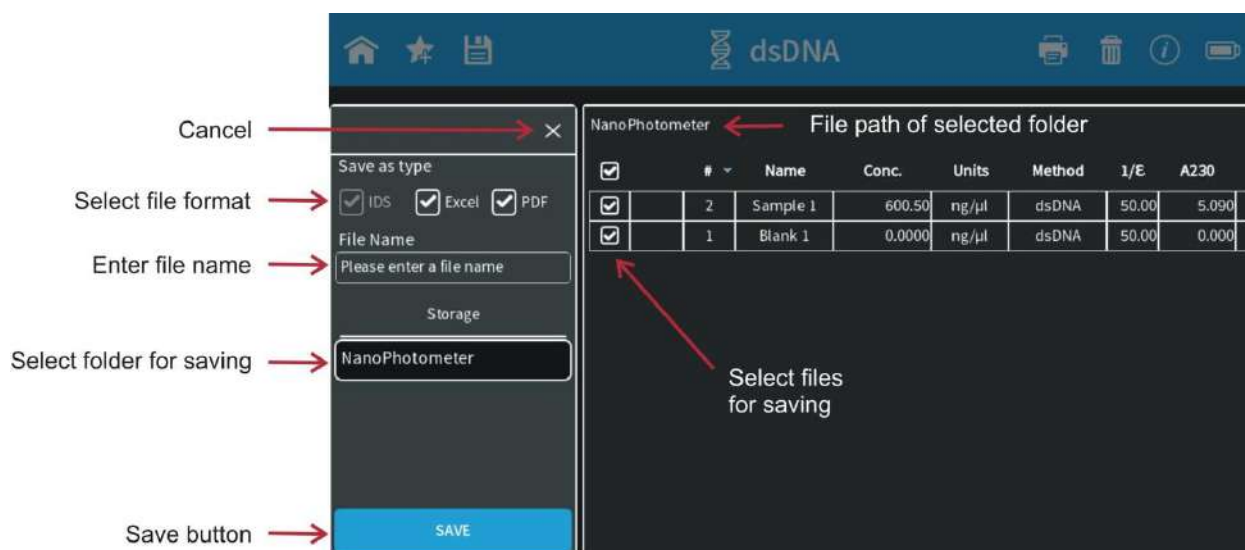
▪ English Print

Option to change the language of the printout from the selected language setting to English.

Note: English Print can also be selected in Preferences. When this setting is enabled in preferences, the toggle switch present in the print dialog has no function.

SAVE

Selecting the save data icon () opens a full screen overlay dialog window with various save options.



By default all samples are ticked in the first column of the table and will be saved. It is possible to select samples for saving by using the tick boxes. The header tick box selects/deselects all samples.

Note: In the smartphone app there are always all measurements saved, no selection possible.

Save as Type

With the Save as Type option it is possible to specify the file type for saving. File type options include Excel, PDF and Implen Document Source (IDS). It is possible to save different file formats simultaneously.

Note: IDS files cannot be saved on control devices. IDS files can only be saved in the NanoPhotometer® storage, defined network folder or on a USB flash drive. It is not possible to save IDS files from opened data. In these cases the IDS tick box is greyed out.

Note: PDF and Excel files cannot be opened on the NanoPhotometer®. The files need to be transferred to a computer or device where Excel or PDF reader is available.

Note: All data saved on the NanoPhotometer® are stored on an internal micro SD card. It is recommended to back up the data regularly to a hard drive of a computer or network. In the rare case that the micro SD card crashes data loss cannot be ruled out.

Implen Document Source File

The Implen Document Source (IDS) file is a specific file format, which can only be opened with the NPOS software. It is a hardcopy file which cannot be changed. This file type contains all measurement information including raw data, results, values, and parameters.

Note: The saved files contain only the selected samples at the time the file is saved.

Excel File

Measurement data can be saved as Excel file. This file type contains all measurement information including raw data, results, and parameters.

Note: The saved files contain only the selected samples at the time the file is saved.

PDF File

Measurement data can be saved as PDF file. This file type contains all measurement information including raw data, results, and parameters.

It is possible to configure the table columns for PDFs and printouts in preferences (page 133 Report Configuration).

Note: The saved files contain only the selected samples at the time the file is saved.

▪ File Name

Enter the name for the file. Allowed characters are: A...Z a...z 0...9 , . - () @ ! = _ ~ ; [] { } ‘

Note: Blank character is not allowed.

▪ Storage


Shows folder directory to select the save location. Options include: NanoPhotometer®, USB flash drive (if connected), Network folder (if defined) and Control Device. If Control Device is selected the data will be transferred to the control device that is currently connected with the NanoPhotometer®.

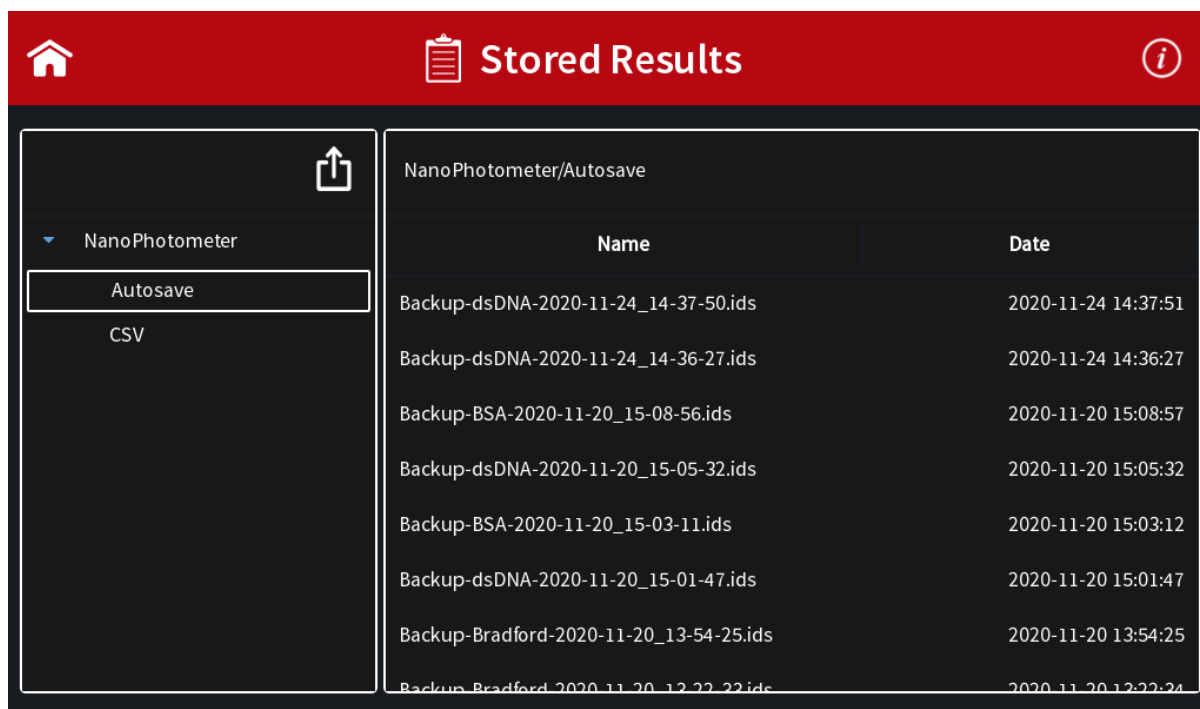
Note: It is not possible to save IDS files to a control device like computer (PC/Mac), tablets or smartphones.

▪ Auto Save

To prevent data loss, all measurements are automatically stored as IDS file on the internal memory of the NanoPhotometer®. These backup copies can be found in the Autosave folder of the NanoPhotometer® (Stored Results/NanoPhotometer/Autosave) for up to ten days. Files contain the base name Backup, the method name, and a time/date stamp.

After ten days the autosave files are automatically moved to an autosave archive folder. The autosave archive folder can only be accessed via NanoPhotometer® file server (see page 56 Data Transfer via File Server). Data in the autosave archive folder are not automatically deleted.

The content of the Autosave Archive folder can be deleted via the action button  in Stored Results:

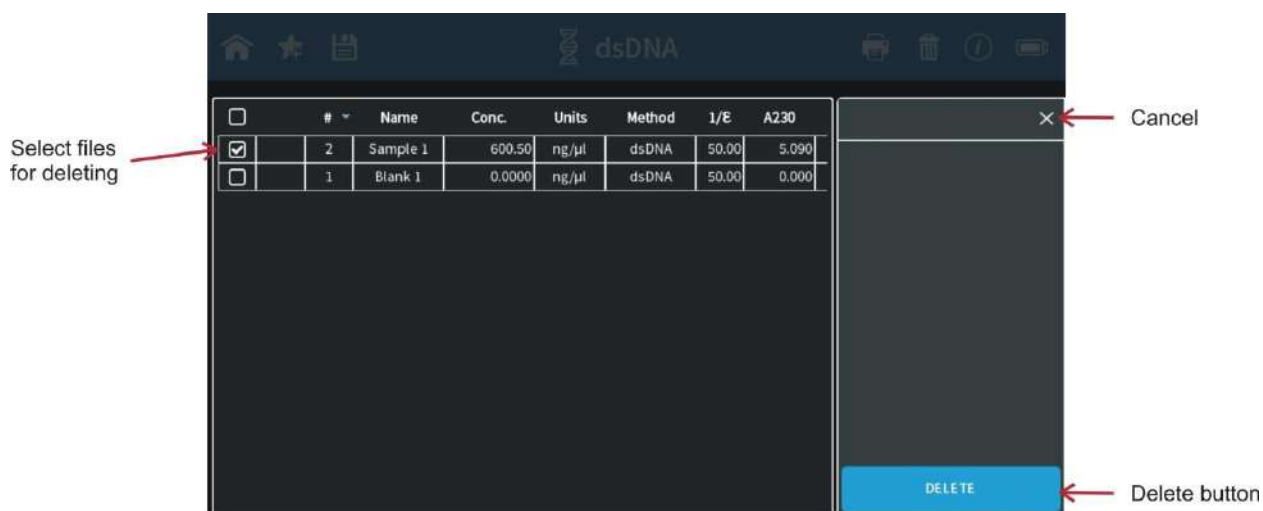


DELETE

Selecting the delete icon (🗑️) opens a full screen overlay dialog window. All data which are selected (tick) in the first table column will be deleted. The header tick box selects/deselects all samples.

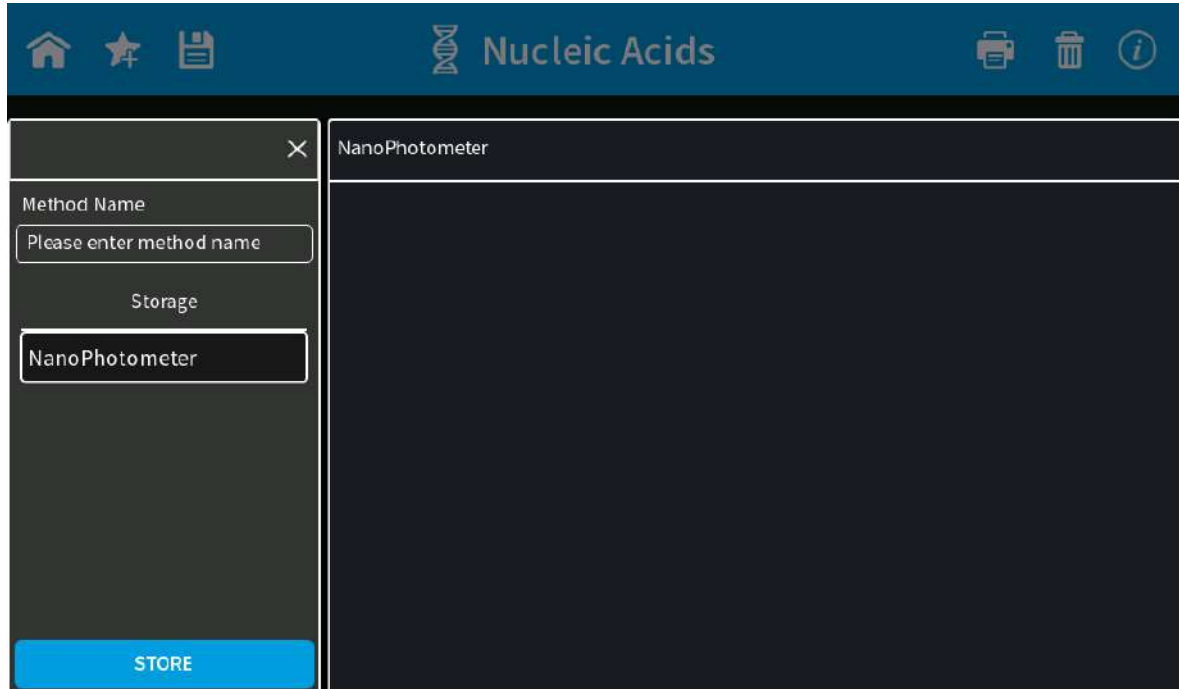
Initiate the deletion with the delete button. Confirm the deletion in the following warning message: “Do you want to delete all/selected files?” select cancel (x) will return to the delete menu screen or confirm with delete to delete the selected data.

Note: The delete function is not available for the software version designed for smartphones.



STORE METHODS

With the favorite icon ★ it is possible in each method to save the parameter settings for easy access of custom defined methods. Select the desired parameter settings and open the Stored Method dialog by pushing o the favorite icon ★.



Enter a method name and select a save location in the folder. Options include: NanoPhotometer®, USB flash drive (if connected) and Network folder (if defined). Push the store button to save the method.

Stored Methods can be opened on the homescreen by opening Stored Methods (see page 122 Stored Methods)

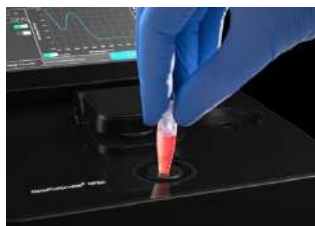
BASIC OPERATION

The NanoPhotometer[®] product line offers a complete solution for NanoVolume (N50/N60/NP80/N120/C40) and standard cuvette volume (C40/NP80) applications. With the NanoPhotometer[®] N50/N60/NP80 NanoVolume applications the sample volume ranges from 0.3 μ l to a maximum sample volume of 2.0 μ l. For the NanoVolume applications with the NanoPhotometer[®] N120 a minimum of 2 μ l sample volume is necessary. Standard cuvette applications can be performed with quartz, glass or plastic cuvettes with a pathlength of 10 mm, 5 mm, 2 mm, 1 mm or 0.5 mm and a center height of 8.5 cm.

Note: NanoVolume application in the NanoPhotometer[®] C40 is only available with the optional accessory “submicroliter cell” from Implen.

NANO VOLUME SINGLE SAMPLE MEASUREMENT BASICS (N50/N60/NP80)

1. Select a method depending on your sample and set the parameters for the measurement.
2. Ensure that the sample window on pedestal and the mirror in the lid arm are clean.
3. Use the integrated vortexer (N60 and NP80 only) to mix the sample to achieve a homogenous sample



4. Raise the lid arm and pipette the appropriate amount of blank solution onto the illuminated sample window on pedestal. The illumination turns automatically off when the lid arm is lowered.



Note: Do not overfill the well.

Note: The low energy red light (LED) illumination can be switched off in Preferences

5. Lower the lid arm and initiate a blank measurement with the blank button
6. Clean the measurement window and mirror on the lid arm with a slightly wet lint-free tissue. Use water, 70% ethanol or isopropanol if needed.



Note: Make sure that the metal contact face (around the measurement window and the mirror) is clean.

Note: Do not use aggressive solvents such as strong acids or bases or organic solvents at any time (see page 55 Solvent Compatibility (N50/N60/N120/NP80/C40 with submicroliter cell). If unsure please contact support@implen.de for detailed information about your specific reagent/solvent.

- It is possible to enter a sample name for each sample in the input window “enter sample name”.

Note: Allowed characters are: A...Z a...z 0...9 , . - () @ ! = _ ~ ; [] { } ‘

Note: Blank character is not allowed.

- Raise the lid arm and pipette the appropriate amount of sample solution onto the illuminated sample window. Upon completion of measurement raise lid arm, clean the surfaces and apply the next sample.

Note: Parameter setting Volume 1 - 2 μ l adjusts the path length automatically. The parameter setting Volume 0.3 μ l measures only the 0.07 mm path length for higher concentrations (dsDNA > 420 ng/ μ l / BSA > 12.6 mg/ml).

Note: The sample window on pedestal must be clean and residual fluff from any cleaning wipe must be removed for optimum performance.

NANOVOLUME MULTI SAMPLE MEASUREMENT BASICS (N120)

Note: The multi sample mode is available for the following methods: Nucleic Acid, Protein UV, Protein Assay and Wavescan.

- Select a multi-channel method depending on your sample and set the parameters for the measurement.
- Sample Map: Option to set sample IDs via the sample ID pop up (📄) or import sample IDs with customized sample names (see page 36 Sample Map / Definition of Sample IDs for further details).



It is not necessary to define sample IDs in the sample map for measurements.

Note: Allowed characters for Sample IDs are: A...Z a...z 0...9 , . - () @ ! = _ ~ ; [] { } ‘
Blank character is not allowed.

- Ensure that the sample spots on pedestal and the mirrors in the lid arm are clean.

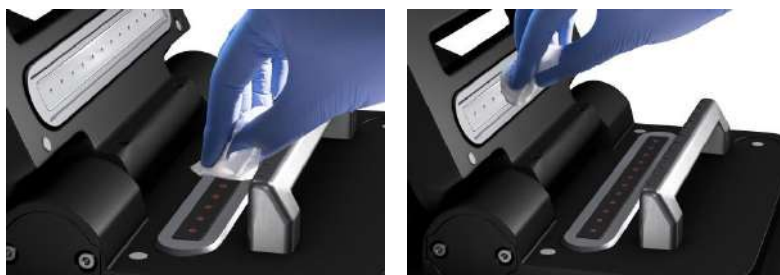
4. Ensure that samples are mixed well to achieve homogenous samples.
5. Raise the lid arm and pipette 2 μ l of blank solution onto the illuminated sample spots on pedestal. Only illuminated sample windows will be measured. The illumination turns off automatically when the lid arm is lowered.



Note: It is recommended to use multi-channel pipettes to avoid evaporation.

Note: The low energy red light (LED) illumination can be switched off in Preferences.

6. Lower the lid arm and initiate a blank measurement with the blank button
7. Clean the measurement spots and mirrors in the lid arm with a slightly wet lint-free tissue. Use water, 70% ethanol or isopropanol if needed.



Note: Make sure that the metal contact face (around the measurement window and the mirror) is clean.

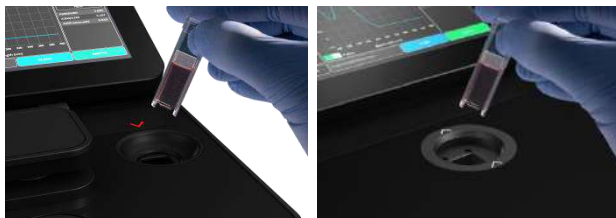
Note: Do not use aggressive solvents such as strong acids or bases or organic solvents at any time (see page 55 solvent compatibility). If unsure please contact support@implen.de for detailed information about your specific reagent/solvent.

8. Raise the lid arm and pipette 2 μ l of sample solution onto the illuminated sample windows. Upon completion of measurement raise lid arm, clean the surfaces and apply the next samples.

Note: The sample window on pedestal must be clean and residual fluff from any cleaning wipe must be removed for optimum performance.

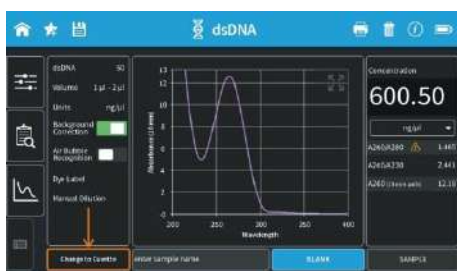
CUVETTE MEASUREMENT BASICS (C40/NP80)

The NanoPhotometer® (NP80 and C40 only) is compatible with standard cuvettes having an 8.5 mm center height. The light path is indicated with a red status LED arrow for the NP80 model and with two white arrows for the C40 model.



Note: For the NP80 the cuvette compartment needs to be activated by the “Change to Cuvette” button in the parameter area. Once the cuvette option is activated the sample compartment door will be opened automatically and a red arrow above the cuvette compartment will appear. The arrow indicates the light path.

1. Select a method depending on your sample and set the parameters for the measurement.
2. Open the automatic cuvette cover by pushing the “Change to Cuvette” button below the parameter area (NP80 only).



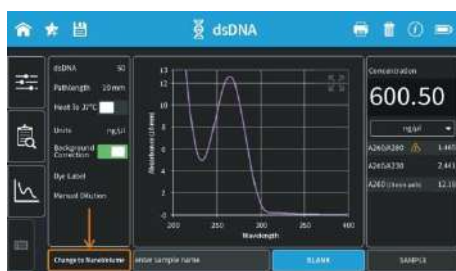
3. Add a blank solution to a cuvette and ensure that the filling volume is sufficient to allow light path to pass through the solution.
4. Insert cuvette into the cell holder.
5. Initiate a blank measurement with the blank button. Upon completion of measurement remove the cuvette.
6. Add sample to a cuvette and ensure that the sample volume is sufficient to allow light to pass through the sample.
7. It is possible to enter a sample name for each sample in the input window “enter sample name”.
Note: Allowed characters are: A...Z a...z 0...9 , . - () @ ! = _ ~ ; [] { } ‘
Note: Blank character is not allowed.
8. Initiate a sample measurement with the sample button. Upon completion of measurement remove the cuvette.
9. Apply further samples.

SUBMICROLITER CELL MEASUREMENTS BASICS (C40)

The Implen submicroliter cell can be used with the NanoPhotometer® C40. Insert the submicroliter cell to the cell holder with the cell windows facing the light beam. We recommend facing the Implen logo to the right side. The light beam is directed from BACK to FRONT as indicated with the white arrows.

Note: For best performance insert the submicroliter cell always in the same direction.

1. Select a method depending on your sample.
2. Enable the NanoVolume application with the “Change to NanoVolume” button below the parameter area.



Note: If there is no “Change to NanoVolume” button available in the method go to preferences/general and enable the NanoVolume option. We guarantee our submicroliter cell specifications only for Implen submicroliter cells which are aligned to the NanoPhotometer® C40 by Implen.

3. Set the parameter for the measurement. Choose the dilution lid depending on your sample concentration.

Lid	dsDNA (ng/µl)	BSA (mg/ml)	absorbance range 10 mm path
5 (optional)	2 – 375		0.05 – 7.5
10	5 – 750	0.15 – 22.5	0.10 – 15
50	25 – 3,750	0.75 – 111	0.50 – 150
100 (optional)	50 – 7,500	1.5 – 225	1.00 – 150
250 (optional)	125 – 18,750	3.75 – 562	2.50 – 375

Note: A new Blank is recommended when changing the dilution lid.

4. Ensure that the sample window and the mirror in the lid are clean.
5. Pipette the appropriate sample volume (blank solution) onto the center of the measuring window. The required volumes are also shown in the parameter area of the software.

Lid	Sample volume	Path length	Dilution
5 (optional)	3.5 – 5 µl	2 mm	1:5
10	1 – 3 µl	1 mm	1:10
50	0.3 – 2 µl	0.2 mm	1:50
100 (optional)	0.3 – 2 µl	0.1 mm	1:100
250 (optional)	0.3 – 2 µl	0.04 mm	1:250

Note: Do not overfill the well.

Note: A new Blank is recommended when changing the dilution lid.

6. For measurements, ensure that the lid fits exactly onto the positioning supports mounted to the body of the cell and initiate a blank measurement with the blank button.

Note: Possible UV exposure. Only initiate a measurement when the lid is closed.
7. Clean the measurement window and mirror in the lid with a slightly wet lint-free tissue. Use water, 70% EtOH or isopropanol, if needed.

Note: Do not use aggressive solvents such as strong acids or bases or organic solvents at any time (see page 55 solvent compatibility). If unsure please contact support@implen.de for detailed information about your specific reagent/solvent.
8. It is possible to enter a sample name for each sample in the input window “enter sample name”.

Note: Allowed characters are: A...Z a...z 0...9 , . - () @ ! = _ ~ ; [] { } ‘

Note: Blank character is not allowed.
9. Mix the sample very well to achieve a homogenous sample.
10. Apply the appropriate amount of sample solution onto the measurement window and initiate the sample measurement with the sample button. Upon completion of measurement remove the lid, clean the surfaces and apply the next sample.

SAMPLE HANDLING TIPS

▪ NanoVolume Methods (NP80/N60/N50)



- The NanoPhotometer® includes an integrated vortexer (N60/NP80 only) to assure sample homogeneity. It is recommended to vortex every sample right before the measurement.
- The sample window on pedestal is illuminated (NP80/N60/N50 only) with a low energy red light to assist with accurate sample application. The red light is switched off once the lid arm is closed. It is possible to disable the illumination feature in preferences of the NPOS software.
- The minimum volume that can be used for NanoVolume samples is 0.3 µl (NP80/N60/N50 dsDNA > 420 ng/µl and BSA > 12.6 mg/ml and for submicroliter cell in the C40 dsDNA > 25 ng/µl and BSA > 0.7 mg/ml). For automatic path length setting at least 1µl is needed (NP80/N60/N50 only).
- The maximum volume that can be used for NanoVolume samples is 2.0 µl (NP80/N60/N50) and 5 µl for the submicroliter cell with lid 5.
- The sample can be fully recovered after measurement with a pipette if desired.

Note: Minimal cross contamination cannot be avoided on molecular level.
- Proper cleaning is important to ensure accurate measurements. In most cases a dry lint-free laboratory wipe is sufficient to clean the sample quartz surfaces. In the case of

highly concentrated samples or certain proteins, the recommended procedure for cleaning is to use a slightly wet lint-free laboratory wipe (with water or 70% EtOH depending on sample type) to thoroughly clean the sample surface.

- It is mandatory that the metal contact face around the measurement window and the mirror is clean.

▪ NanoVolume Methods (N120)



- The sample windows on pedestal are illuminated with a low energy red light to assist with accurate sample application. Depending on selected samples, the red lights are turned on for measurement spots, which will be measured. Apply samples on illuminated measurement spots.

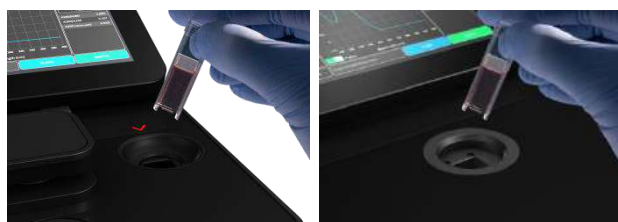
The red light is switched off once the lid arm is closed. It is possible to disable the illumination feature in preferences of the NPOS software.

e.g. 12 samples selected:



- For blank and sample measurements 2 μ l of sample volume are necessary. Pathlength will be set automatically, if necessary.
- Proper cleaning is important to ensure accurate measurements. In most cases a dry lint-free laboratory wipe is sufficient to clean the sample quartz surfaces. In the case of highly concentrated samples or certain proteins, the recommended procedure for cleaning is to use a slightly wet lint-free laboratory wipe (with water or 70% EtOH depending on sample type) to thoroughly clean the sample surface.
- It is mandatory that the metal contact face around the measurement window and the mirror is clean.
- Close the lid arm carefully. Do not push the lid arm down forcefully. Do not touch the lid arm during measurements.

▪ Cuvette Methods



- The cuvette holder (NP80/C40 only) is compatible with standard 10 mm path length quartz, glass and plastic cuvettes with an optical height of 8.5 mm.

- It is also possible to use cuvettes with 5 mm, 2 mm, 1 mm or 0.5 mm path lengths, but there may be an adapter necessary. Please ask your cuvette supplier for a suitable adapter.
- The minimum volume for accurate measurements depends on the cuvette type used; it is necessary that the light passes through the sample for accurate measurements. Center height is 8.5 mm.

Note: The cuvette holder is not removable. Do not pour any cleaning solution into the cuvette holder as larger amounts of liquids can get into the instrument and cause damage.

SOLVENT COMPATIBILITY (N50/N60/N120/NP80/C40 WITH SUBMICROLITER CELL)

Most solvents typically used in life science laboratories are compatible with the NanoPhotometer® NanoVolume sample surfaces. The following solvents are compatible for use with the NanoPhotometer® models N50, N60, N120 and NP80 at room temperature:

- Acetone ($\leq 5\%$)
- Acetonitrile
- Benzene
- Butanol
- Carbon tetrachloride
- Chloroform
- Ethanol
- Ether
- HEPES
- Hexane
- Isopropanol
- MES
- Methanol
- Methylene chloride
- MOPS
- Phenol ($\leq 1\%$)
- N-propanol
- Toluene
- Phosphate containing buffers
- PBS (pH 4-10)
- Citrate
- Borate
- Chloride salts
- Acids $> \text{pH } 2$
- Bases $< \text{pH } 10$

Note: Highly concentrated acids and bases are not recommended. It is recommended to wipe the sample surface with a lint-free laboratory wipe immediately upon completion of each measurement. For more information about compatibility of specific solvents not listed above, please contact the Implen support team (support@implen.de) to check the compatibility.

DATA TRANSFER VIA FILE SERVER

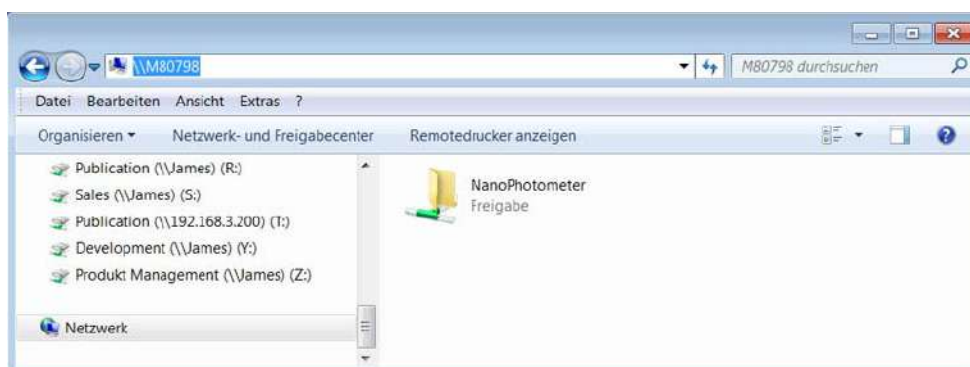
All data saved on the NanoPhotometer® can easily be accessed from and transferred to a computer via the NanoPhotometer® file server. Connection options are LAN/WLAN, USB cable or WiFi Hotspot.

It is possible to create user accounts for password secured file server access. User accounts for file server access can be activated in Preferences see page 130 File Server Access.

▪ File Server Access via LAN/WLAN

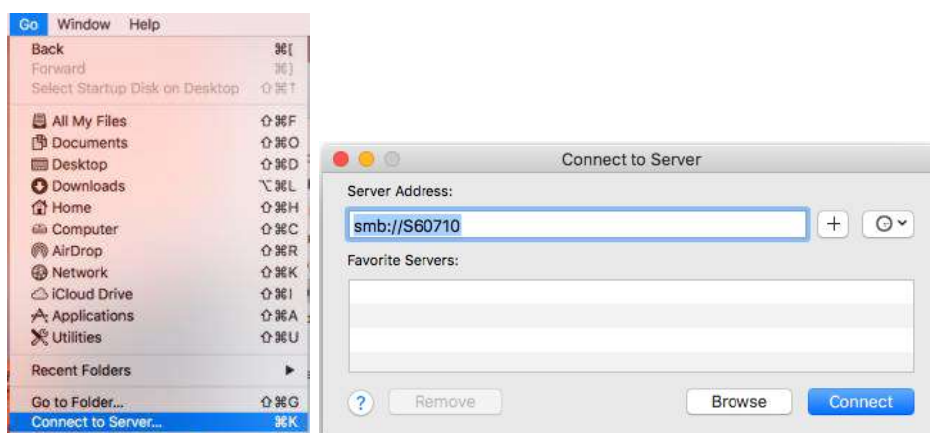
For the file server access via LAN/WLAN it is necessary that both the computer and the NanoPhotometer® are connected to the same LAN/WLAN network. For connection of the NanoPhotometer® to LAN/WLAN see page 128 Network.

For **Windows computer** open the Windows explorer and enter the serial number or the NanoPhotometer® IP in the address bar of the Windows Explorer (e.g. \\M80798\ or \\Assigned IP Address\).



Note: Serial number and IP address of the NanoPhotometer® can be found in the NanoPhotometer® software under Preferences/General/About.

For a **MAC computer** open the "Connect to Server" dialog in the "Go" menu of the Mac OS X Finder and enter the NanoPhotometer® serial number or the active NanoPhotometer® IP address in the server address field to connect.



Note: Serial number and IP address of the NanoPhotometer® can be found in the NanoPhotometer® software under Preferences/General/About.

- **File Server Access via USB cable**

For file server access via USB cable connection, connect the NanoPhotometer® with a USB A/B cable to the computer and open the Windows Explorer or Connect to Server option for Mac (see file server access via LAN/WLAN) and enter \\192.168.7.1\ for connection.

- **File Server Access via WiFi Hotspot**

For file server access via WiFi Hotspot the WiFi Hotspot needs to be active on the NanoPhotometer®. For activation see page 129 WLAN Settings. The computer needs to be connected to the NanoPhotometer® WiFi Hotspot (SSID: NanoPhotometer® serial number; password: Implenuser).

Open the Windows Explorer or Connect to Server option for Mac (see file server access via LAN/WLAN) and enter \\192.168.8.1\ for connection.

BATTERY OPERATION

The optional battery operation is only available for the NanoPhotometer® N120/NP80/N60/C40 models (N120-Mobile, NP80-Mobile, N60-Mobile and C40-Mobile) and not for the NanoPhotometer® N50. A full battery pack can be operated for approximately 8 hours (NP80/N60/C40) and 3 hours (N120) within normal usage, i.e. 20 - 30 measurements per hour.

Note: Screen saver activation will influence the time for battery operation. To prolong battery operation, switch the screen saver off.

When the battery gets low there will be a first warning message “Battery low” and a beep is sounded. At this status it is possible to use the instrument under standard conditions for at least one hour. It is recommended to charge the battery at this status. If the battery is not charged there will be a second warning message when the battery is empty and several beep sounds before the instrument is automatically shutting down within 10 to 15 seconds.

Note: Battery operation time is depending on display usage. Activating the screen saver video may reduce the battery operation time.

Note: Unsaved data can be found in the auto save folder in Stored Results. If the NanoPhotometer® is shut down during a measurement process the data of this measurement are lost.

The battery pack is designed for 800 full charging cycles. Afterwards the capacity and operation time of the battery may vary. A fully charged battery pack will self-discharge (switched off NanoPhotometer®) over a time of 10 – 14 days. To avoid deep discharging of the battery pack charge the battery pack at least once a month even if the NanoPhotometer® is not in use.

Note: Recharging of deep discharged batteries is not possible they need to be replaced by new ones.

Charging time for an empty battery pack is 3 hours.

4. NANOPHOTOMETER® APPLICATIONS

The NanoPhotometer® comes with pre-programmed applications as well as the ability to create custom applications. Any application method can be selected by tapping the icon once or pushing on the icon (computer based software).


Note: Within each method selection of NanoVolume or cuvette mode (NP80/C40) is possible. The “Change to Cuvette” or “Change to NanoVolume” button is located at the bottom of the parameter area.



NUCLEIC ACIDS

METHOD OVERVIEW

Nucleic acids in solution absorb light with a peak in the ultraviolet region of 260 nm. For determination of nucleic acid concentration in solution the absorbance at wavelength 260 nm is used along with the Beer-Lambert law. In addition to calculating concentrations of nucleic acids, absorbance measurements are also useful for estimating purity of nucleic acids by calculating the 260/280 nm and 260/230 nm ratios. Further, it is possible to determine the degree of labeling of nucleic acids with probes including fluorescent dyes.

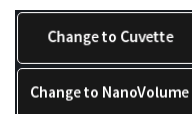
Sample Control™ gives useful information about sample conditions. It recognizes air bubbles, sample impurities, turbidity, lint residues and potential contaminations. If Sample Control™ detects any inconstancy an alert icon  is shown in the result/table area. A push on the alert icon shows additional information about the inconstancy.

MEASUREMENT PROTOCOL SINGLE SAMPLE

1. Select the Nucleic Acids icon on the home screen.



2. To change between NanoVolume and cuvette application (NP80/C40 only) use the Change to Cuvette/NanoVolume button below the parameter area.



3. To change the nucleic acid type push on dsDNA and a list with available options opens on the right side.

Options are: dsDNA, ssDNA, RNA miRNA, miRNA Sequence, Oligo, Oligo Sequence and Custom (see Table 1 on page 66).

- The miRNA sequence and Oligo sequence options allow the sequence to be entered and the extinction coefficient will be automatically calculated. A minimum of 4 bases is required.
- The default setting for Custom is 50 and allows changing the extinction coefficient ($1/\epsilon$) in a range of 15 - 150. It is possible to enter a name for the custom nucleic acid factor for documentation.

dsDNA	50	dsDNA	50	ssDNA	37
Volume	1 µl - 2 µl	RNA	40	miRNA	33
Units	ng/µl	miRNA Seq.	<input type="text" value="Please enter miRNA sequence"/>		
Background Correction		Oligo	33		
Air Bubble Recognition	<input type="checkbox"/>	Oligo Seq. DNA	<input type="text" value="Please enter DNA Oligo sequence"/>		
Dye Label		Oligo Seq. RNA	<input type="text" value="Please enter RNA Oligo sequence"/>		
Manual Dilution		Custom	<input type="text" value="Please enter Name"/>		
			<input type="text" value="Please enter Factor"/>		

4. For NanoVolume application:

Select the volume of sample to be applied.

dsDNA	50	1-2 µl sample volume	Autoranging
Volume	1 µl - 2 µl	0.3 µl sample volume	Dilution 140 / 0.07 mm path

Note: 1 - 2 µl (default): automatic path length setting; 0.3 µl measures only the 0.07 mm path length (for samples with concentrations > 420 ng/µl dsDNA)

For cuvette application:

Select the path length depending on the used cuvette.
Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm

dsDNA	50	Pathlength	0.5 mm
Pathlength	10 mm	Pathlength	1 mm
Heat To 37°C	<input type="checkbox"/>	Pathlength	2 mm
Units	ng/µl	Pathlength	5 mm
		Pathlength	10 mm

If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

Select a Lid depending on your sample concentration for concentration ranges of different lids (see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

dsDNA	50	Lid 5	3.5 µl - 5 µl sample volume
Lid	50	Lid 10	1 µl - 3 µl sample volume
Units	ng/µl	Lid 50	0.3 µl - 2 µl sample volume
Background Correction		Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Select the Units in which the concentration should be calculated. Options are ng/µl (default), µg/µl and µg/ml. pmol/µl if a nucleic acid sequence is entered for nucleic acid factor calculation.

dsDNA	50	ng/µl
Volume	1 µl - 2 µl	µg/µl
Units	ng/µl	µg/ml

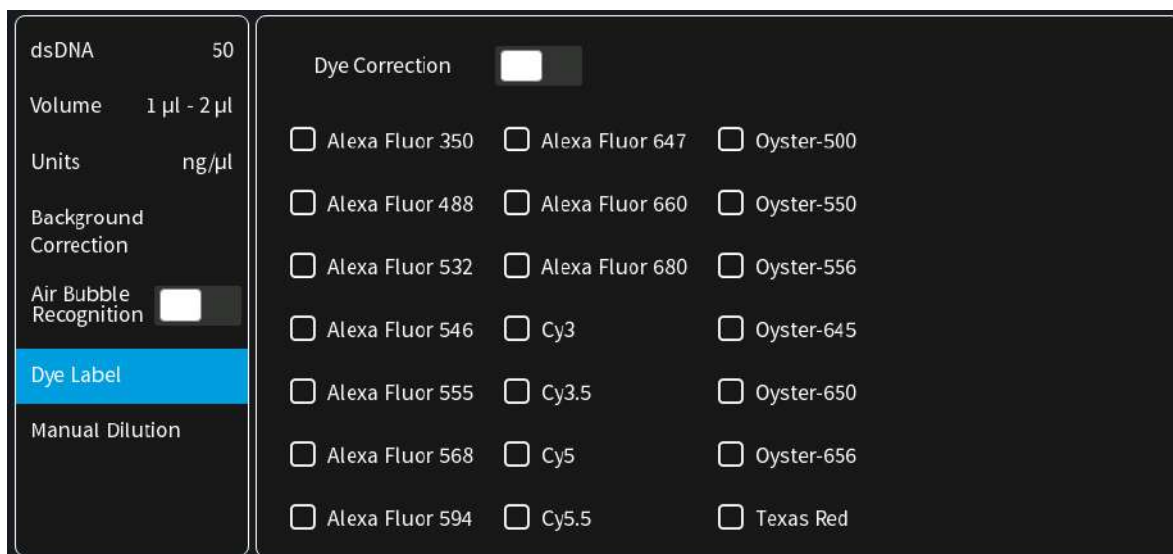
- The background correction is enabled at 320 nm by default. Selection options are 320 nm, 340 nm or any wavelength in the range of 220 – 350 nm. The background correction can be disabled with the toggle switch.

dsDNA	50	Background Correction	<input checked="" type="checkbox"/>
Volume	1 µl - 2 µl	Background Correction at 320 nm	
Units	ng/µl	Background Correction at 340 nm	
Background Correction		Background Correction at	<input type="text" value="320 nm"/>

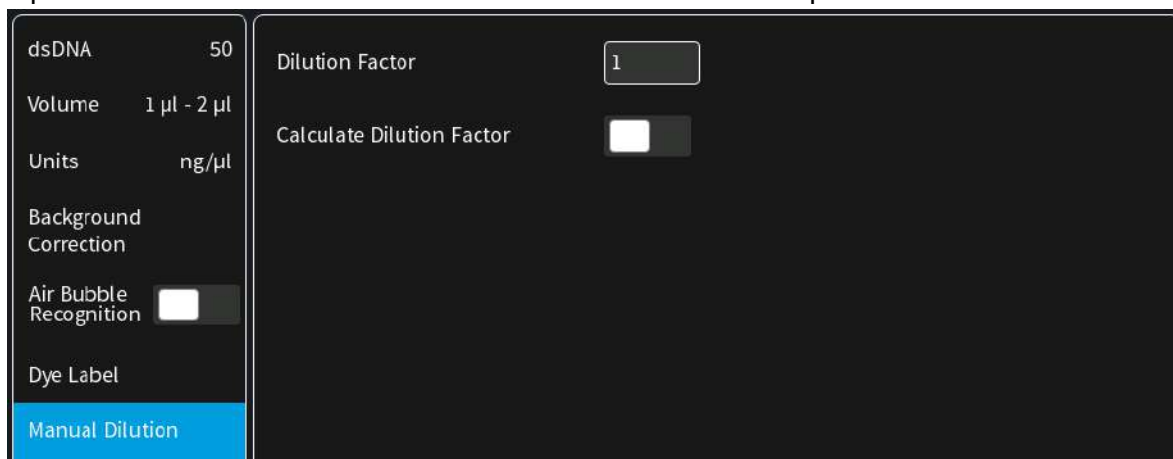
- Air bubble recognition is disabled by default. Setting to on detects air bubbles, lint residues and bad sample conditions of the sample.
Note: Lint residues and bad sample conditions are detected even if the air bubble recognition is set to off.



- For dye labelled samples tick the dye labels in the list, which should be used for result calculation.
Note: If the used dye is not available in the list, please go to preferences and add a custom dye to the dye list.
There is an option for dye correction which can be enabled/disabled with a toggle switch.
Note: Dye correction is only available for single dye selection.



9. Option to set/calculate a dilution factor for manual diluted samples.



10. Apply the blank ddH₂O or buffer to the illuminated sample window on the pedestal and close the lid arm for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in the preferences.

11. Use a lint-free laboratory wipe to clean both the sample window on pedestal and mirror in lid arm prior to applying the next sample.



Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

12. Apply sample to the sample window on pedestal and press the sample button to initiate the measurement.



If auto sample is active the sample measurement is automatically started after closing the lid arm.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.

MEASUREMENT PROTOCOL MULTI SAMPLE (N120)

1. Select the Nucleic Acids icon on the home screen.



2. To change between single and multi-sample application use the toggle switch Single Sample in the parameter area.



3. Parameter setting Nucleic Acid type:

To change the nucleic acid type, select either dsDNA, ssDNA, RNA, miRNA, miRNA Sequence, Oligo, Oligo Sequence or Custom (see Table 1 on page 66).

- The miRNA sequence and Oligo sequence options allow the sequence to be entered and the extinction coefficient will be automatically calculated. A minimum of 4 bases is required.
- The default setting for Custom is 50. The extinction coefficient (1/ε) can be set in a range of 15 - 150. It is possible to enter a name for the custom nucleic acid factor for documentation.

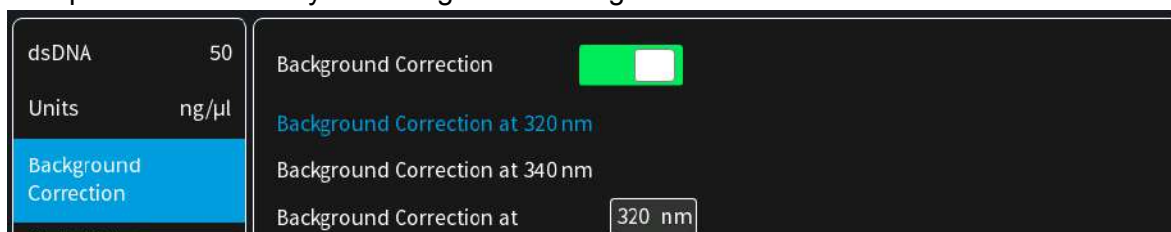
dsDNA	50	dsDNA	50	ssDNA	37
Units	ng/μl	RNA	40	miRNA	33
Background Correction		miRNA Seq.	Please enter miRNA sequence		
Air Bubble Recognition	<input type="checkbox"/>	Oligo	33		
Dye Label		Oligo Seq. DNA	Please enter DNA Oligo sequence		
Sample Loading		Oligo Seq. RNA	Please enter RNA Oligo sequence		
Single Sample	<input type="checkbox"/>	Custom	Please enter Name		
			Please enter Factor		

4. Select the Units in which the concentration should be calculated. Options are ng/μl, μg/μl and μg/ml. pmol/μl if a nucleic acid sequence is entered for nucleic acid factor calculation.

dsDNA	50	ng/μl
Units	ng/μl	μg/μl
Background Correction		μg/ml

5. The background correction is enabled at 320 nm by default. It is possible to disable the background correction with the toggle switch. Selection options are 320 nm, 340 nm. It is

also possible to add any wavelength in the range of 220 – 350 nm



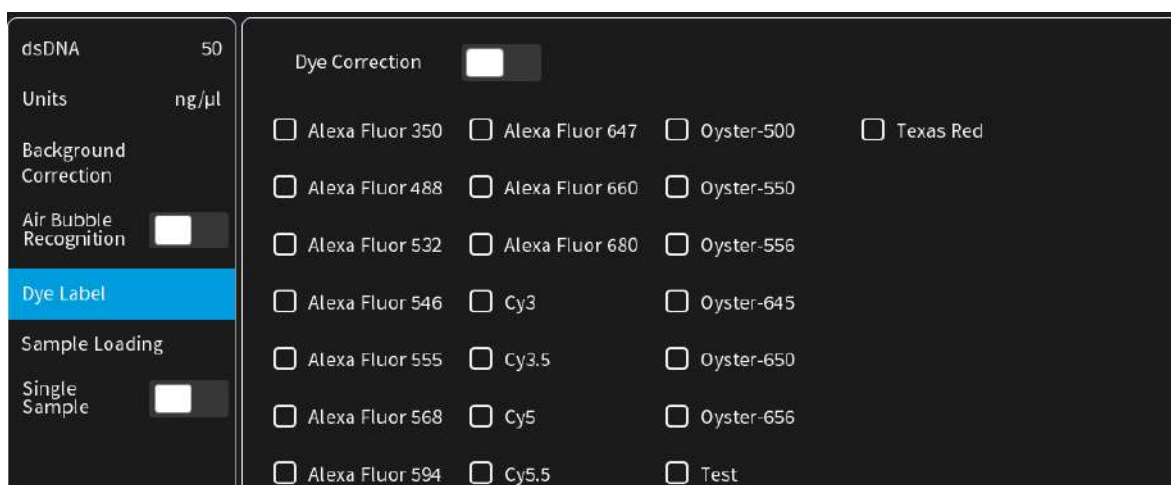
- Air bubble recognition is disabled by default. Set to on it detects air bubbles, lint residues and bad sample conditions of the sample.
Note: Lint residues and bad sample conditions are detected even if the air bubble recognition is set to off.



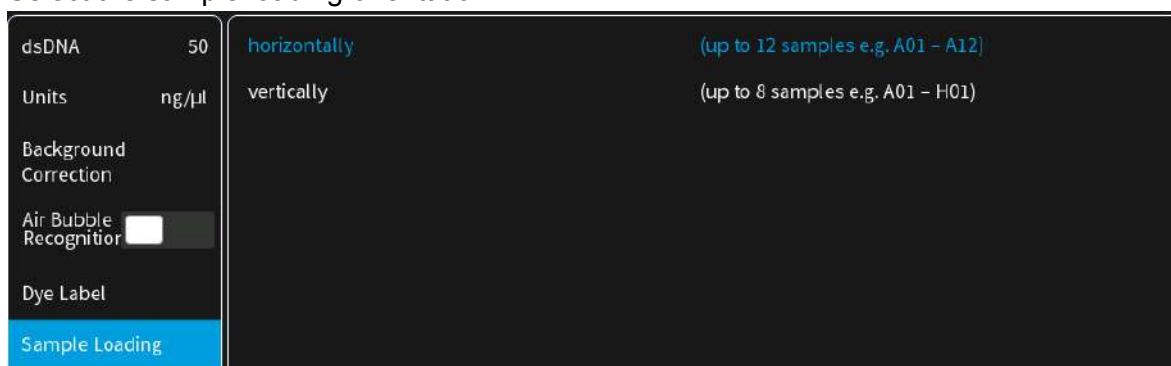
- For dye labelled samples tick the dye labels in the list, which should be used for result calculation.
Note: If the used dye is not available in the list, please go to preferences and add a custom dye to the dye list (see page 125 Dyes).

There is an option for dye correction which can be enabled/disabled with a toggle switch.

Note: Dye correction is only available for single dye selection.



- Select the sample loading orientation.



There are two options:

Horizontally: Measure up to 12 samples (A01 – A12 shown in sample map as row)

	1	2	3	4	5	6	7	8	9	10	11	12
A	→											
B												
C												
D												

Vertically: Measure up to 8 samples (A01 – H01 shown in sample map as column)

	1	2	3	4
A	↓			
B				
C				
D				
E				
F				
G				
H				

- To confirm the parameter setting, swipe the screen to the left and the sample map screen is shown.



The sample map offers the possibility to select cells and define sample IDs for measurements. It is not necessary to make any selection. If cells are left empty without defined sample IDs for horizontal or vertical sample loading, all 12 positions for horizontal loading or all 8 positions for vertical loading are measured.

To measure a defined amount of samples select a cell pattern starting at A01. Sample IDs can be defined by pushing on the sample ID icon (▮▮▮) in the navigation bar. The opened pop up offers options to define, import, export or delete sample IDs.



Further information about cell selection and sample ID definition can be found in chapter 3 NanoPhotometer® Basics / Sample Map on page 36

10. Apply the blank ddH₂O or buffer to the illuminated sample windows on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in preferences.

Note: If the blank and sample buttons are not shown please either open the lid arm, push the ▲ on the right bottom corner or swipe from the bottom edge in the screen.

11. Use a lint-free laboratory wipe to clean both the sample windows on pedestal and mirrors in lid arm prior to applying the next sample.



Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

12. Apply samples to the illuminated sample windows on pedestal and press the sample button to initiate the measurement.



If auto sample is active the sample measurement is automatically started after closing the lid arm.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.

CALCULATIONS

▪ Nucleic Acid Concentration

To determine the concentration of nucleic acids in solution, the absorbance is measured at a wavelength of 260 nm. The function describing the relationship between concentration and absorbance is a modification of the Beer-Lambert law equation. The concentration of the

nucleic acid samples can be calculated with or without background correction depending on enabled/disabled background correction parameter.

Without background correction:

$$C = A_{260} * \text{Factor}_{\text{nuc}} * \text{D}$$

With background correction:

$$C = (A_{260} - A_{\text{BKG}}) * \text{Factor}_{\text{nuc}} * \text{D}$$

C Concentration in ng/μl

A₂₆₀ Absorbance at 260 nm (10 mm path)

A_{BKG} Absorbance at selected background wavelength (10 mm path)

D Manual dilution factor

Factor_{nuc} Nucleic acid factor in ng*cm/μl

Table 1. Nucleic acids factors (Factor_{nuc})

Type	Factor _{nuc}
dsDNA	50 ng*cm/μl
ssDNA	37 ng*cm/μl
RNA	40 ng*cm/μl
miRNA	33 ng*cm/μl
Oligo	33 ng*cm/μl
miRNA Seq.	calculated via extinction coefficient of constituent nucleotides entered
Oligo Seq.	calculated via extinction coefficient of constituent nucleotides entered
Custom	Option to enter any factor between 15 and 150 ng*cm/μl

▪ **Dye-labeled Nucleic Acid Concentration**

For dye-labeled nucleic acids, the concentration of the nucleic acid is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye and a certain dye-specific correction factor at 260 nm (see Table 2 on page 68). The concentration of a dye-labeled nucleic acid is calculated with or without background/dye correction as follows:

With background and with dye correction:

$$C = \left[(A_{260} - A_{\text{BKG}}) - (cf_{\text{dye}} * (A_{\text{max, dye}} - A_{\text{BKG}})) \right] * \text{Factor}_{\text{nuc}} * \text{D}$$

With background and without dye correction:

$$C = (A_{260} - A_{\text{BKG}}) * \text{Factor}_{\text{nuc}} * \text{D}$$

Without background and with dye correction:

$$C = \left[A_{260} - (cf_{\text{dye}} * A_{\text{max, dye}}) \right] * \text{Factor}_{\text{nuc}} * \text{D}$$

Without background and without dye correction:

$$C = A_{260} * \text{Factor}_{\text{nuc}} * \text{D}$$

C	Concentration in ng/μl
A ₂₆₀	Absorbance at 260 nm (10 mm path)
A _{BKG}	Absorbance at selected background wavelength (10 mm path)
A _{max, dye}	Absorbance value at the absorbance maximum of the dye (10 mm path)
Factor _{nuc}	Nucleic acid factor in ng*cm/μl
cf _{dye}	Dye-dependent correction factor at 260 nm
D	Manual dilution factor

Note: Dye correction is only available for single dye selection.

- **Dye Concentration**

For dye-labeled nucleic acids, the concentration of the dye is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye, the dye-specific extinction coefficient (see Table 2 on page 68). The dye concentration is calculated with or without background correction as follows:

With background correction:

$$C = \frac{(A_{\text{max, dye}} - A_{\text{BKG}}) * \text{D}}{m\epsilon_{\text{dye}} * 10^{-6}}$$

Without background correction:

$$C = \frac{A_{\text{max, dye}} * \text{D}}{m\epsilon_{\text{dye}} * 10^{-6}}$$

C	Concentration in pmol/μl
A _{max, dye}	Absorbance value at the absorbance maximum of the dye (10 mm path)
A _{BKG}	Absorbance at 320 nm (10 mm path)
mε _{dye}	Molar extinction coefficient of dye in M ⁻¹ *cm ⁻¹
D	Manual dilution factor

- **Frequency of Incorporation (FOI)**

FOI is the degree of labeling based on dye incorporation in a labeled nucleic acid sample. It is generally expressed as the number of dye molecules incorporated per 1000 nucleotides. FOI can be calculated with or without background/dye correction as follows:

With background correction with dye correction:

$$\text{FOI} = \frac{324.5 * (A_{\text{max, dye}} - A_{\text{BKG}})}{m\epsilon_{\text{dye}} * 10^{-6} * (A_{260} - A_{\text{BKG}} - \text{cf}_{\text{dye}} * (A_{\text{max, dye}} - A_{\text{BKG}})) * \text{Factor}_{\text{nuc}}}$$

With background correction without dye correction:

$$FOI = \frac{324.5 * (A_{\max, \text{dye}} - A_{\text{BKG}})}{m\epsilon_{\text{dye}} * 10^{-6} * (A_{260} - A_{\text{BKG}}) * \text{Factor}_{\text{nuc}}}$$

Without background correction and with dye correction:

$$FOI = \frac{324.5 * A_{\max, \text{dye}}}{m\epsilon_{\text{dye}} * 10^{-6} * (A_{260} - c_{\text{dye}} * A_{\max, \text{dye}}) * \text{Factor}_{\text{nuc}}}$$

Without background correction and without dye correction:

$$FOI = \frac{324.5 * A_{\max, \text{dye}}}{m\epsilon_{\text{dye}} * 10^{-6} * A_{260} * \text{Factor}_{\text{nuc}}}$$


FOI	Frequency of Incorporation (dye per 1,000 bases)
A_{260}	Absorbance at 260 nm (10 mm path)
A_{BKG}	Absorbance at selected background wavelength (10 mm path)
$A_{\max, \text{dye}}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
$m\epsilon_{\text{dye}}$	molar extinction coefficient of dye in $\text{M}^{-1} * \text{cm}^{-1}$
$\text{Factor}_{\text{nuc}}$	Nucleic acid factor in $\text{ng} * \text{cm} / \mu\text{l}$
c_{dye}	Dye-dependent correction factor at 260 nm

Table 2. Dye Types, Absorbance Max, Extinction coefficient, and dye-dependent correction factors

NanoPhotometer® Model	Dye Type	Absorbance maximum of Dye (nm)	molar ext. coeff. of dye $m\epsilon_{\text{dye}}$ in $\text{M}^{-1} * \text{cm}^{-1}$	Dye-dependent correction factor at 260 nm c_{dye}
NP80/N60/N50/C40	Alexa Fluor 350	345	18,400	0.25
NP80/N60/N50/C40	Alexa Fluor 488	492	62,000	0.30
NP80/N60/N50/C40	Alexa Fluor 532	525	82,300	0.24
NP80/N60/N50/C40	Alexa Fluor 546	555	104,000	0.21
NP80/N60/N50/C40	Alexa Fluor 555	555	150,000	0.04
NP80/N60/N50/C40	Alexa Fluor 568	576	93,000	0.45
NP80/N60/N50/C40	Alexa Fluor 594	588	80,400	0.43
NP80/N60/C40	Alexa Fluor 647	650	239,000	0.00
NP80/N60/C40	Alexa Fluor 660	660	107,000	0.00
NP80/N60/C40	Alexa Fluor 680	680	164,000	0.00
NP80/N60/N50/C40	Cy3	550	150,000	0.08
NP80/N60/N50/C40	Cy3.5	581	150,000	0.08
NP80/N60/C40	Cy5	649	250,000	0.05

NP80/N60/C40	Cy5.5	675	250,000	0.05
NP80/N60/N50/C40	Oyster-500	503	78,000	0.29
NP80/N60/N50/C40	Oyster-550	553	150,000	0.05
NP80/N60/N50/C40	Oyster-556	560	155,000	0.03
NP80/N60/C40	Oyster-645	649	220,000	0.05
NP80/N60/C40	Oyster-650	653	200,000	0.04
NP80/N60/C40	Oyster-656	660	200,000	0.04
NP80/N60/N50/C40	Texas Red	593	85,000	0.23

▪ Ratios

Reactions utilizing nucleic acids often require minimum purity standards. Common contaminants of nucleic acid samples include: proteins, organic compounds, and other. Based on the common contaminants of nucleic acid samples, the 260/280 and 260/230 ratios are calculated for nucleic acids to give an indication of the purity of the samples. Pure DNA and RNA preparations have expected 260/280 ratios of ≥ 1.8 and ≥ 2.0 respectively. An elevated absorbance at 230 nm can indicate the presence of impurities as well; 230 nm is near the absorbance maximum of peptide bonds and also indicates buffer contamination since TRIS, EDTA and other buffer salts absorb at 230nm. When measuring RNA samples, the 260/230 ratio should be > 2.0 ; a ratio lower than this is generally indicative of contamination with guanidinium thiocyanate, a reagent commonly used in RNA purification and which absorbs over the 230-260 nm range. If a ratio is detected out of the acceptable range an alert icon  is shown in the results/table area. A push on the alert icon shows additional information. The ranges for acceptable ratio values can be defined in preferences. The ratios are calculated with or without background correction according to if the background correction is activated during the measurements or not as follows:

Without background correction:

$$260/280 \text{ ratio} = \frac{A_{260}}{A_{280}}$$

$$260/230 \text{ ratio} = \frac{A_{260}}{A_{230}}$$

With background correction:

$$260/280 \text{ ratio} = \frac{A_{260} - A_{BKG}}{A_{280} - A_{BKG}}$$

$$260/230 \text{ ratio} = \frac{A_{260} - A_{BKG}}{A_{230} - A_{BKG}}$$



PROTEIN UV

METHOD OVERVIEW


The Protein UV method exploits the inherent absorbance of proteins at 280 nm in combination with the Beer-Lambert Law, where each protein is characterized by a protein specific extinction coefficient (ϵ) which can be used to determine total protein concentration of a solution. The intrinsic absorbance of proteins is due to the presence of aromatic amino acids in their structure, primarily tryptophan and tyrosine, as well as cysteine (oxidized cysteine residues in a disulphide bond). The aromatic amino acid residues in a protein containing tryptophan and tyrosine exhibit strong intrinsic absorbance at 280 nm, with a lesser contribution by phenylalanine. Therefore, it is the aromatic amino acid residues which dictate the extinction coefficient at 280 nm for a protein.

The most straightforward method to determine concentration of a purified, homogenous protein with a known extinction coefficient (ϵ) is by direct measurement of UV280 provided as long as the protein contains no prosthetic groups with strong absorption in the same region. However, for unknown proteins including homogenous protein mixtures, it is possible to make direct A_{280} measurements using a composite ϵ value derived from comparison of many proteins, although this will only provide an approximate but close estimate of the true protein concentration.

The NanoPhotometer® determines protein concentration by performing calculations based on specific ϵ values, either pre-programmed in the instrument or entered manually by the user. Extinction coefficient (ϵ) values at 280 nm vary greatly for different proteins due to their particular aromatic amino acid content. Fixed ϵ values are pre-programmed in the software for certain proteins (see Table 3 on page 79). However, if the protein of interest is not included in the pre-programmed methods it is also possible to manually enter the specific ϵ for the protein of interest using the custom Mol. Ext. Coefficient, custom Ext. Coefficient or custom $1/\epsilon$ protein factor option. For correct calculation, it is necessary to supply either: a) the molar extinction coefficient (ϵ_M in $M^{-1} \cdot cm^{-1}$) and the molecular weight expressed in molar mass units (g/mol); b) the mass extinction coefficient (ϵ in $l/g \cdot cm$) or c) the protein factor $1/\epsilon$ of the protein.

To determine the degree of dye labelling of a protein, the absorbance measured at the wavelength corresponding to the absorbance maximum of the fluorescence dye is used (see Table 3 on page 79). The corresponding extinction coefficient of the dye is used along with the Beer-Lambert Law to determine the dye concentration.

Note: It is important to ensure the extinction coefficient and units entered are correct in order to ensure that calculations are performed properly for accurate concentration values.

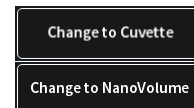
Sample Control™ gives useful information about air bubbles, sample impurities, turbidity, lint residues and potential contaminations. If the Sample Control™ detects any inconstancy an alert icon  is shown in the result/table area. A push on the alert icon shows additional information about the inconstancy.

MEASUREMENT PROTOCOL SINGLE SAMPLE

1. Select the Protein UV icon on the home screen.



2. To change between NanoVolume and Cuvette application (NP80/C40 only) use the Change to Cuvette/NanoVolume button below the parameter area.



3. To change the protein type push on BSA and a list with available options opens on the right side.

Options are: BSA, SA Mouse, SA Human, IgG Mouse, IgG Human, IgE Human, Lysozyme, OD1, Custom (Molar Extinction Coefficient), Custom (Extinction Coefficient) and Custom (1/ε).

- For Custom (Mol. Ext. Coefficient) enter Molecular Weight in g/mol and the Mol. Ext. Coefficient in $M^{-1} \cdot cm^{-1}$
- For Custom (Ext. Coefficient) enter the Ext. Coefficient in $l/g \cdot cm$
- For Custom (1/ε) enter the calculated protein factor 1/ε

BSA	1.499	BSA	1.499	SA Mouse	1.493	SA Human	1.718
Volume	1 μl - 2 μl	IgG Mouse	0.714	IgG Human	0.735	IgE Human	0.654
Wavelength	280 nm	Lysozyme	0.379	OD1	1.000	Custom (Mol. Ext. Coeff.)	Please enter MW (g/mol)
Units	mg/ml	Custom (Ext. Coeff.)	Please enter Ext. Coeff. (l/g*cm)	Custom (1/ε)	Please enter Protein Factor (1/ε)		
Background Correction							
Air Bubble Recognition	<input type="checkbox"/>						
Dye Label							
Manual Dilution							

4. For NanoVolume application:

Select the volume of sample to be applied.

BSA	1.5	1-2 μl sample volume	Autoranging
Volume	1 μl - 2 μl	0.3 μl sample volume	Dilution 140 / 0.07 mm path

Note: 1-2 μl (default): automatic path length setting; 0.3 μl measures only the 0.07 mm path length (possible for samples with concentrations e.g. BSA > 12.6 mg/ml)

For cuvette application:

Select the path length depending on the used cuvette.

Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm

BSA	1.5	Pathlength	0.5 mm
Pathlength	10 mm	Pathlength	1 mm
Heat To 37°C	<input type="checkbox"/>	Pathlength	2 mm
Wavelength	280 nm	Pathlength	5 mm
		Pathlength	10 mm

If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

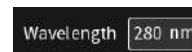
Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

BSA	1.5	Lid 5	3.5 µl - 5 µl sample volume
Lid	50	Lid 10	1 µl - 3 µl sample volume
Wavelength	280 nm	Lid 50	0.3 µl - 2 µl sample volume
Units	mg/ml	Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

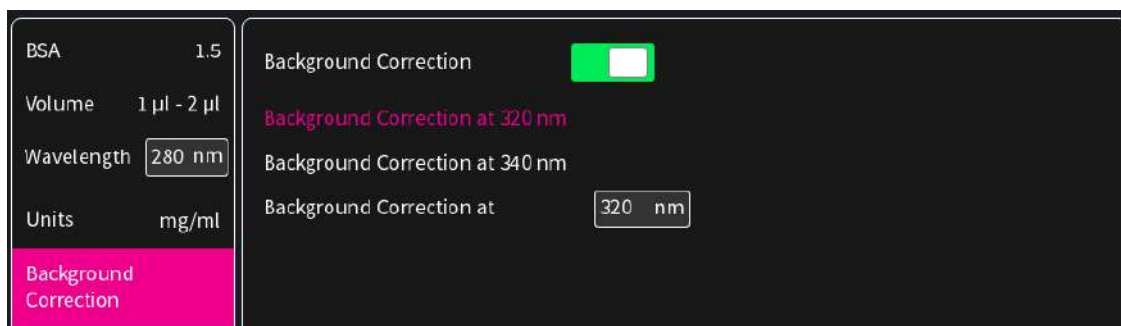
Note: A new Blank is recommended when changing the dilution lid.

- The wavelength for protein measurements can be changed in the range of 200 – 330nm depending on the wavelength peak of the protein. Default setting is 280 nm
- Select the Units in which the concentration should be calculated. Options are ng/µl, µg/µl, µg/ml and mg/ml (default).



BSA	1.499	ng/µl
Volume	1 µl - 2 µl	µg/µl
Wavelength	330 nm	µg/ml
Units	mg/ml	mg/ml

- The background correction is enabled at 320 nm by default. Selection options are 320 nm, 340 nm or any wavelength in the range of 220 – 350 nm. The background correction can be disabled with the toggle switch.



- 8. Air bubble recognition is disabled by default. When enabled it detects air bubbles, lint residues and poor conditions of the sample.



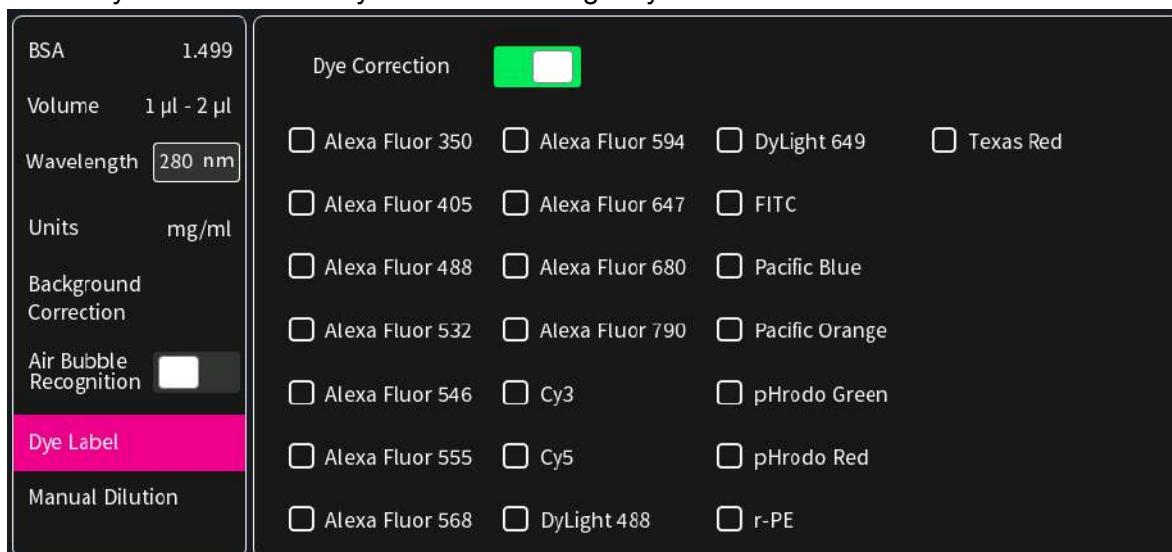
Note: Lint residues and bad sample conditions are detected even if the air bubble recognition is set to off.

- 9. For dye labelled samples, tick the dye labels in the list which should be used for result calculation.

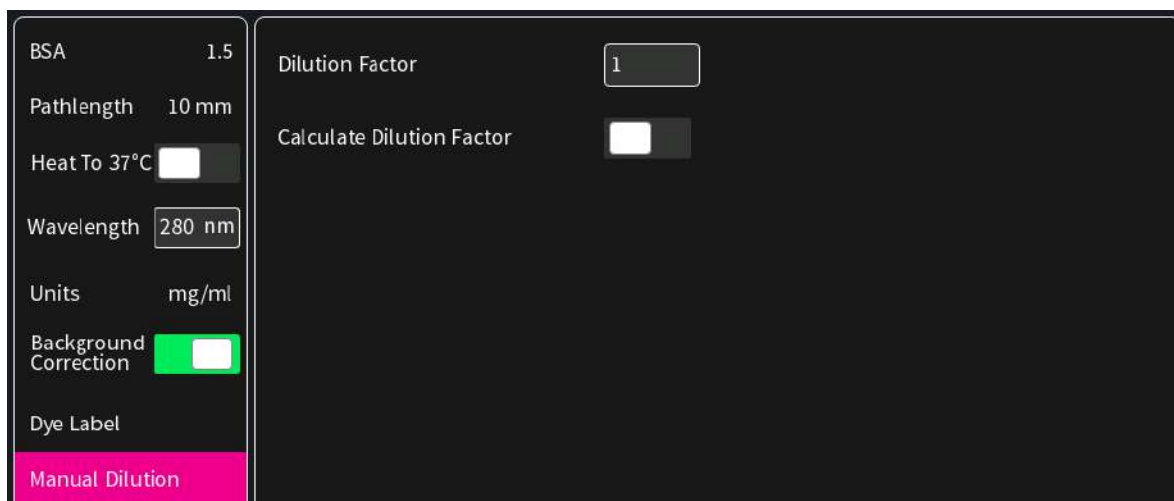
Note: If the used dye is not available in the list, please go to preferences and add a custom dye to the dye list.

There is an option for dye correction which can be enabled/disabled with a toggle switch.

Note: Dye correction is only available for single dye selection.



- 10. Option to set/calculate a dilution factor for manual diluted samples.



- Apply the blank ddH₂O or buffer to the illuminated sample window on pedestal for the reference measurement and select blank to initiate the reading.

Note: The illumination of the sample window can be switched off in the preferences.



- Use a lint-free laboratory wipe to clean both the sample window on pedestal and mirror in the lid arm prior to applying the next sample.

Note: It can be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.



- Apply sample to the sample window on pedestal and press the sample button to initiate the measurement. If auto sample is active the sample measurement is automatically started after closing the lid arm.

Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.



MEASUREMENT PROTOCOL MULTI SAMPLE

- Select the Protein UV icon on the home screen.



- To change between single and multi-sample application use the toggle switch Single Sample in the parameter area.



- To change the protein type push on BSA and a list with available options opens on the right side.

Options are: BSA, SA Mouse, SA Human, IgG Mouse, IgG Human, IgE Human, Lysozyme, OD1, Custom (Molar Extinction Coefficient), Custom (Extinction Coefficient) and Custom (1/ε).

- For Custom (Mol. Ext. Coefficient) enter Molecular Weight in g/mol and the Mol. Ext. Coefficient in M⁻¹*cm⁻¹
- For Custom (Ext. Coefficient) enter the Ext. Coefficient in l/g*cm
- For Custom (1/ε) enter the calculated protein factor 1/ε

BSA	1.499	BSA	1.499	SA Mouse	1.493	SA Human	1.718
Wavelength	280 nm	IgG Mouse	0.714	IgG Human	0.735		
Units	mg/ml	IgE Human	0.654				
Background Correction		Lysozyme	0.379				
Air Bubble Recognition	<input type="checkbox"/>	OD1	1.000				
Dye Label		Custom (Mol. Ext. Coeff.)	<input type="text" value="Please enter MW (g/mol)"/>				
Sample Loading		Custom (Ext. Coeff.)	<input type="text" value="Please enter Mol.Ext.Coeff.(M-1 *cm-1)"/>				
Single Sample	<input type="checkbox"/>	Custom (1/ε)	<input type="text" value="Please enter Ext. Coeff. (l/g*cm)"/>				
			<input type="text" value="Please enter Protein Factor (1/ε)"/>				

4. The wavelength for protein measurements can be changed in the range of 200 – 330nm depending on the wavelength peak of the protein. Default setting is 280 nm.

Wavelength

5. Select the Units in which the concentration should be calculated. Options are ng/μl, μg/μl, μg/ml and mg/ml (default).

BSA	1.5	ng/μl
Wavelength	280 nm	μg/μl
Units	mg/ml	μg/ml
Background Correction		mg/ml

6. The background correction is enabled at 320 nm by default. It is possible to disable the background correction with the toggle switch. Selection options are 320 nm, 340 nm or any wavelength in the range of 220 – 350 nm. The background correction can be disabled with the toggle switch.

BSA	1.5	Background Correction	<input checked="" type="checkbox"/>
Wavelength	280 nm	Background Correction at 320 nm	
Units	mg/ml	Background Correction at 340 nm	
Background Correction		Background Correction at	<input type="text" value="320 nm"/>

- Air bubble recognition is disabled by default. When activated it detects air bubbles, lint residue and bad sample conditions.



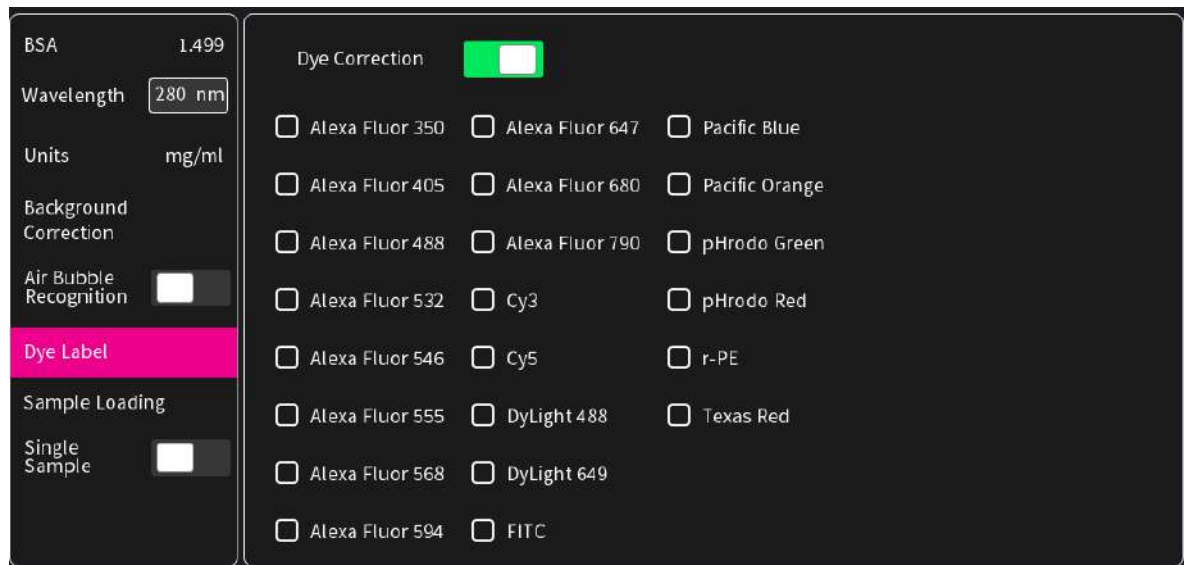
Note: Lint residue and bad sample conditions are detected even if the air bubble recognition is off.

- For dye labelled samples tick the dye labels in the list, which should be used for result calculation.

Note: If the used dye is not available in the list, please go to preferences and add a custom dye to the dye list.

There is an option for dye correction which can be enabled/disabled with a toggle switch.

Note: Dye correction is only available for single dye selection.



- Select the sample loading orientation.



There are two options:

Horizontally: Measure up to 12 samples (A01 – A12 shown in sample map as row)

	1	2	3	4	5	6	7	8	9	10	11	12
A	→											
B												
C												
D												

Vertically: Measure up to 8 samples (A01 – H01 shown in sample map as column)

	1	2	3	4
A	↓			
B				
C				
D				
E				
F				
G				
H				

- To confirm the parameter setting, swipe the screen to the left and the sample map screen is shown.

🏠 ★ 📄 🌿 Sample Map ☰ 🖨️ 🗑️ ℹ️												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

The sample map offers the possibility to select cells and define sample IDs for measurements. It is not necessary to make a selection. If cells are left empty without defined sample IDs for horizontal and vertical sample loading, all 12 positions for horizontal loading and all 8 positions for vertical loading are measured. To measure a defined amount of samples select a cell pattern starting at A01. Sample IDs can be defined by pushing on the sample ID icon (☰) in the navigation bar.

The opened pop up offers options to define, import, export or delete sample IDs.



Further information about cell selection and sample ID definition can be found in chapter 3 NanoPhotometer® Basics / Sample Map on page 36.

11. Apply the blank ddH₂O or buffer to the illuminated sample windows on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in the preferences.

Note: If the blank and sample buttons are not shown please either open the lid arm, push on ▲ on the right bottom corner or swipe from the bottom edge in the screen.

12. Use a lint-free laboratory wipe to clean both the sample windows on pedestal and mirrors in lid arm prior to applying the next sample.



Note: It could be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

13. Apply samples to the illuminated sample windows on pedestal and press the sample button to initiate the measurement.



If auto sample is active the sample measurement is automatically started after closing the lid arm.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.

CALCULATIONS

▪ Protein UV280 Concentration

The protein concentration in the Protein UV method is calculated with the absorbance value of the sample at 280 nm or entered wavelength in the range of 220-350 nm along with the extinction coefficient defined by the user. The protein concentration is calculated with or without background correction as follows:

With background correction:

$$C = (A_{280} - A_{BKG}) * \text{Factor}_{\text{prot}} * D$$

Without background correction:

$$C = A_{280} * \text{Factor}_{\text{prot}} * D$$

C Concentration (mg/ml)

A_{280} Absorbance at 280 nm (10 mm path) or entered wavelength

A_{BKG} Absorbance at selected background wavelength (10 mm path)

$\text{Factor}_{\text{prot}}$ Protein factor in $\text{g}^* \text{cm} / \text{l}$ (1/Ext. Coeff. or MW/Mol. Ext. Coeff.)

D Dilution factor

Table 3. Protein extinction coefficients

Type	$\text{Factor}_{\text{prot}}$ [$\text{g}^* \text{cm} / \text{l}$]	Ext. Coeff. [$1 / \text{g}^* \text{cm}$]	Mol. Ext. Coeff. [$\text{M}^{-1} * \text{cm}^{-1}$]	MW [g / mol]
BSA	1.499	0.6670	44,289	66,400
SA Mouse	1.493	0.6700	44,220	66,000
SA Human	1.718	0.5820	40,370	69,365
IgG Mouse	0.714	1.4000	224,000	160,000
IgG Human	0.735	1.3600	204,000	150,000
IgE Human	0.654	1.5300	290,700	190,000
Lysozyme	0.379	2.6400	37,984	14,388
OD1	1.000	N/A	N/A	N/A

- **Dye-labeled Protein UV280 Concentration**

For dye-labeled proteins, the concentration of the protein is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye, and a certain dye-specific correction factor at 280 nm (see Table 4 on page 81). The dye concentration is calculated with or without background/dye correction for are as follows:

With background and with dye correction:

$$C = \left[A_{280} - A_{BKG} - \left(\text{cf}_{\text{dye}} * (A_{\text{max, dye}} - A_{BKG}) \right) \right] * \text{Factor}_{\text{prot}} * D$$

With background and without dye correction:

$$C = (A_{280} - A_{BKG}) * \text{Factor}_{\text{prot}} * D$$

Without background and with dye correction:

$$C = \left(A_{280} - \left(\text{cf}_{\text{dye}} * A_{\text{max, dye}} \right) \right) * \text{Factor}_{\text{prot}} * D$$

Without background and without dye correction:

$$C = A_{280} * \text{Factor}_{\text{prot}} * D$$

C	Concentration in mg/ml
A ₂₈₀	Absorbance at 280 nm (10 mm path)
A _{BKG}	Absorbance at selected background wavelength (10 mm path)
A _{max, dye}	Absorbance value at the absorbance maximum of the dye (10 mm path)
Factor _{prot}	Protein factor in g*cm/l (1/Ext. Coeff. or MW/Mol. Ext. Coeff.)
cf _{dye}	Dye-dependent correction factor at 280 nm
Đ	Dilution factor

▪ Dye Concentration

For dye-labeled proteins, the concentration of the dye is calculated using a modified form of the Beer-Lambert equation. For these calculations, the instrument considers the absorption maximum of the dye, and a dye-specific extinction coefficient (see Table 4 on page 81). The dye concentration is calculated with or without background correction for are as follows:

With background correction:

$$C = \frac{((A_{\text{max, dye}} - A_{\text{BKG}}) * \text{Đ})}{m\epsilon_{\text{dye}} * 10^{-6}}$$

Without background correction:

$$C = \frac{(A_{\text{max, dye}} * \text{Đ})}{m\epsilon_{\text{dye}} * 10^{-6}}$$

C	Concentration in pmol/μl
A _{max, dye}	Absorbance at the max dye absorbance value (10 mm path)
A _{BKG}	Absorbance at selected background wavelength (10 mm path)
mε _{dye}	Molar Extinction coefficient of dye in M ⁻¹ *cm ⁻¹
Đ	Dilution factor

▪ Degree of Labeling (DOL)

DOL is the degree of labeling based on the average number of dye molecules coupled to a protein molecule. The degree of labeling can be determined from the absorption spectrum of the labeled antibody with or without background/dye correction as follows:

With background and with dye correction:

$$\text{DOL} = \frac{(A_{\text{max, dye}} - A_{\text{BKG}}) * m\epsilon_{\text{prot}}}{((A_{280} - A_{\text{BKG}}) - (\text{cf}_{\text{dye}} * (A_{\text{max, dye}} - A_{\text{BKG}}))) * m\epsilon_{\text{dye}}}$$

With background and w/o dye correction:

$$\text{DOL} = \frac{(A_{\text{max, dye}} - A_{\text{BKG}}) * m\epsilon_{\text{prot}}}{(A_{280} - A_{\text{BKG}}) * m\epsilon_{\text{dye}}}$$

W/o background and with dye correction:

$$DOL = \frac{A_{\max, \text{dye}} * m\epsilon_{\text{prot}}}{(A_{280} - (cf_{\text{dye}} * A_{\max, \text{dye}})) * m\epsilon_{\text{dye}}}$$

W/o background and w/o dye correction:


$$DOL = \frac{A_{\max, \text{dye}} * m\epsilon_{\text{prot}}}{A_{280} * m\epsilon_{\text{dye}}}$$

DOL	Degree of labeling/dye per protein ratio
$A_{\max, \text{dye}}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
A_{280}	Absorbance at 280 nm (10 mm path)
A_{BKG}	Absorbance at selected background wavelength (10 mm path)
$m\epsilon_{\text{dye}}$	Molar extinction coefficient of dye ($M^{-1} * cm^{-1}$)
$m\epsilon_{\text{prot}}$	Molar extinction coefficient of protein ($M^{-1} * cm^{-1}$)
cf_{dye}	Dye-dependent correction factor at 280 nm

Table 4. Dye Types, Absorbance Max, Extinction coefficients, and dye-dependent correction factors

NanoPhotometer® Models	Dye Type	Absorbance maximum of Dye (nm)	Molar ext. coeff. of Dye $m\epsilon_{\text{dye}}$ in $M^{-1} * cm^{-1}$	Dye-dependent correction factor at 280 nm cf_{dye}
NP80/N60/N50/C40	Alexa Fluor 350	346	19,000	0.19
NP80/N60/N50/C40	Alexa Fluor 405	401	34,500	0.70
NP80/N60/N50/C40	Alexa Fluor 488	494	71,000	0.11
NP80/N60/N50/C40	Alexa Fluor 532	530	81,000	0.09
NP80/N60/N50/C40	Alexa Fluor 546	554	104,000	0.12
NP80/N60/N50/C40	Alexa Fluor 555	555	150,000	0.08
NP80/N60/N50/C40	Alexa Fluor 568	577	91,300	0.46
NP80/N60/N50/C40	Alexa Fluor 594	590	73,000	0.56
NP80/N60/C40	Alexa Fluor 647	650	239,000	0.03
NP80/N60/C40	Alexa Fluor 680	679	184,000	0.05
NP80/N60/C40	Alexa Fluor 790	785	260,000	0.08
NP80/N60/N50/C40	Cy3	550	150,000	0.05
NP80/N60/C40	Cy5	649	250,000	0.05
NP80/N60/C40	DyLight 649	654	250,000	0.04
NP80/N60/N50/C40	DyLight 488	493	70,000	0.15
NP80/N60/N50/C40	FITC	495	68,000	0.30
NP80/N60/N50/C40	Pacific Blue	409	30,000	0.20
NP80/N60/N50/C40	Pacific Orange	397	24,500	0.60
NP80/N60/N50/C40	pHrodo Green	505	75,000	0.20
NP80/N60/N50/C40	pHrodo Red	560	65,000	0.12
NP80/N60/N50/C40	r-PE	566	200,000	0.18
NP80/N60/N50/C40	Texas Red	595	80,000	0.18

▪ Ratios

Protein samples e.g. from whole cell lysates may contain nucleic acids. To check the purity of the isolated protein, the 260/280 ratio is calculated to give an indication of the nucleic acid contamination. A pure protein preparation has an expected 260/280 ratios of ~ 0.57. If a ratio is detected out of the acceptable range an alert icon  is shown in the results/table area. A push on the alert icon shows additional information. The ranges for acceptable ratio values can be defined in preferences.

The ratio is calculated with or without background correction according to if the background correction is activated during the measurements or not as follows:

Without background correction:

$$260/280 \text{ ratio} = \frac{A_{260}}{A_{280}}$$

With background correction:

$$260/280 \text{ ratio} = \frac{A_{260} - A_{BKG}}{A_{280} - A_{BKG}}$$



PROTEIN ASSAYS

METHOD OVERVIEW

Protein concentration may be measured using colorimetric assays, in which certain reagents are added to the protein solution to generate a colored product; either a protein-cupric ion chelate as in the Biuret, Lowry, BCA assays or a protein-dye complex as in the Bradford assay. In these colorimetric assays, the absorbance is measured in the visible range at the appropriate wavelength for each assay and compared against a standard curve prepared by serial dilution of a protein standard of known concentration. A linear, linear zero or 2nd order regression analysis of the calibration standard data points is calculated by the NanoPhotometer®. A correlation coefficient (R^2) in the range of 0.95 to 1.00 indicates a good fit to a straight line.

▪ Bradford Assay

Method depends on quantifying the binding of a dye, Coomassie Brilliant Blue, to an unknown protein and comparing this binding to that of a standard curve prepared from a set of known protein of known concentrations at 595 nm. This standard is usually BSA (bovine serum albumin).

▪ Biuret Assay

Method depends on a reaction between cupric ions and peptide bonds in an alkali solution, resulting in the formation of a complex absorbing at 546 nm.

Note: Biuret is only available for cuvette measurements (NP80 and C40).

▪ BCA Assay

Method depends on a reaction between cupric ions and peptide bonds coupled with the detection of cuprous ions using bicinchoninic acid (BCA), giving an absorbance maximum at 562 nm. The BCA process is less sensitive to the presence of detergents used to solubilize membranes.

▪ Lowry Assay

Method is based on the Biuret reaction. Under alkaline conditions the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. The bound reagent changes color from yellow to blue. This binding is compared with that obtained with a standard protein at 750 nm; this is usually BSA (bovine serum albumin).

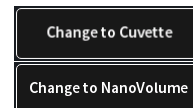
Note: Detailed protocols are customarily supplied with these assay kits, and must be closely followed to ensure that accurate results are obtained.

MEASUREMENT PROTOCOL SINGLE SAMPLE

1. Select protein assays icon from home screen.



2. To change between NanoVolume and cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).



3. To change the assay type push on Bradford and a list with available options opens on the right side.

Options are: BCA Assay, Biuret Assay (cuvette only), Bradford Assay, Lowry Assay (not for N50)

Bradford	BCA	562 nm
Dilution 15	Biuret	546 nm
Baseline Correction	Bradford	595 nm
Curve Fit	Lowry	750 nm

4. For Cuvette Application:

Select the path length depending on the used cuvette.

Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm

Bradford	Pathlength	0.5 mm
Pathlength 10 mm	Pathlength	1 mm
Heat To 37°C <input type="checkbox"/>	Pathlength	2 mm
Baseline Correction	Pathlength	5 mm
	Pathlength	10 mm

If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume Application:

Select the dilution depending on the sample concentration

Bradford	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Dilution 15	Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

Note: There is no automatic path length setting available in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm) depending on your sample concentration.

For NanoVolume application with submicroliter cell (C40 only):

Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

Bradford	Lid 5	3.5 µl - 5 µl sample volume
Lid 50	Lid 10	1 µl - 3 µl sample volume
Baseline Correction	Lid 50	0.3 µl - 2 µl sample volume
Curve Fit	Lid 100	0.3 µl - 2 µl sample volume
	Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Default values for the baseline correction are depending on selected protein assay type and instrument version:

BCA default value 750 nm (N50 off)

Biuret default off

Bradford default value 750 nm (N50: 350 nm)

Lowry default value 405 nm

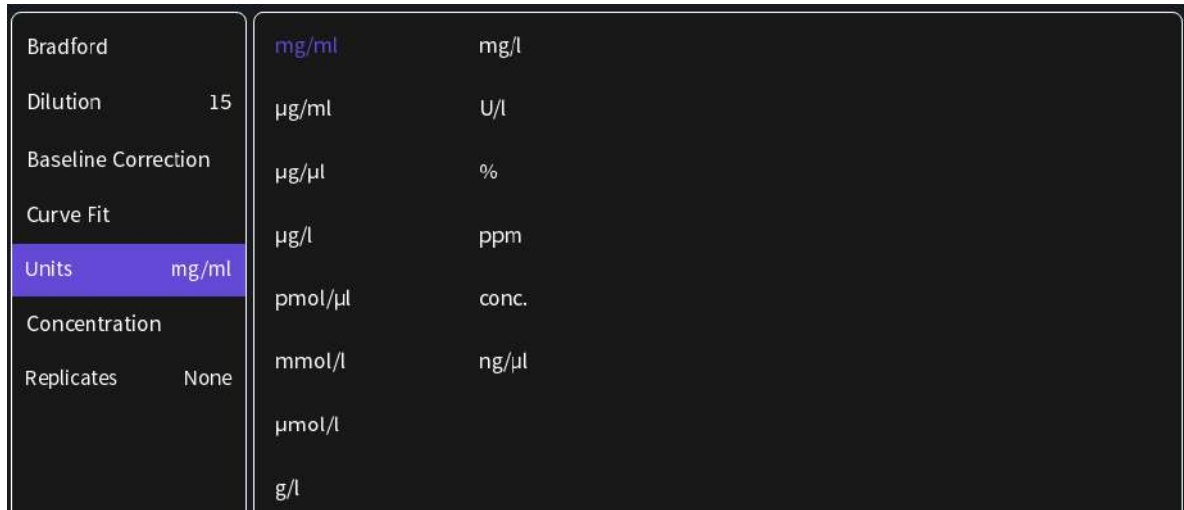
Bradford	Baseline Correction	<input checked="" type="checkbox"/>
Dilution 15	Baseline Correction at 377 nm	
Baseline Correction	Baseline Correction at 604 nm	
Curve Fit	Baseline Correction at 650 nm	
Units mg/ml	Baseline Correction at 770 nm	
Concentration	Baseline Correction at 823 nm	
Replicates None	Baseline Correction at	<input type="text" value="750 nm"/>

Note: It is recommended to use the default baseline for each assay.

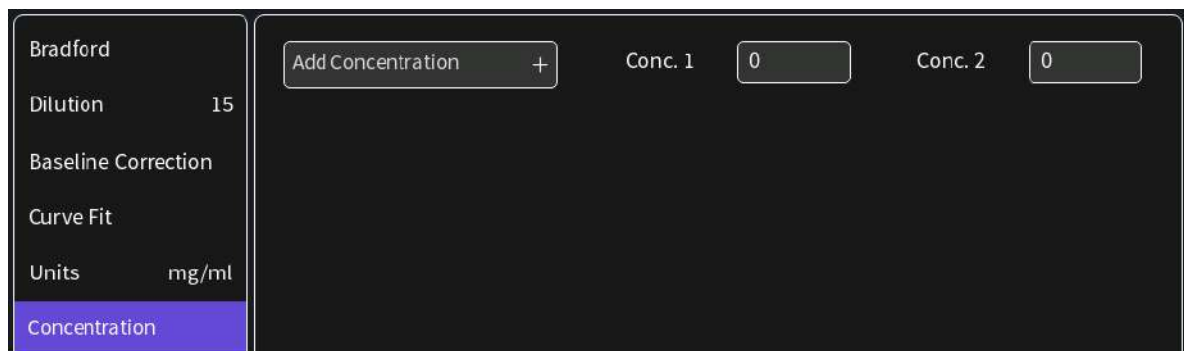
- Select curve fit type: Options are linear regression, zero regression (forces the straight line through the origin) and 2nd order regression

Bradford	Regression Linear
Dilution 15	Zero Regression Linear
Baseline Correction	Regression 2nd Order
Curve Fit	

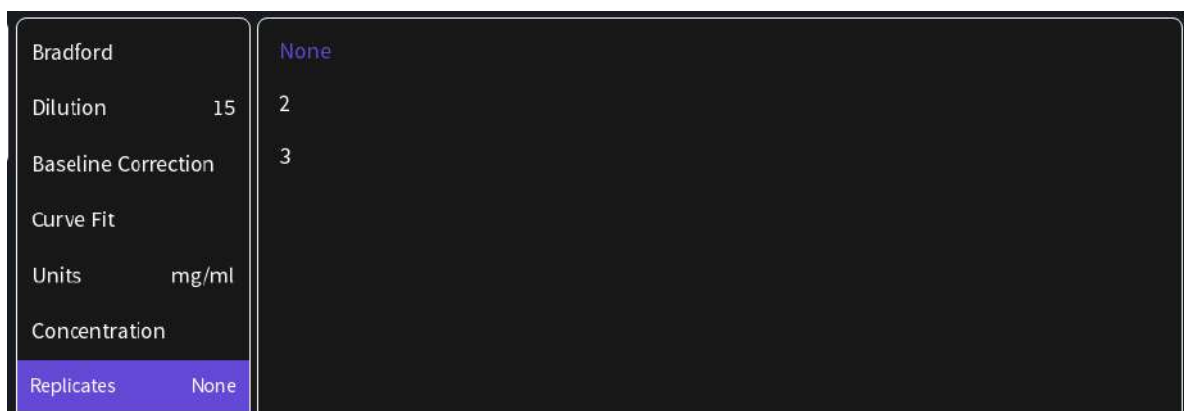
7. Select Unit



8. Add up to 20 Concentrations by pushing on the Add Concentration button. Added concentrations can be deleted with ⊗
Enter the concentrations of the standards for the standard curve.



9. Select Replicates none, 2 or 3



10. Measure a blank and depending on the replicate selection, all required concentrations. Absorbances of the replicates will be shown in the results area and if replicates are selected a mean value for each standard. It is possible to exclude single measurements from the curve calculation by switching the toggle switch off.

Note: After the first sample measurement the standard curve can no longer be altered.



11. Once a Standard curve is created or loaded from a stored method it will be used for concentration calculations of measured samples. It may be necessary to do a blank measurement.



12. Apply sample and press the sample button to initiate the sample measurement.

If auto sample is active the sample measurement is automatically started after closing the lid arm.

Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.



Note: Once the sample measurement is initiated it is no longer possible to change the standard curve.

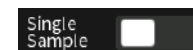
MEASUREMENT PROTOCOL MULTI SAMPLE

The Protein Assay multi-channel method is an auto ranging method. There is no need to set any Dilution/Pathlength.

1. Select protein assays icon from home screen.



2. To change between single and multi-sample application use the toggle switch Single Sample in the parameter area.



3. To change the assay type push on Bradford and a list with available options opens on the right side.

Options are: BCA Assay, Bradford Assay and Lowry Assay

Bradford	BCA	562 nm
Baseline Correction	Bradford	595 nm
Curve Fit	Lowry	750 nm

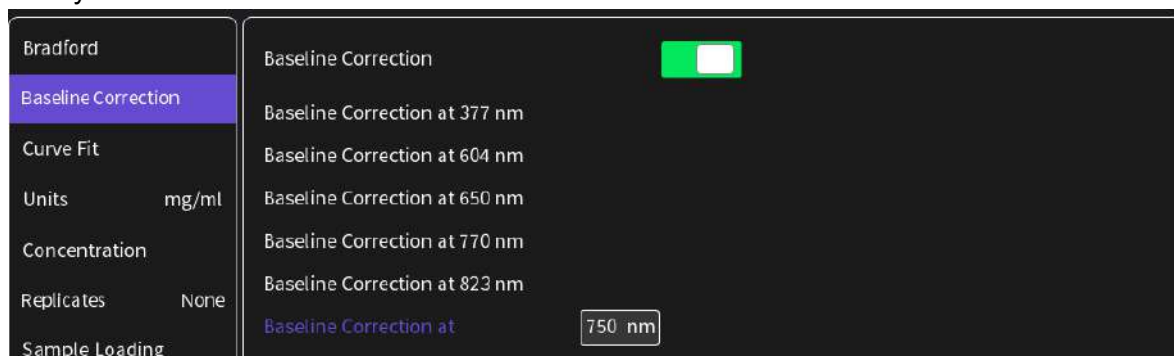
4. Default values for the baseline correction are depending on selected protein assay type and

instrument version:

BCA default value 750 nm

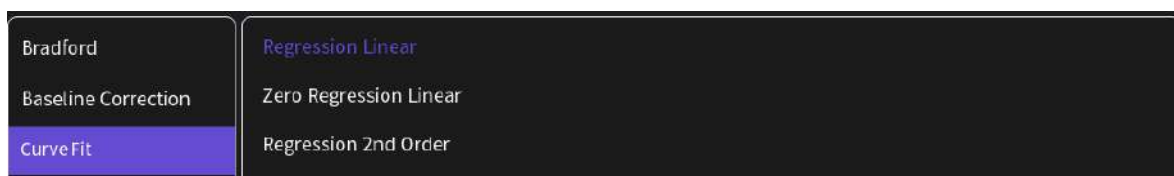
Bradford default value 750 nm

Lowry default value 405 nm

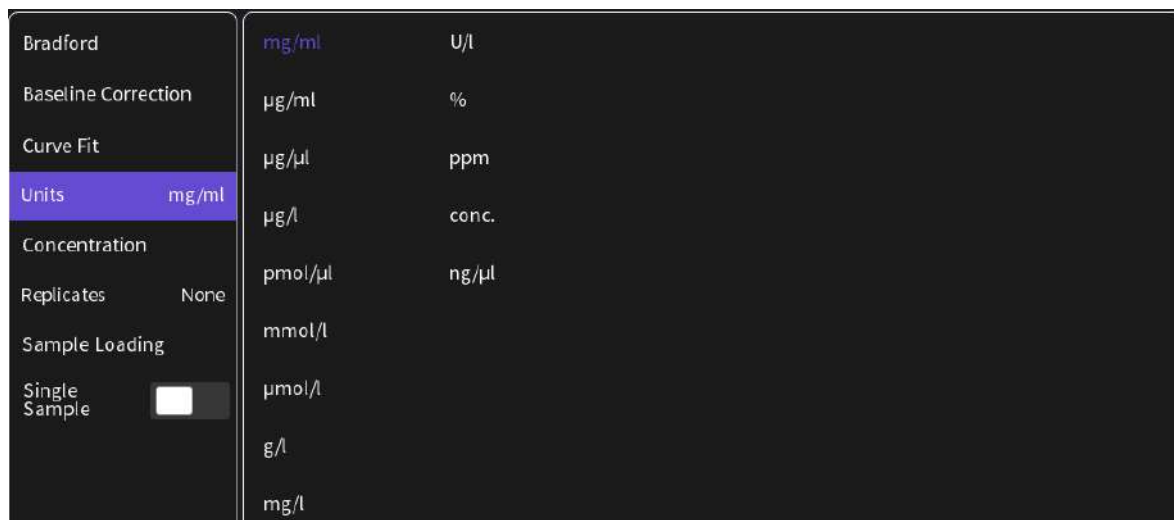


Note: It is recommended to use the default baseline for each assay.

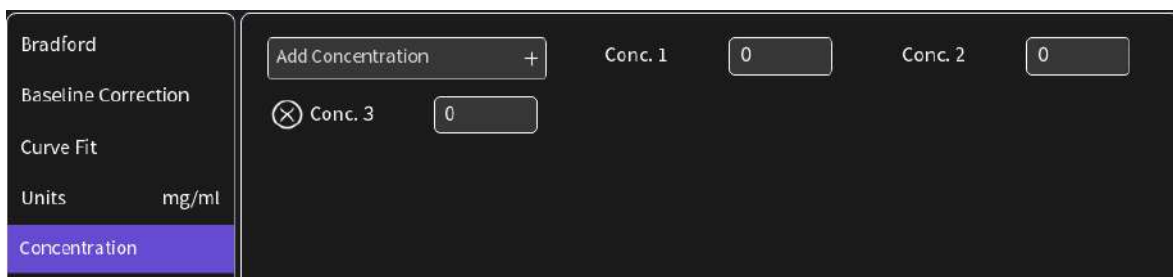
5. Select curve fit type: Options are linear regression, zero regression (forces the straight line through the origin) and 2nd order regression



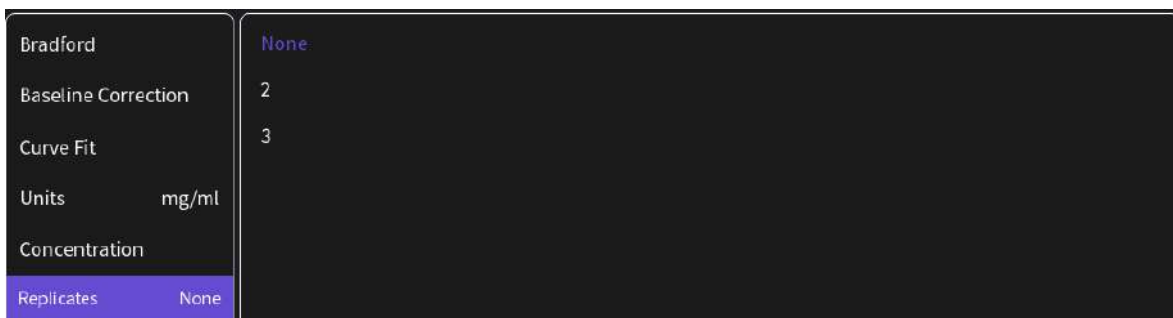
6. Select Unit



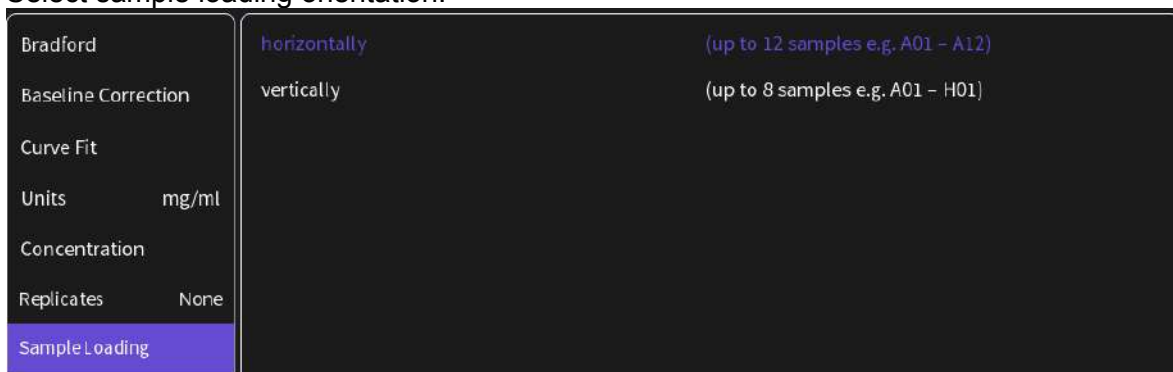
7. Add up to 20 Concentrations by pushing on the Add Concentration button. Added concentrations can be deleted with ⊗
Enter the concentrations of the standards for the standard curve.



8. Select Replicates none, 2 or 3



9. Select sample loading orientation.



10. To confirm the parameter setting, swipe the screen to the left and the standard map screen is shown. The standard map is created automatically depending on the parameter settings (sample orientation, concentration and replicates). It is not possible to change the Sample IDs for standards.

e.g. horizontally 5 standards and 2 replicates defined:

	1	2	3	4	5	6	7	8	9	10	11	12
SA	BLK01	BLK02	BLK03	BLK04	BLK05	BLK06	BLK07	BLK08	BLK09	BLK10	BLK11	BLK12
SB	STD01-1	STD02-1	STD03-1	STD04-1	STD05-1							
SC	STD01-2	STD02-2	STD03-2	STD04-2	STD05-2							
SD												
	Position	Sample ID	Content	Conc.	Units	Abs.	Dilution					

e.g. vertically 6 standards and 3 replicates defined:

	1	2	3	4	Position	Sample ID	Content	Conc.	Units	Abs.	Dilution
SA	BLK01	STD01-1	STD01-2	STD01-3							
SB	BLK02	STD02-1	STD02-2	STD02-3							
SC	BLK03	STD03-1	STD03-2	STD03-3							
SD	BLK04	STD04-1	STD04-2	STD04-3							
SE	BLK05	STD05-1	STD05-2	STD05-3							
SF	BLK06	STD06-1	STD06-2	STD06-3							
SG	BLK07										
SH	BLK08										

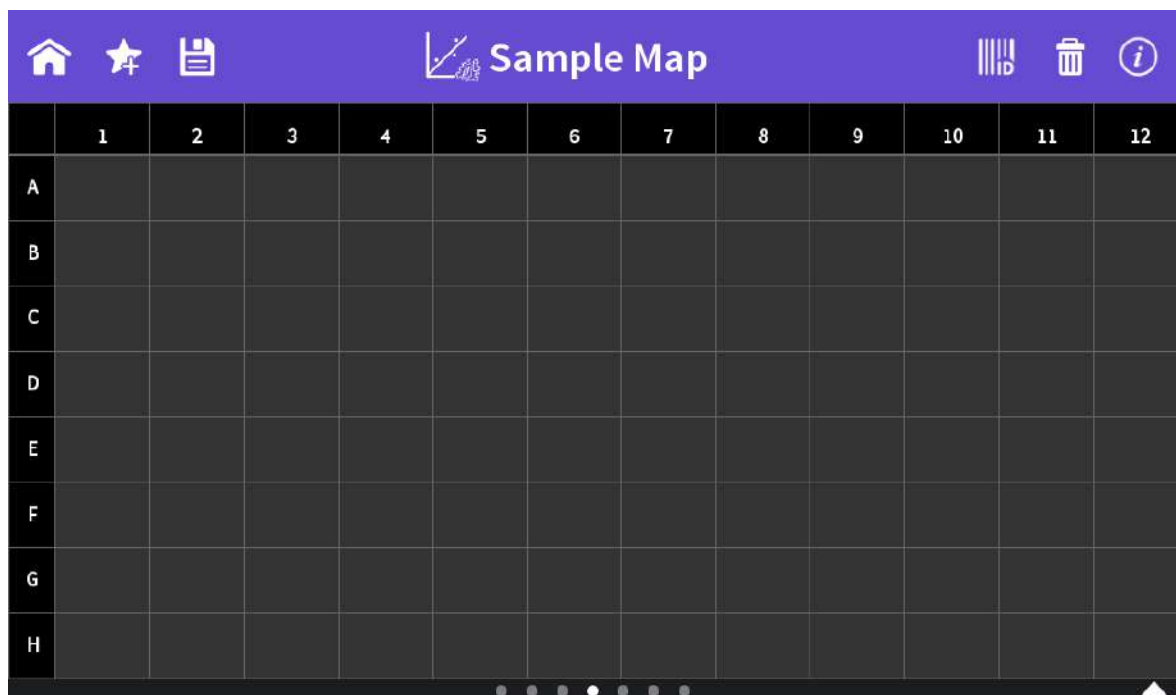
Note: It is necessary to measure all defined blanks (horizontally 12 and vertically 8) as they might be needed for sample measurements.

11. Measure blanks and standards as defined by initiating the blank button for blank measurements and sample button for the standard measurements. Results will be shown in the table of the standard map. Once all standards are measured the screen changes automatically to the standard curve screen.
12. In the graph screen the standard curve is shown and if replicates are defined the results and mean calculation of each standard. It is possible to exclude single standard measurements with the toggle switches and standard curve is recalculated.

Note: After the first sample measurement the standard curve can no longer be altered.



14. After the standard curve is finished, swipe the screen to the left and the sample map screen is shown.



The sample map offers the possibility to select cells and define sample IDs for measurements. It is not necessary to make a selection. If cells are left empty without defined sample IDs for horizontal and vertical sample loading, all 12 positions for horizontal loading and all 8 positions for vertical loading are measured.

To measure a defined amount of samples select a cell pattern starting at A01. Sample IDs can be defined by pushing on the sample ID icon (☰) in the navigation bar. The opened pop up offers options to define, import, export or delete sample IDs.



Further information about cell selection and sample ID definition can be found in chapter 3 NanoPhotometer® Basics / Sample Map on page 36

13. Depending on measuring or loading a standard curve a blank reading is required. To initiate a blank apply the blank and push the blank button.



14. Apply sample and press the sample button to initiate the sample measurement.



If auto sample is active the sample measurement is automatically started after closing the lid arm.




Note: Auto sample function is only available NanoVolume methods and

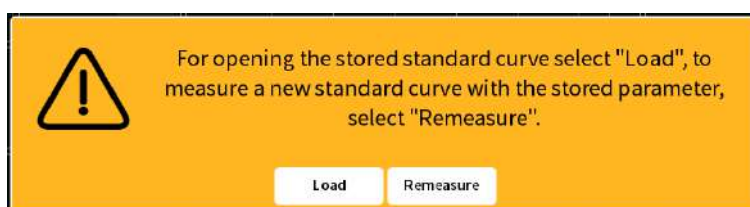
new NanoPhotometer® versions it may not be available after updating an older firmware version.

Note: Once the sample measurement is initiated it is no longer possible to change the standard curve.

SAVING AND LOADING STANDARD CURVES

It is possible to save measured standard curves as a Stored Method. To save the standard curve push the store method button  and enter a method name, select a folder and save with the Store button.

Methods can be opened in the Stored Methods menu on the homescreen. Opening a saved Protein Assay method shows a message with the option to load or remeasure the standard curve.



CALCULATIONS

Protein concentration is determined using the standard curve by correlating absorbance values of samples with known concentration to calculate the concentration of the unknown sample. In order to maintain accuracy and precision please ensure that the R^2 value of the standard curve is 0.95 or greater.



KINETICS

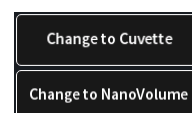
METHOD OVERVIEW

The Kinetics application is useful for: measuring initial rates of enzyme-catalyzed reactions, performing progress-curve analysis for complete reactions, calculating basic Michaelis-Menten parameters for single-substrate reactions, and measuring enzyme inhibition. Simple kinetics studies, where the change in absorbance is followed as a function of time at a fixed wavelength, can be readily performed with the NanoPhotometer®. The rate of a chemical reaction can be measured using spectrophotometric methods by studying the change in absorbance at a fixed wavelength as a function of time. These changes in absorbance reflect corresponding changes in the concentration of reactants or products as the reaction progresses. The rate of many chemical reactions can be markedly accelerated by the presence of catalysts, which remain chemically intact during the reaction. Catalysts in the case of biochemical reactions are generally represented by enzymes, which are specialized protein catalysts. However, a few examples of special reactions catalyzed by RNA molecules also exist. Studying the kinetics of a reaction can reveal important details of the catalytic mechanism involved in terms of sequence steps, transition state of reactants or nature of enzyme inhibitors.

MEASUREMENT PROTOCOL

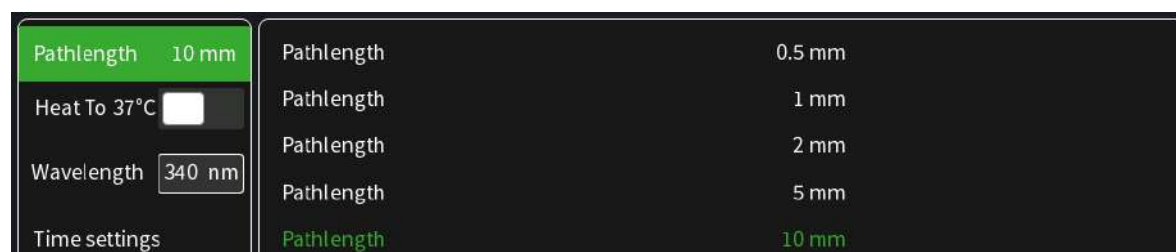
Note: If a kinetic is started from a control device via WiFi connection (tablet or smartphone) set the auto lock of the tablet or smartphone to never. Otherwise the kinetics will be interrupted when the smartphone or tablet is locked, because of losing the WiFi connection.

1. Select the Kinetics icon on the home screen
2. To change between NanoVolume and cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).



3. **For Cuvette Application:**

Select the path length depending on the used cuvette.
Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm



If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume Application:

Select the dilution depending on the sample concentration.

Dilution	15	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Wavelength	340 nm	Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

Note: There is no automatic path length setting available in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).

For NanoVolume application with submicroliter cell (C40 only):

Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is listed to the right.

Lid	50	Lid 5	3.5 µl - 5 µl sample volume
Wavelength	340 nm	Lid 10	1 µl - 3 µl sample volume
Time settings		Lid 50	0.3 µl - 2 µl sample volume
		Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Default wavelength (λ) is 340 nm but can be changed in the range of 200–900 nm (N50: 200–650 nm), depending on the application.

λ	340 nm
-----------	--------

- Time settings:

- Enter the duration time in minutes over which measurements are to be taken. Possible range is 1–3000 minutes.
- Enter the interval time between measurements in seconds. Possible interval times are 5–3,600 seconds (N50: 10–3,600 sec.), depending on the duration time.
- Enter the delay time in seconds before the first measurement is taken. Possible delay time is between 0–3,600 seconds, depending on the duration time.

Dilution	15	Duration (min)	10
Wavelength	340 nm	Interval (sec)	10
Time settings		Delay Time (sec)	0

Note: A maximum of 500 samples is possible. Please consider this when choosing the duration and interval time.

6. Insert cuvette with the reference sample and select blank button to initiate the measurement.



7. Insert cuvette with the sample and select the sample button to initiate the measurements. Once the kinetic is started the Blank button turns to a Pause/Continue button and the Sample button to a Stop button.



Note: While the kinetics is running it is not possible to change the parameters, save data or delete data. Change of parameters is only possible before starting the kinetic readings. Save and delete data is only available after the kinetic session is stopped.

Note: Auto print and cryo label print is not available in Kinetics.

CALCULATIONS

All absorbance values are normalized to a 10 mm path.

A_0 = absorbance of start value (10 mm path)

A_n = absorbance of actual value time n (10 mm path)

dA = absorbance of actual value – absorbance of start value

Slope = linear regression fit of all actual measurement points

Final A = absorbance of final value

$R^2 = R^2 = \sum_{i=1}^n \frac{(O_i - E_i)^2}{E_i}$ [O_i = observed slope value; E_i = expected slope value]



OD600

METHOD OVERVIEW

The growth of bacteria in liquid culture media is commonly monitored by measuring the optical density at 600 nm (OD600) in small samples taken from the cultures. OD600 measurements are typically used to determine the stage of growth of the bacterial culture, thereby ensuring that cells are harvested at an optimum point that corresponds to an appropriate density of live cells. Growth of bacterial cells typically progresses through a series of consecutive phases including: lag, log, stationary and decline (see Figure 1 on page 97). In general, cells should be harvested towards the end of the log phase using the optical density of the samples to determine when this point has been reached. Since optical density in the case of OD600 measurements results from light scattering rather than light absorption, this value varies depending on the type of bacterial cells in the culture in terms of size and shape. Cells are routinely grown until the absorbance at 600 nm (known as OD 600) reaches approximately 0.4 prior to induction or harvesting. A linear relationship exists between cell number (density) and OD 600 up to an absorbance value of 0.6, approximately.

As mentioned above, for turbid samples such as cell cultures, the absorbance measured is due to light scattering, and not the result of molecular absorption. Since the extent of scattering is affected by the optics of the system (distance between the cell holder and instrument exit slit, monochromator optics, slit geometry, etc.), different spectrophotometer types will tend to give different OD 600 readings for the same turbid sample. Therefore, if results from different spectrophotometers are to be compared, they must be normalized first using appropriate calibration curves. For more information see Technical Note #8 OD 600 which can be downloaded on the Implen webpage: www.implen.de/scientific-publications/

A calibration curve can be constructed by comparing measured OD 600 to expected OD 600. Expected OD 600 is determined by counting cell number using an alternative technique (for example microscope slide method) and converting to OD 600 using the rule of thumb that $1 \text{ OD } 600 = 5 \times 10^8 \text{ cells/ml}$ for *E. coli*.

The NanoPhotometer® comes with a correction factor of 1 as standard. To compare OD 600 values between different spectrophotometers, it is necessary to determine the constant deviation or ratio between the absorbance values for the same sample from each instrument and use this factor within the setting “correction factor” of your NanoPhotometer® Software.

Note: The use of 10 mm path length disposable cuvettes is recommended for optical density measurements of cell culture solutions. The amount of cells is reflected in the reading and the likelihood of fluctuating amount of cells in a drop from sample to sample can be considered as extremely significant. It is therefore recommended to use cuvettes since the amount of error in a bigger volume is not as significant. The cuvette measurements provide a bigger average and therefore more reproducible readings. Also, to prevent the suspension settling too quickly and giving an OD reading that changes with time, glycerol should be added to the sample.

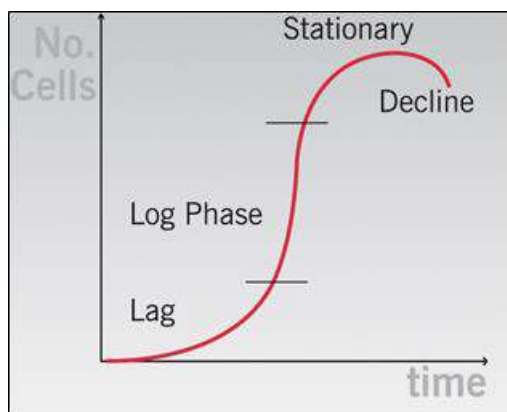


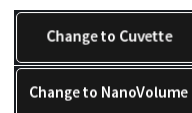
Figure 1 Bacterial growth curve

MEASUREMENT PROTOCOL

1. Select the OD600 icon on the home screen



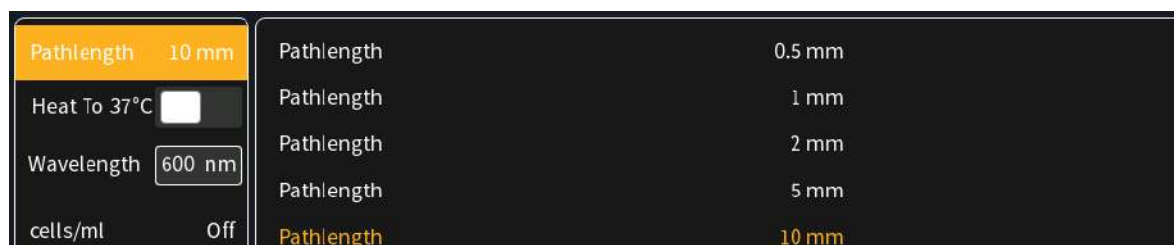
2. To change between NanoVolume and cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).



3. For **Cuvette Application**:

Select the path length depending on the used cuvette.

Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm



If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For **NanoVolume Application**:

Select the dilution depending on the sample concentration



Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)

For **NanoVolume application with submicroliter cell** (C40 only):

Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

Lid	50	Lid 5	3.5 µl - 5 µl sample volume
Wavelength	600 nm	Lid 10	1 µl - 3 µl sample volume
cells/ml	Off	Lid 50	0.3 µl - 2 µl sample volume
Correction		Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Default wavelength is 600 nm but the wavelength can be changed in the range of 200–900 nm (N50: 200–650 nm), depending on the application.



- Toggle switch cells/ml is disabled by default. Enable cells/ml to get the cells/ml calculated. Enter the cell specific factor and multiplier (e.g. 1 OD600 = 5 x 10⁸ cells/ml)

Pathlength	10 mm	cells/ml	<input checked="" type="checkbox"/>
Heat To 37°C	<input type="checkbox"/>	Factor	5
Wavelength	600 nm	Multiplier	1,000
cells/ml	On		1,000,000
Correction			100,000,000

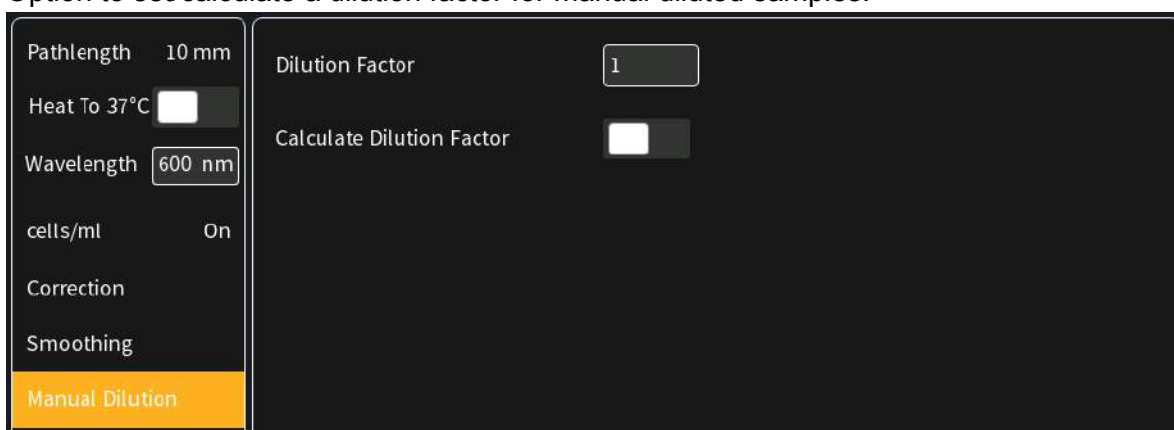
- Enter the correction factor to compensate for different optical configurations between the NanoPhotometer® and other instruments.

Pathlength	10 mm	Correction Factor	1
Heat To 37°C	<input type="checkbox"/>		
Wavelength	600 nm		
cells/ml	On		
Correction			

- Option to smooth the graph with different boxcars. Options: Off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61



8. Option to set/calculate a dilution factor for manual diluted samples.



9. Insert cuvette with the reference sample and select blank button to initiate the measurement



10. Insert cuvette with the sample and select the sample button to initiate the measurement.



CALCULATIONS

$$OD_{600} = A_{600} * D * cf$$

- OD₆₀₀ Optical density at 600 nm
- A₆₀₀ Absorbance at 600 nm (10 mm path)
- D Dilution factor
- cf Correction factor for spectrophotometer

$$\text{Cells/ml} = A_{600} * D * cf * \text{multiplier}$$

- A₆₀₀ Absorbance at 600 nm (10 mm path)
- D Dilution factor
- cf Correction factor for spectrophotometer
- multiplier Multiplier of sample



MORE APPS

The More Apps icon located on the home screen opens another menu screen with access to icons for additional applications available on the NanoPhotometer®. The applications featured in this menu include: wavelength, concentration, wavescan, absorbance/ratio, standard curve and custom applications.




MORE APPS: WAVELENGTH

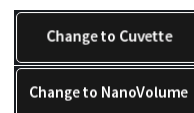
METHOD OVERVIEW

In the wavelength application it is possible to measure simple absorbance (A) and % transmittance (%Trans. / only in cuvette mode) of a sample at specific wavelengths. It is possible to add up to 20 different wavelengths.

The wavelength method includes a calculation tool to define and calculate customer defined formulas.

MEASUREMENT PROTOCOL

1. Select the More Apps icon  from the home screen and the Wavelength icon from the More Apps screen.
2. To change between NanoVolume and Cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).
3. **For NanoVolume application:**
Select the dilution depending on the sample concentration.

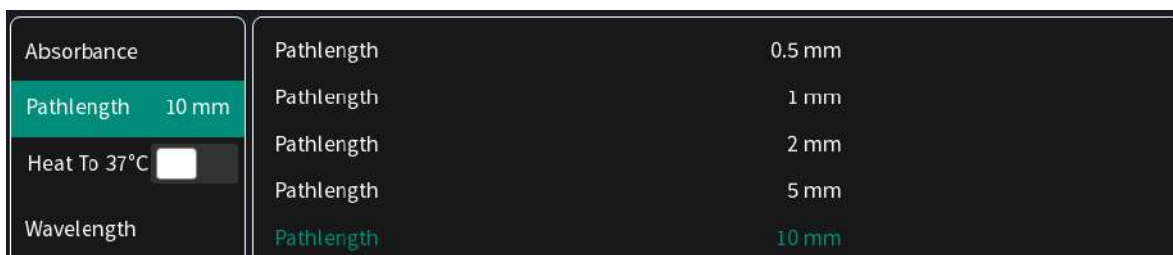


Dilution	15	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Wavelength		Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).

For cuvette application:

Select the path length depending on the used cuvette.
Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm



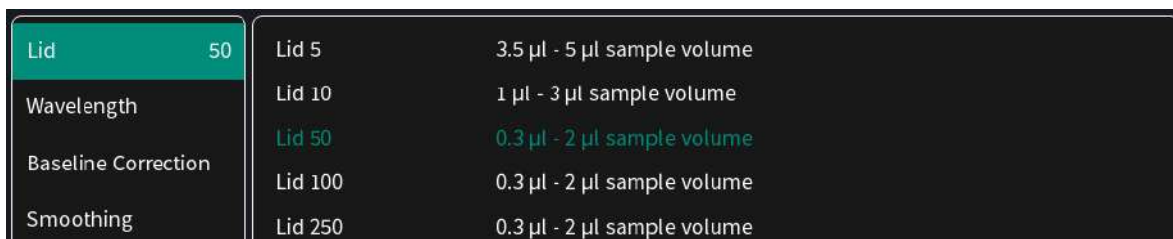
If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

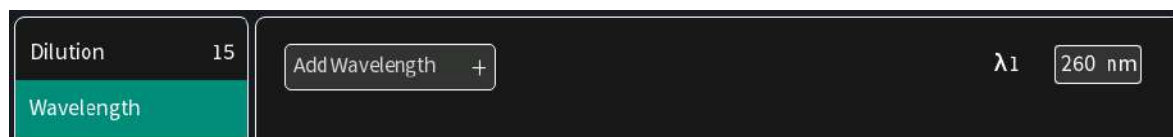
Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

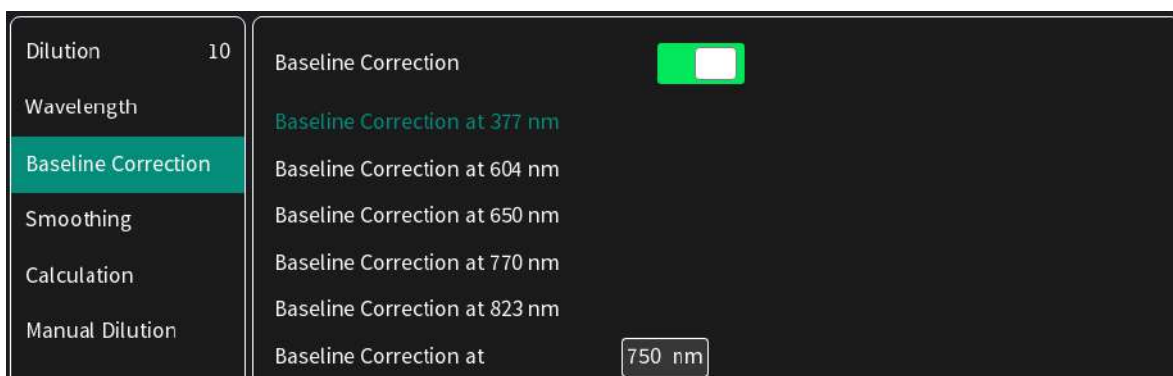


Note: A new Blank is recommended when changing the dilution lid.

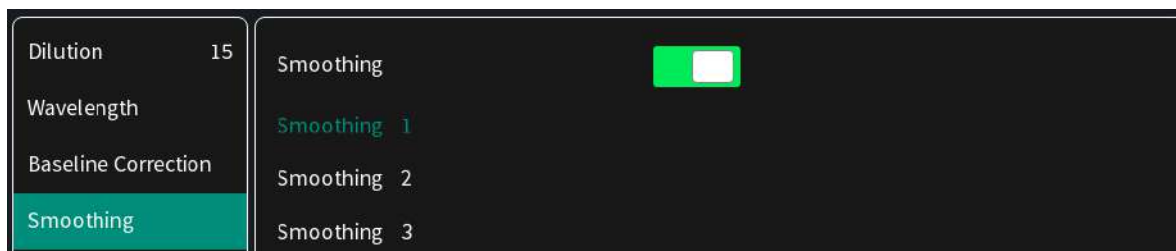
4. Enter desired wavelength (λ) to be measured. It is possible to measure up to 20 wavelengths simultaneously. More wavelength (λ) options can be added by selecting the Add Wavelength button. Added wavelength can be deleted with ⊗



5. Baseline correction is set off by default. Enabling the baseline corrections shows a list with different wavelength options: 377 nm, 604 nm, 650 nm, 770 nm (N/A N50) and 823 nm (N/A N50). Option to enter any wavelength between 200 nm and 900 nm (N50: 650 nm).



6. Option to smooth the graph with different boxcars. Options: Off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61



7. Option to enter formulas to calculate



It is possible to enter up to 5 different custom formulas for result calculation.

Formula Acceptance Guidelines:

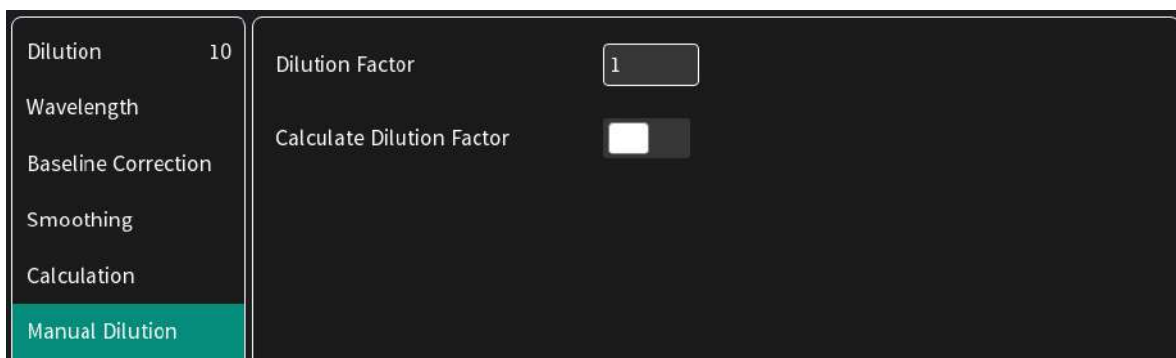
1. Numbers:
 - Up to 20 significant figures if no decimal separator is used
 - Up to 4 significant figures if a decimal separator (period ".") is used
2. Numeric Operations:
 - + (add), - (subtract), * (multiply), / (divide) and parentheses ()
3. Absorbance:
 - Axxx e.g. for absorbance at 260 nm: A260

Note: Do not use blank character.

Example:

Nucleic Acid (dsDNA) concentration calculation with background correction at 320 nm:
 $(A_{260} - A_{320}) * 50$

8. Option to set/calculate a dilution factor for manual diluted samples.



9. Apply the blank ddH₂O or buffer to the illuminated sample window on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in preferences.

10. Use a lint-free laboratory wipe to clean both the sample window on pedestal and mirror in lid arm prior to applying the next sample.



Note: It can be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

11. Apply sample to the sample window on pedestal and press the sample button to initiate the measurement.

If auto sample is active the sample measurement is automatically started after closing the lid arm.

Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.




CALCULATIONS

▪ Formula Calculation:

Is depending on the entered formula in the parameter concentration.

▪ Absorbance Calculation:

Absorbance is formally defined as the decimal logarithm (base 10) of the reciprocal of transmittance:

$$A = \log\left(\frac{1}{T}\right) = -\log T$$

$$T = 10^{(-A)}$$

Note: Corresponding absorbance value e.g. Absorbance value ($\lambda = 230$) etc. normalized to 10 mm path length

▪ %Transmittance Calculations (cuvette mode only)

In the wavelength application it is possible to measure the absorbance (A) and % transmittance (%T) of a sample with respect to a reference at a specific wavelength. Transmittance is the ratio of light intensity remaining after it has passed through the sample (I) to the initial incident light intensity (I₀):

$$T = \frac{I}{I_0} \qquad \%T = \frac{I}{I_0} \times 100$$



MORE APPS: WAVESCAN

METHOD OVERVIEW

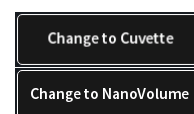
Using the wavescan application it is possible to obtain the complete spectral scan for a defined wavelength range between 200-900 nm (NP80/N60/C40) or from 200-650 nm (N50).

MEASUREMENT PROTOCOL SINGLE SAMPLE

1. Select the More Apps icon from the Home screen and the Wavescan icon from the More Apps screen



2. To change between NanoVolume and Cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).



3. **For NanoVolume application:**

Select the dilution depending on the sample concentration

Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)

Dilution	15	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Wavelength Range		Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

For cuvette application:

Select the path length depending on the used cuvette.

Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm

Absorbance	Pathlength	0.5 mm
Pathlength	Pathlength	1 mm
Heat To 37°C <input type="checkbox"/>	Pathlength	2 mm
Wavelength Range	Pathlength	5 mm
	Pathlength	10 mm

If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

Lid	50	Lid 5	3.5 µl - 5 µl sample volume
Wavelength Range		Lid 10	1 µl - 3 µl sample volume
Baseline Correction		Lid 50	0.3 µl - 2 µl sample volume
Smoothing		Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Set Start and End Wavelength to define the scan range.

Dilution	15	Start Wavelength (nm)	<input type="text" value="200 nm"/>
Wavelength Range		End Wavelength (nm)	<input type="text" value="900 nm"/>

Note: If samples with a different wavelength range are selected, the graphs are shown on full scan range of 200–900 nm (N50: 200-650 nm).

- Baseline correction is set off by default. Enabling the baseline corrections shows a list with different wavelength options: 377 nm, 604 nm, 650 nm, 770 nm (N/A N50) and 823 nm (N/A N50). Option to enter any wavelength between 200 nm and 900 nm (N50: 650 nm)

Dilution	15	Baseline Correction	<input checked="" type="checkbox"/>
Wavelength Range		Baseline Correction at 377 nm	
Baseline Correction		Baseline Correction at 604 nm	
Smoothing		Baseline Correction at 650 nm	
Manual Dilution		Baseline Correction at 770 nm	
		Baseline Correction at 823 nm	
		Baseline Correction at	<input type="text" value="750 nm"/>

- Option to smooth the graph with different boxcars. Options: Off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

Dilution	15	Smoothing	<input checked="" type="checkbox"/>
Wavelength Range		Smoothing 1	
Baseline Correction		Smoothing 2	
Smoothing		Smoothing 3	

- Option to set/calculate a dilution factor for manual diluted samples.



- Apply the blank ddH₂O or buffer to the illuminated sample window on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in the preferences.

- Use a lint-free laboratory wipe to clean both the sample window on pedestal and mirror in lid arm prior to applying the next sample.




Note: It can be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

- Apply sample to the sample window on pedestal and press the sample button to initiate the measurement. If auto sample is active the sample measurement is automatically started after closing the lid arm.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.

MEASUREMENT PROTOCOL MULTI SAMPLE

- Select the More Apps icon  from the Home screen and the Wavescan icon from the More Apps screen

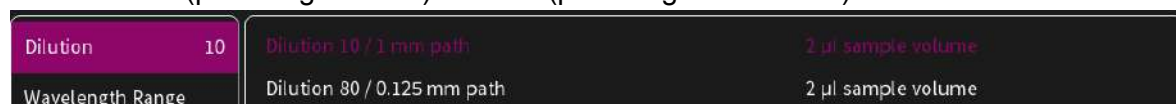


- To change between single and multi-sample application use the toggle switch Single Sample in the parameter area.



- Select the dilution depending on the sample concentration

Note: There is no automatic path length setting in this method. Select either a virtual dilution of 10 (path length 1 mm) or of 80 (path length 0.125 mm)



- Set Start and End Wavelength to define the scan range.

Dilution	10	Start Wavelength (nm)	<input type="text" value="200 nm"/>
Wavelength Range		End Wavelength (nm)	<input type="text" value="900 nm"/>

- Baseline correction is set off by default. Enabling the baseline corrections shows a list with different wavelength options: 377 nm, 604 nm, 650 nm, 770 nm and 823 nm. Option to enter any wavelength between 200 nm and 900 nm.

Dilution	10	Baseline Correction	<input checked="" type="checkbox"/>
Wavelength Range		Baseline Correction at 377 nm	
Baseline Correction		Baseline Correction at 604 nm	
Smoothing		Baseline Correction at 650 nm	
Sample Loading		Baseline Correction at 770 nm	
Single Sample	<input type="checkbox"/>	Baseline Correction at 823 nm	
		Baseline Correction at	<input type="text" value="750 nm"/>

- Option to smooth the graph with different boxcars. Options: Off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

Dilution	10	Smoothing	<input checked="" type="checkbox"/>
Wavelength Range		Smoothing 1	
Baseline Correction		Smoothing 2	
Smoothing		Smoothing 3	

Select the sample loading orientation:

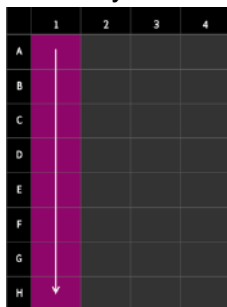
Dilution	10	horizontally	(up to 12 samples e.g. A01 – A12)
Wavelength Range		vertically	(up to 8 samples e.g. A01 – H01)
Baseline Correction			
Smoothing			
Sample Loading			

There are two options:

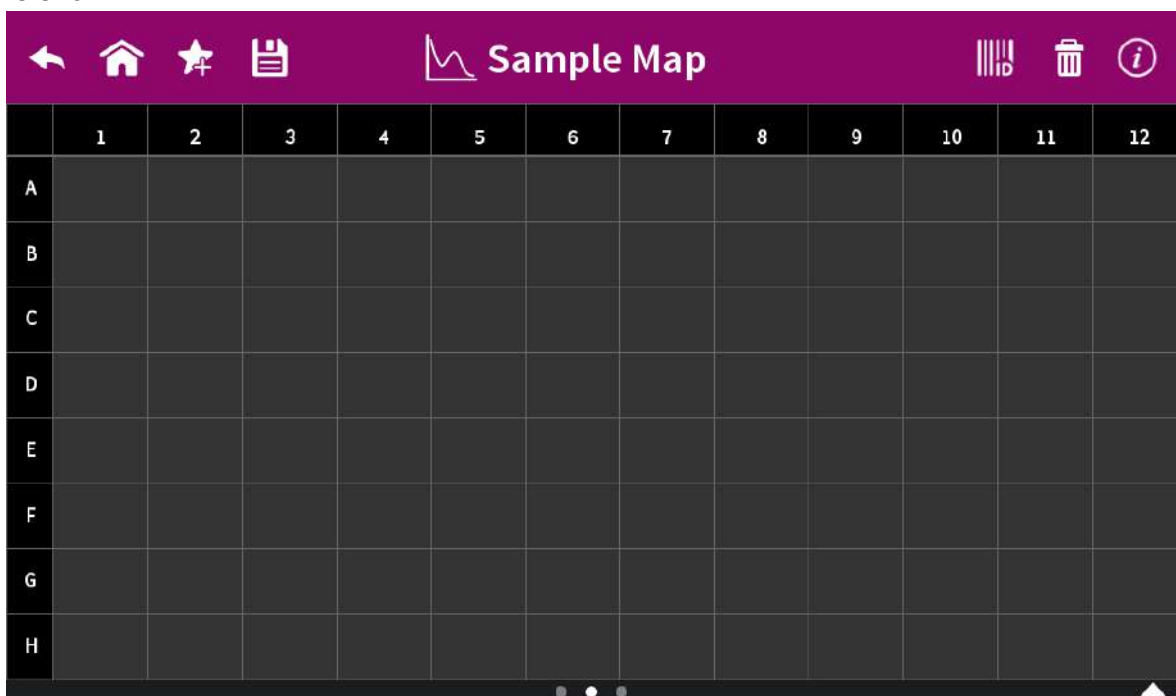
Horizontally: Measure up to 12 samples (A01 – A12 shown in sample map as row)

	1	2	3	4	5	6	7	8	9	10	11	12
A	→											
B												
C												
D												

Vertically: Measure up to 8 samples (A01 – H01 shown in sample map as column)



- To confirm the parameter setting, swipe the screen to the left and the sample map screen is shown.



The sample map offers the possibility to select cells and define sample IDs for measurements. It is not necessary to make a selection. If cells are left empty without defined sample IDs for horizontal and vertical sample loading, all 12 positions for horizontal loading and all 8 positions for vertical loading are measured.

To measure a defined amount of samples select a cell pattern starting at A01.

Sample IDs can be defined by pushing on the sample ID icon (≡) in the navigation bar.

The opened pop up offers options to define, import, export or delete sample IDs.



Further information about cell selection and sample ID definition can be found in chapter 3 NanoPhotometer® Basics / Sample Map on page 36.

- Apply the blank ddH₂O or buffer to the illuminated sample windows on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in the preferences.

Note: If the blank and sample buttons are not shown please either open the lid arm, push on ▲ on the right bottom corner or swipe from the bottom edge in the screen.

- Use a lint-free laboratory wipe to clean both the sample windows on pedestal and mirrors in lid arm prior to applying the next sample.



Note: It can be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

- Apply samples to the illuminated sample windows on pedestal and press the sample button to initiate the measurement.



If auto sample is active the sample measurement is automatically started after closing the lid arm.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.

CALCULATIONS

No calculations necessary: values are reported based on 10 mm path length.

The results show prominent peaks with wavelength and absorbance values. For cuvette measurements it is possible to change to the %Transmittance mode. If a peak of interest is not shown in the results the peak can be selected by pushing on the graph. The peak can then be added to the results by tapping on the Add Peak button in the pop-up window.



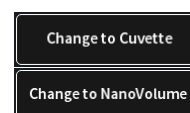
MORE APPS: ABSORBANCE RATIO

METHOD OVERVIEW

In this mode, it is possible to determine simple absorbance ratios for a given sample by measuring the absorbance at two wavelengths specified in the parameters of the method relative to a blank.

MEASUREMENT PROTOCOL

1. Select the More Apps icon from the home screen and the Absorbance/Ratio icon from the More Apps screen
2. To change between NanoVolume and Cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).
3. **For NanoVolume application:**
Select the dilution depending on the sample concentration



Dilution	15	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Ratio		Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).

For cuvette application:

Select the path length depending on the used cuvette. Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm

Pathlength	10 mm	Pathlength	0.5 mm
Heat To 37°C	<input type="checkbox"/>	Pathlength	1 mm
Ratio		Pathlength	2 mm
Baseline Correction		Pathlength	5 mm
		Pathlength	10 mm

If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics). Required sample volume for each lid is shown.

Lid	50	Lid 5	3.5 µl - 5 µl sample volume
Ratio		Lid 10	1 µl - 3 µl sample volume
Baseline Correction		Lid 50	0.3 µl - 2 µl sample volume
Smoothing		Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Enter desired wavelengths (λ 1-1 and λ 1-2) for ratio calculation. It is possible to measure up to 20 absorbance/ratios simultaneously. More wavelengths for ratio calculation can be added by selecting the Add Ratio button. Added ratios can be deleted with ⊗.

Dilution	15	AddRatio	+	Ratio 1	λ 1-1	260 nm	λ 1-2	280 nm
Ratio								

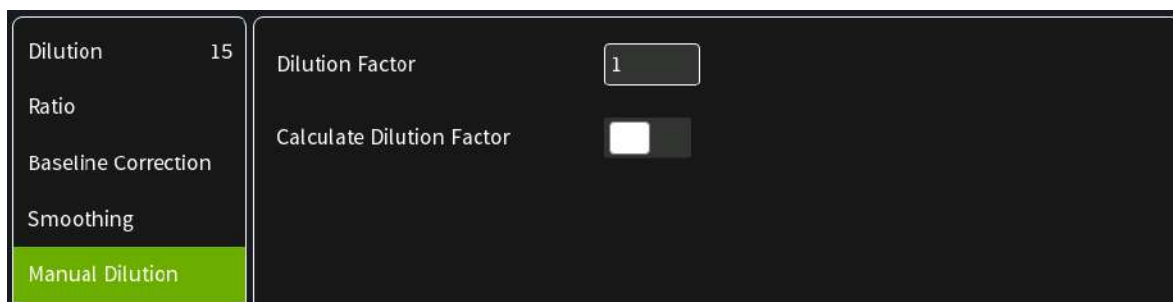
- Baseline correction is set off by default. Enabling the baseline corrections shows a list with different wavelength options: 377 nm, 604 nm, 650 nm, 770 nm (N/A N50) and 823 nm (N/A N50). Option to enter any wavelength between 200 nm and 900 nm (N50: 650 nm)

Dilution	15	Baseline Correction	<input checked="" type="checkbox"/>
Ratio		Baseline Correction at 377 nm	
Baseline Correction		Baseline Correction at 604 nm	
Smoothing		Baseline Correction at 650 nm	
Manual Dilution		Baseline Correction at 770 nm	
		Baseline Correction at 823 nm	
		Baseline Correction at	750 nm

- Option to smooth the graph with different boxcars. Options: Off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61

Dilution	15	Smoothing	<input checked="" type="checkbox"/>
Ratio		Smoothing 1	
Baseline Correction		Smoothing 2	
Smoothing		Smoothing 3	

- Option to set/calculate a dilution factor for manual diluted samples.



- Apply the blank ddH₂O or buffer to the illuminated sample window on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in the preferences.

- Use a lint-free laboratory wipe to clean both the sample window on pedestal and mirror in lid arm prior to applying the next sample.



Note: It can be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.

- Apply sample to the sample window on pedestal and press the sample button to initiate the measurement.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.



CALCULATIONS

The absorbance ratio is calculated from the two path lengths specified by the user in the parameters.

$$\lambda_1 : \lambda_2 = \frac{\lambda_1}{\lambda_2}$$

$\lambda_1 : \lambda_2$ = Absorbance Ratio

λ_1 = Absorbance 1 corresponding absorbance value 1 selected normalized to 10 mm path

λ_2 = Absorbance 2 corresponding absorbance value 2 selected normalized to 10 mm path




MORE APPS: CONCENTRATION

METHOD OVERVIEW

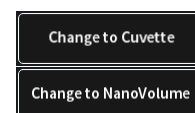
In this mode, concentration can be calculated for a sample by determining the absorbance at a specific wavelength relative to a reference. The concentration is then obtained by multiplying the measured absorbance by a specific factor. This factor may be known in advance and entered by the user, or it may be calculated by the instrument by measuring a set of standard (standard curve method) with known concentrations to create a standard curve.

MEASUREMENT PROTOCOL

1. Select the More Apps icon  from the home screen and the Concentration icon from the More Apps screen.

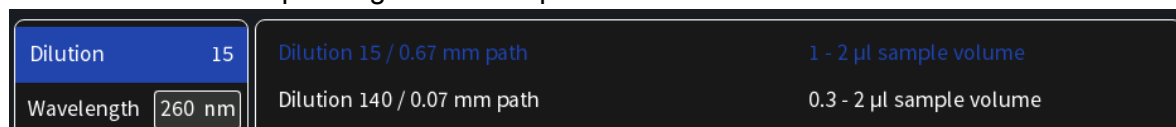


2. To change between NanoVolume and Cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).



3. **For NanoVolume application:**

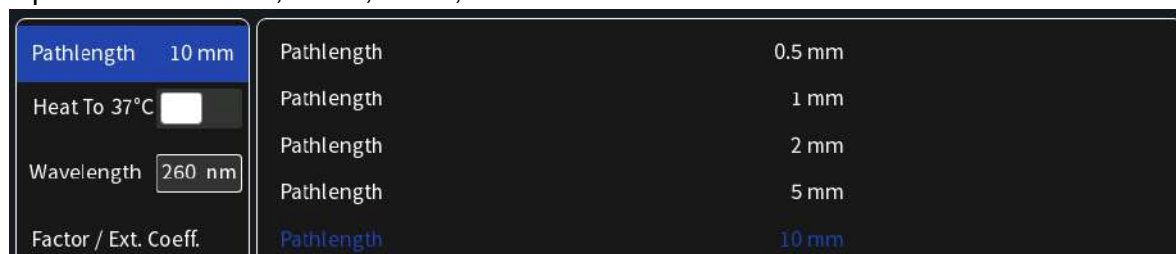
Select the dilution depending on the sample concentration.



Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).

For cuvette application:

Select the path length depending on the used cuvette. Options are: 0.5 mm, 1 mm, 2 mm, 5 mm and 10 mm



If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

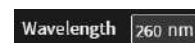
Select Lid depending on your sample concentration (for concentration ranges of different

lids see page 52 Submicroliter Cell Measurements Basics). Required sample volume for each lid is shown.

Lid	50	Lid 5	3.5 µl - 5 µl sample volume
Wavelength	260 nm	Lid 10	1 µl - 3 µl sample volume
Factor / Ext. Coeff.		Lid 50	0.3 µl - 2 µl sample volume
Units	mg/ml	Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

- Default wavelength is 260 nm but can be changed in the range of 200-900 nm (N50: 200–650 nm), depending on the sample/application.
- Enter a factor for concentration calculation.

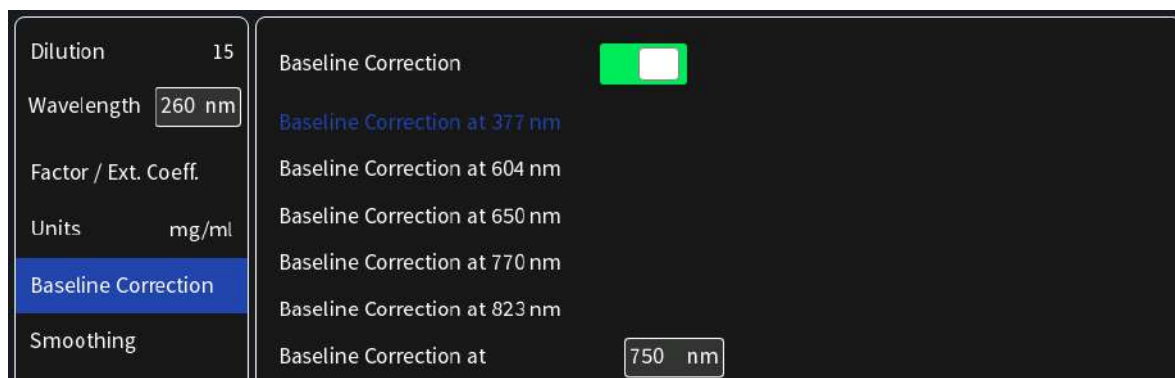


Dilution	15	Factor (1/ε)	50
Wavelength	260 nm		
Factor / Ext. Coeff.			

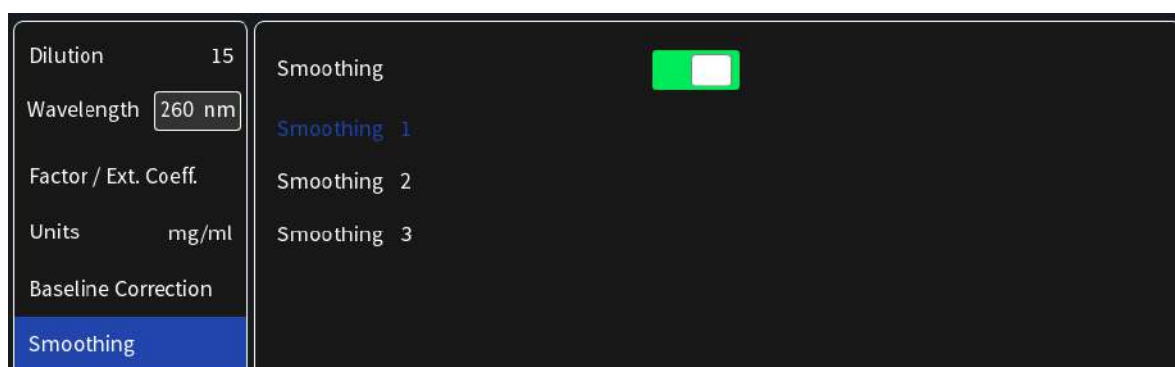
- Unit selection

Dilution	15	mg/ml	mg/l
Wavelength	260 nm	µg/ml	U/l
Factor / Ext. Coeff.		µg/µl	%
Units	mg/ml	µg/l	ppm
Baseline Correction		pmol/µl	conc.
Smoothing		mmol/l	ng/µl
Manual Dilution		µmol/l	
		g/l	

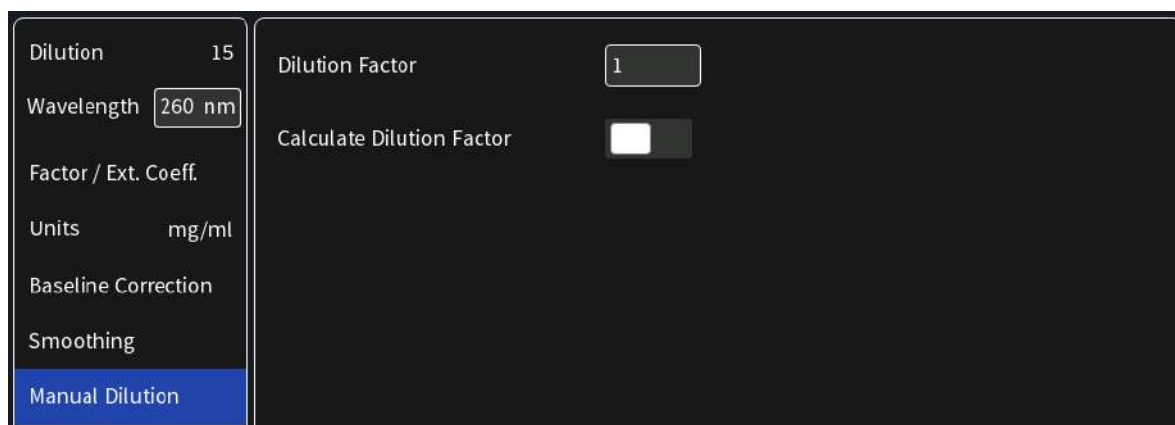
- Baseline correction is set off by default. Enabling the baseline corrections shows a list with different wavelength options: 377 nm, 604 nm, 650 nm, 770 nm (N/A N50) and 823 nm (N/A N50). Option to enter any wavelength between 200 nm and 900 nm (N50: 650 nm)



8. Option to smooth the graph with different boxcars. Options: Off, 1 = boxcar 11 (default), 2 = boxcar 21 and 3 = boxcar 61



9. Option to set/calculate a dilution factor for manual diluted samples.



10. Apply the blank ddH₂O or buffer to the illuminated sample window on pedestal for the reference measurement and select blank to initiate the reading.



Note: The illumination of the sample window can be switched off in the preferences.

11. Use a lint-free laboratory wipe to clean both the sample window on pedestal and mirror in lid arm prior to applying the next sample.

Note: It can be helpful to apply the blank a second time and read it as a sample to ensure a proper blank.



12. Apply sample to the sample window on pedestal and press the sample button to initiate the measurement.

If auto sample is active the sample measurement is automatically started after closing the lid arm.

Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.



CALCULATIONS

In this method, the concentration of the sample is calculated based on the Beer-Lambert law given the user specified wavelength of interest and user defined extinction coefficient. The equations for calculating concentration without background correction are as follows:

Without background correction:

$$C = A_n * \epsilon * D$$

C	Concentration (ng/μl)
A_n	Absorbance at user specified path length n (10 mm path)
D	Dilution factor
ϵ	extinction coefficient/factor




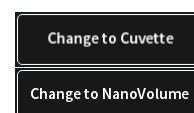
MORE APPS: STANDARD CURVE

METHOD OVERVIEW

The construction of a calibration curve from multiple standards of known concentrations can be created and stored on the NanoPhotometer®. The standard curve can be used to quantify samples of the same type with unknown concentrations. This application provides an extremely useful tool with which to integrate, expedite and simplify the measurement and calculations involved in determining the concentration of analytes in unknown samples. If a zero concentration standard is required, include it in the number of standards to be entered using a reagent blank and entering 0.00 for concentration.

MEASUREMENT PROTOCOL

1. Select the More Apps icon  from the home screen and the Standard Curve icon from the More Apps screen.
2. To change between NanoVolume and Cuvette application, use the Change to Cuvette/NanoVolume button below the parameter area (NP80/C40 only).
3. **For NanoVolume application:**
Select the dilution depending on the sample concentration



Dilution	15	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Wavelength	260 nm	Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).

For cuvette application:

Select the path length depending on the used cuvette.

Pathlength	10 mm	Pathlength	0.5 mm
Heat To 37°C	<input type="checkbox"/>	Pathlength	1 mm
Wavelength	260 nm	Pathlength	2 mm
Baseline Correction		Pathlength	5 mm
		Pathlength	10 mm

If it is desired to heat the sample to 37°C use the toggle switch to turn on cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (NP80 and C40).

For NanoVolume application with submicroliter cell (C40 only):

Select Lid depending on your sample concentration (for concentration ranges of different lids see page 52 Submicroliter Cell Measurements Basics).

Required sample volume for each lid is shown.

Wavelength	260 nm	Lid 5	3.5 µl - 5 µl sample volume
Lid	50	Lid 10	1 µl - 3 µl sample volume
Baseline Correction		Lid 50	0.3 µl - 2 µl sample volume
Curve Fit		Lid 100	0.3 µl - 2 µl sample volume
		Lid 250	0.3 µl - 2 µl sample volume

Note: A new Blank is recommended when changing the dilution lid.

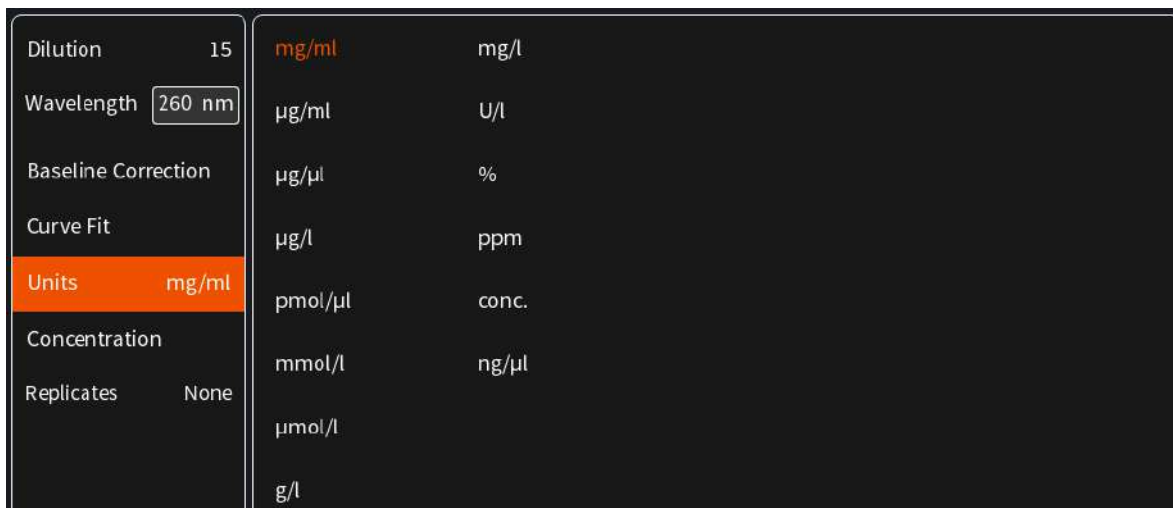
- Baseline correction is set off by default. Enabling the baseline corrections shows a list with different wavelength options: 377 nm, 604 nm, 650 nm, 770 nm (N/A N50) and 823 nm (N/A N50). Option to enter any wavelength between 200 nm and 900 nm (N50: 650 nm)

Dilution	15	Baseline Correction	<input checked="" type="checkbox"/>
Wavelength	260 nm	Baseline Correction at 377 nm	
Baseline Correction		Baseline Correction at 604 nm	
Curve Fit		Baseline Correction at 650 nm	
Units	mg/ml	Baseline Correction at 770 nm	
Concentration		Baseline Correction at 823 nm	
		Baseline Correction at	750 nm

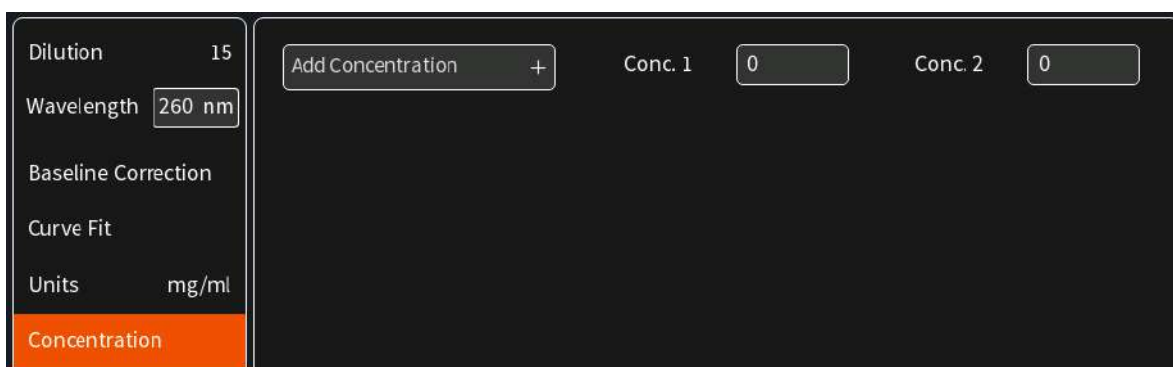
- Select the curve fit type: Options are linear regression, zero regression (forces the straight line through the origin) and 2nd order regression.

Dilution	15	Regression Linear
Wavelength	260 nm	Zero Regression Linear
Baseline Correction		Regression 2nd Order
Curve Fit		

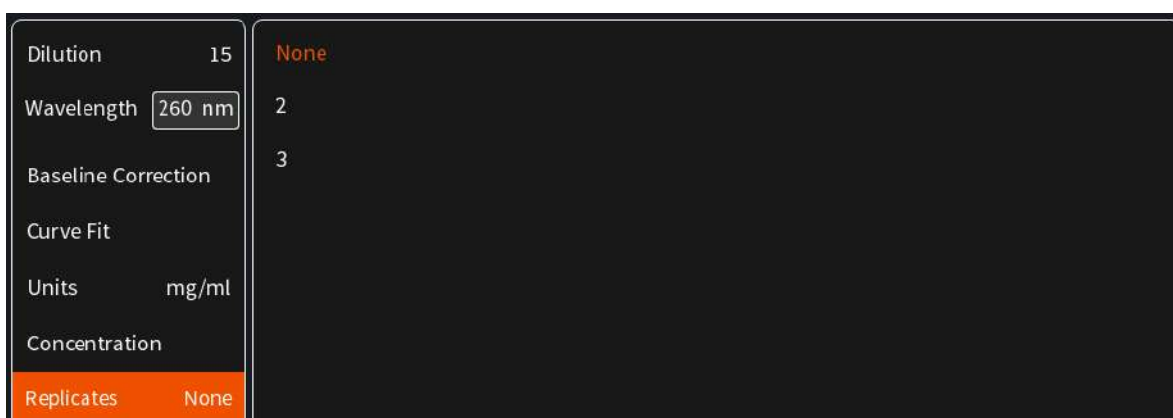
- Select Unit



7. Add up to 20 Concentrations by pushing on the Add Concentration button. Added concentrations can be deleted with ⊗
Enter the concentrations of the standard curve.



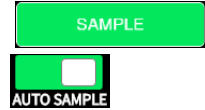
8. Select Replicates none, 2 or 3



9. Once Standard curve is created or loaded it will be used for concentration calculations in the method. It might be necessary to do a blank measurement.



10. Apply sample and press the sample button to initiate the measurement. If auto sample is active the sample measurement is automatically started after closing the lid arm.



Note: Auto sample function is only available NanoVolume methods and new NanoPhotometer® versions it may not be available after updating an older firmware version.

Note: Once the sample measurement is initiated it is not possible to make changes to the standard curve.

CALCULATIONS

Concentration is determined via the absorbance values provided by the standard curve based on the curve fit selection including the following options: linear regression, zero regression and 2nd order regression.



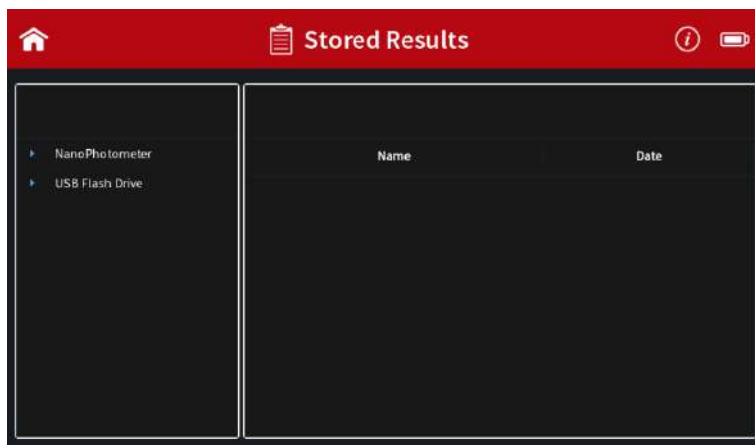
CUSTOM APPS



There is an option for designing customer specific Custom Apps which can be loaded to the NanoPhotometer®. For more information about designing custom applications to suit individual research needs please contact Implen directly for assistance.



STORED RESULTS

The Stored Results icon opens a directory of folders containing files of results that have been previously saved.




On the left side of the screen all available directories/storages are shown: NanoPhotometer®, Control Device, Network and/or USB flash drive (depending on availability). Pushing a storage folder shows the subfolders of this storage folder on the left side and the individual files on the right side. On the right side of the screen all saved result files of the selected folder are shown and can be opened by a long or double click. Folders can be deleted, renamed, moved or copied by pushing on the  icon. It is also possible to delete, rename, move or copy files by pushing on the  icon. The file path of the selected folder is shown on the top of the right file area.

Note: PDF and Excel files cannot be opened on the NanoPhotometer®. Files need to be transferred to a computer or device where Excel or a PDF reader is installed.

Note: Control device is only available on computer, tablets and smartphones not on the NanoPhotometer® version of the software.

For data transfer via Ethernet or WiFi see page 56 Data Transfer.

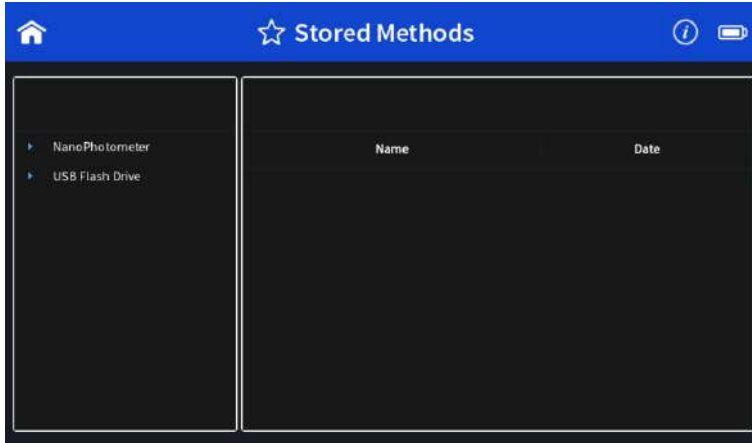
Backup copies are saved in the Autosave folder of the NanoPhotometer® (Stored Results/NanoPhotometer/Autosave) for up to ten days. After ten days the autosave files are automatically moved to an autosave archive folder. The autosave archive folder can only be accessed via NanoPhotometer® file server. Data in the autosave archive folder are not automatically deleted.



The content of the Autosave Archive folder can be deleted via the action button  in Stored Results. Make sure that a backup is created before deleting the Autosave Archive folder content.




STORED METHODS

The stored methods icon opens the directories of folders containing methods stored by the user.



On the left side of the screen all available directories/storages are shown: NanoPhotometer®, Control Device, Network and/or USB flash drive (depending on availability). Pushing a storage folder shows the subfolders of this method folder on the left side and the individual files on the right side. On the right side of the screen all saved method files of the selected folder are shown and can be opened by a long or double click. On the top of the right area the file path of the selected folder is shown. New folders can be created by pushing on **+**. Folders can be deleted, renamed, moved or copied by pushing on the  icon. It is also possible to delete, rename, move or copy methods by pushing on the  icon.

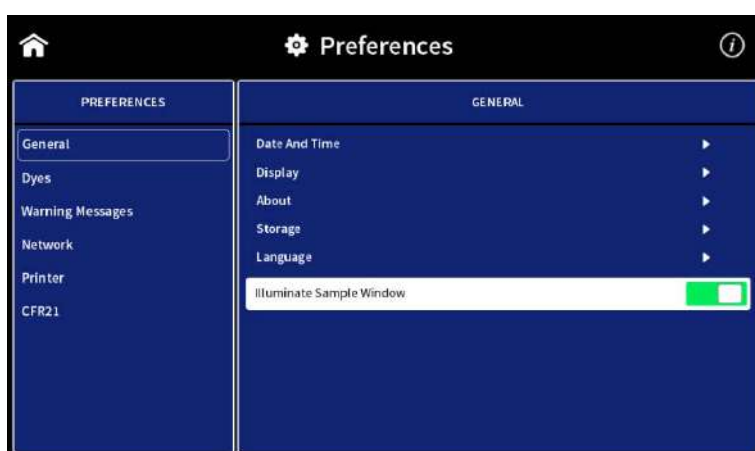
5. PREFERENCES

System preferences can be set by selecting preferences  on the home screen. The preferences menu includes: General, Dyes, Warning Messages, Network, Printer and CFR21. The preference options of the selected menu item are listed in the window on the right.

Note: Preferences are not available on smartphones with screen sizes less than 7 inches.

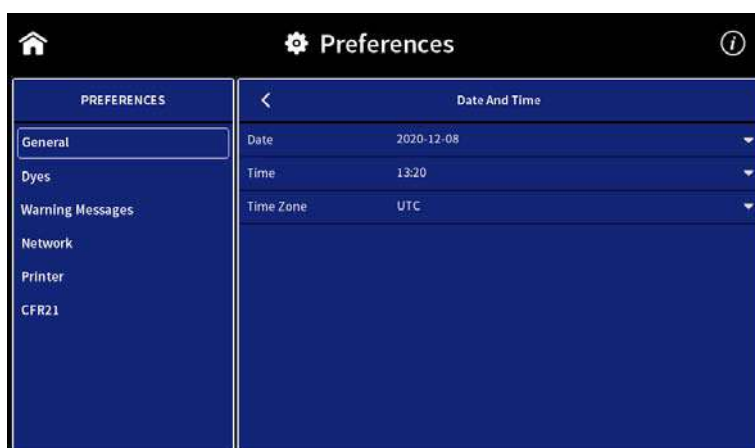
GENERAL

Selecting General in the Preferences menu opens a window to the right of the preferences menu with the following options: Date and Time, Display, About, Storage, Language and Illuminate Sample Window (N120/NP80/N60/N50) or Enable NanoVolume (C40).



DATE AND TIME

Within the Date and Time it is possible to set the actual date and time of the NanoPhotometer® or change the time zone.

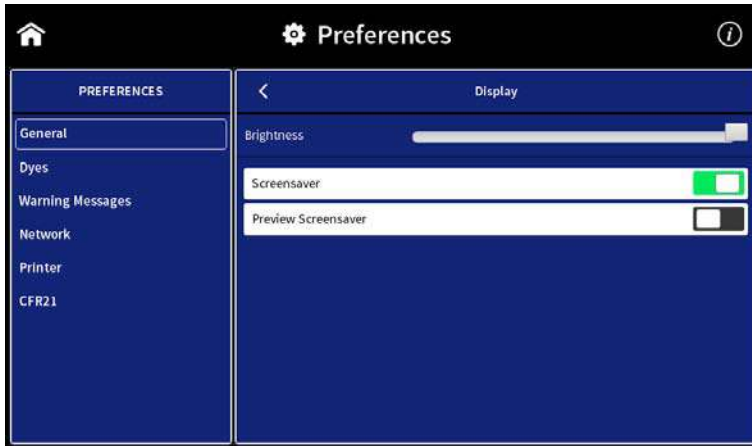


To change the time zone or the date and/or time, push on the appropriate field to open the selection options. The changed settings are shown below the time zone field. To apply the changes, the NanoPhotometer® must be rebooted. Start the reboot by pushing on the Set and Reboot button.

Note: Do not change the date and time at the same time as the time zone. Make sure that the UTC time is set correctly before changing the time zone.

DISPLAY

Brightness: adjustment of the built-in screen brightness



Screensaver: Option to switch the screensaver video on and off. With Preview Screensaver the screensaver can be started immediately.

Note: Screensaver activation will influence the time for battery operation. To prolong battery operation, switch the screensaver off.

Note: The screensaver option is not available when the CFR21 software is enabled.

ABOUT

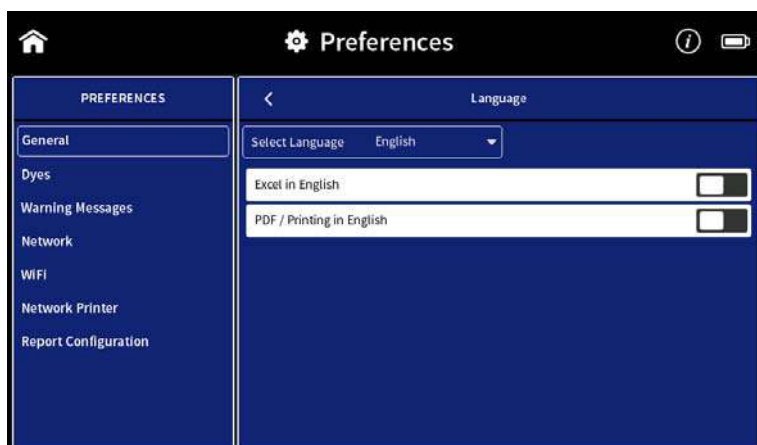
In About the following information of the NanoPhotometer® are shown: NanoPhotometer® Version, Serial Number, Ethernet IP Address, WiFi IP Address, Hardware Version, Firmware Version, Time & Date of Initialization Test and Status of Initialization.

STORAGE

Shows the total storage capacity and the free space of the internal NanoPhotometer® storage.

LANGUAGE

In language settings, the user has the option to change the language of the NanoPhotometer® software. The following languages are available: English, Chinese, French, German, Japanese, Portuguese, Russian or Spanish. The language is changed for the user interface, printouts and saved Excel and PDF files.



Note: The change will affect all methods.

Note: It is possible to work in users' desired language and change the selected language for Excel and PDF Printouts to English. This option can be selected with the toggle switches Excel in English and PDF/Printing in English. This is a global change for all methods. All Excel/PDF files and printouts will be in English. Independent from the language setting utilized in the method.

ILLUMINATION SAMPLE WINDOW

Toggle switch to switch on/off the illumination of the sample window (for N120/NP80/N60/N50 only)

Note: Only available for N120/NP80/N60/N50 models.

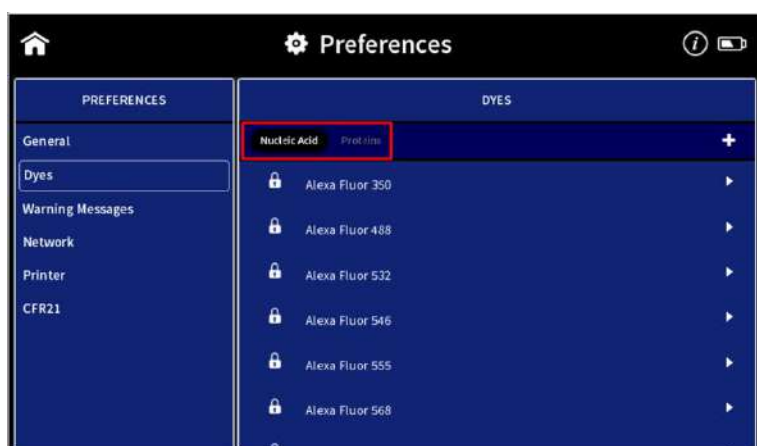
ENABLE NANOVOLUME

Toggle switch to enable the submicroliter cell option for the NanoPhotometer® C40.

Note: Only available for C40 model.

DYES

There is a list of preprogrammed dye-labels for both nucleic acid dyes and protein dyes. To toggle between the nucleic acid and protein list push on the Nucleic Acid/Protein buttons in the header.



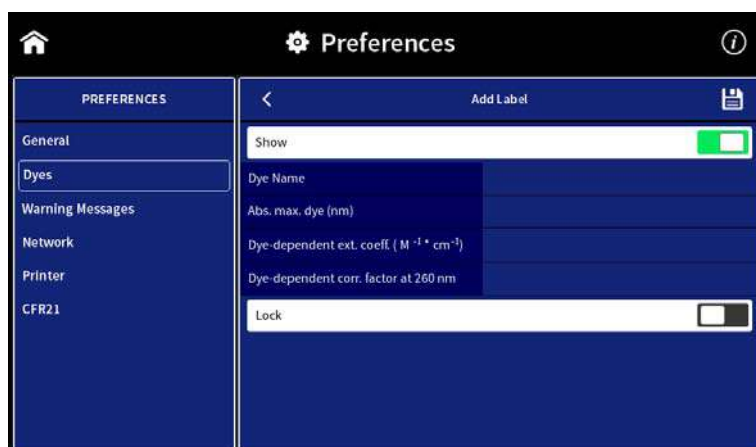
Each dye has either a lock icon (🔒) in front of the dye name indicating that the dye is locked and cannot be changed or a delete symbol (🗑️). The delete option is only available for unlocked and not preprogrammed dyes.

Selecting a dye name opens a new screen with the dye information: dye name, absorbance maximum dye (nm), dye-dependent extinction coefficient ϵ_{dye} ($M^{-1} * \text{cm}^{-1}$), and dye-dependent correction factor as well as the option to show the dye in the parameter list of the application (Nucleic Acid or Protein UV).



Note: It is not possible to delete a dye from the default factory list; custom dyes can be deleted if they are not locked.

It is possible to add a new dye to the list by selecting the + button to add a new dye. A window will open where it is possible to enter the: dye name, dye absorbance maximum (nm), dye-dependent extinction coefficient ϵ_{dye} ($M^{-1} * \text{cm}^{-1}$), and dye-dependent correction factor. There is a toggle switch available to lock the dye to prevent deleting a dye from the dye list accidentally.

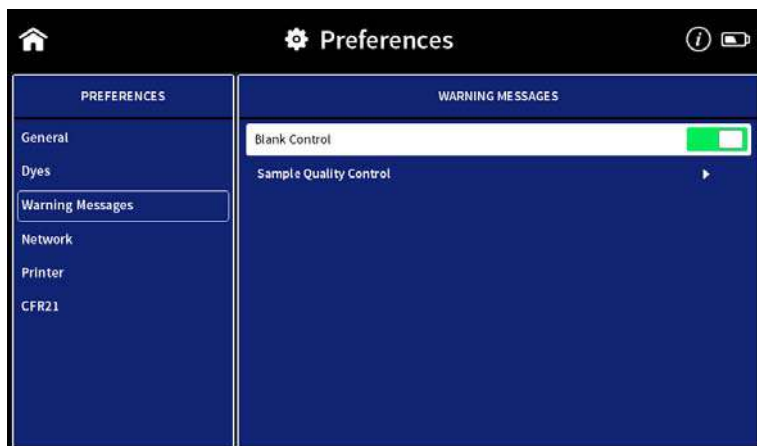


WARNING MESSAGES

BLANK CONTROL

Toggle switch to switch on/off the Blank Control™ of the NanoPhotometer®.

Note: Blank Control™ is available for all NanoVolume methods (for N120/NP80/N60/N50).



SAMPLE QUALITY CONTROL

It is possible to change the upper and the lower limit of the ratio alert warning messages.

Default values for nucleic acid ratios are:

260/230 ratio 1.8 A - 3 A and 260/280 ratio 1.65 A - 2.5 A.

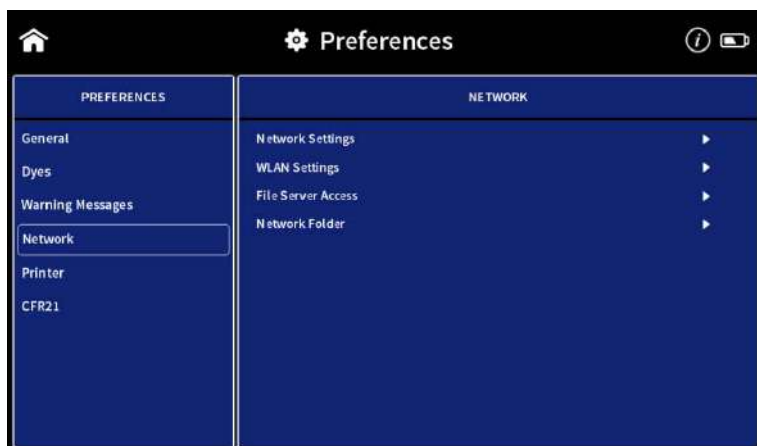
Default value for Protein UV ratio is:

260/280 ratio is 0.7 A.



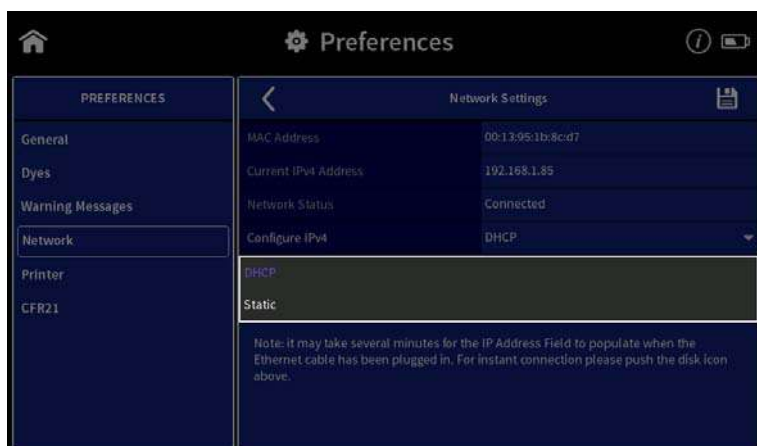
NETWORK

Selecting Network in the Preferences menu opens a window to the right of the preferences menu with the following options: Network Settings, WLAN Settings, File Server Access and Network Folder.



NETWORK SETTINGS

In Network Settings it is possible to choose between a Dynamic Host Configuration Protocol (DHCP) and a static network configuration. The DHCP protocol is used by default. Connect the NanoPhotometer® with the Ethernet and the IP is automatically set during the startup of the NanoPhotometer®. If no IP Address is listed for Current IPv4, push the disk icon (⌘) to search for an available IP address.



For static IP configuration select Static in the Configure IPv4 dropdown and enter the IPv4 address, netmask and gateway then confirm with disk icon (💾).

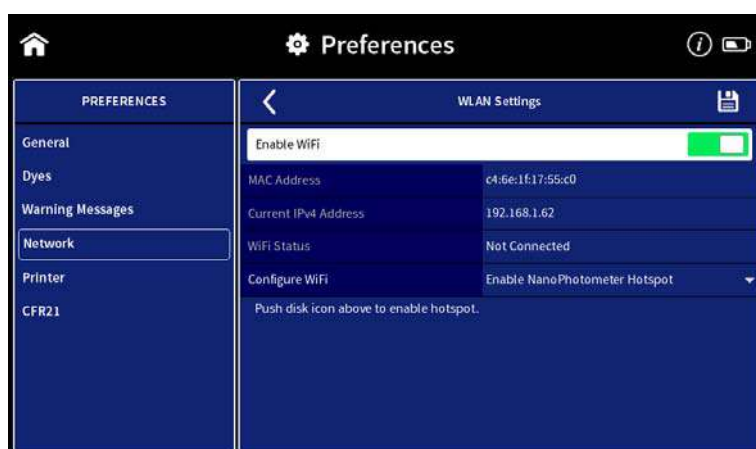


WLAN SETTINGS

The WLAN Setting preferences allow the user to switch off the WiFi or to set up a WiFi Hotspot (WiFi Access Point) or a WiFi network.

To set up a WiFi Hotspot select “Enable NanoPhotometer Hotspot” in the dropdown and push on the save icon.

The WiFi Hotspot can be used to connect a computer, Android smartphone/tablet, iPad or iPhone via WiFi to the NanoPhotometer®.

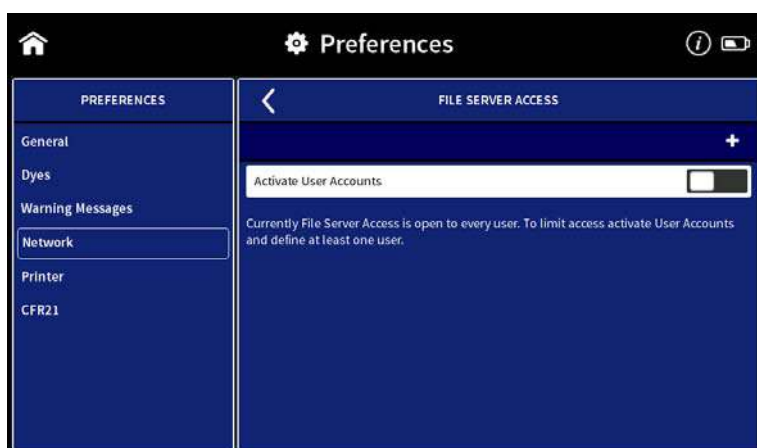


To set up a WiFi network select “Connect to WiFi Network” in the dropdown. Select an available WiFi network from the dropdown list or choose other for hidden networks and enter the password. Confirm with the disk icon (💾).

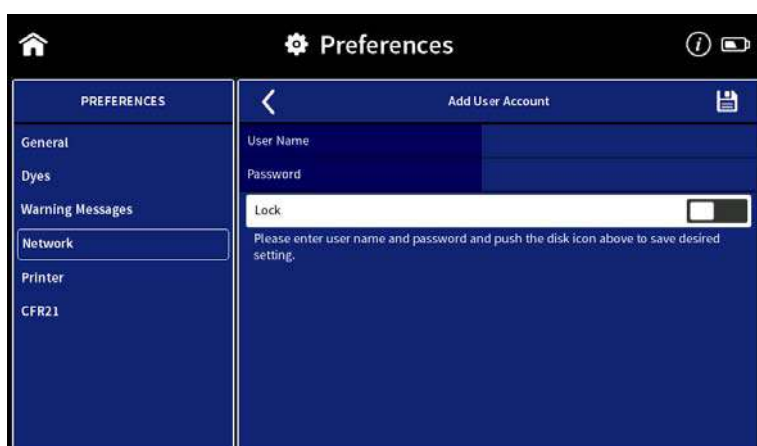


FILE SERVER ACCESS

The File Server Access preferences allow creating user accounts to limit the access to the NanoPhotometer® file server via network. By default, access to the file server is open to any user. It is necessary to create at least one user account to activate the file server access option.



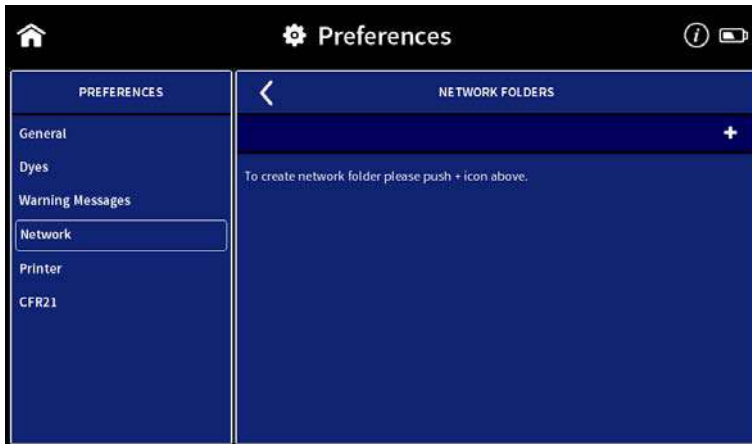
To create a user account push on the + button and enter a user name and password. Confirm with the disk icon (💾).



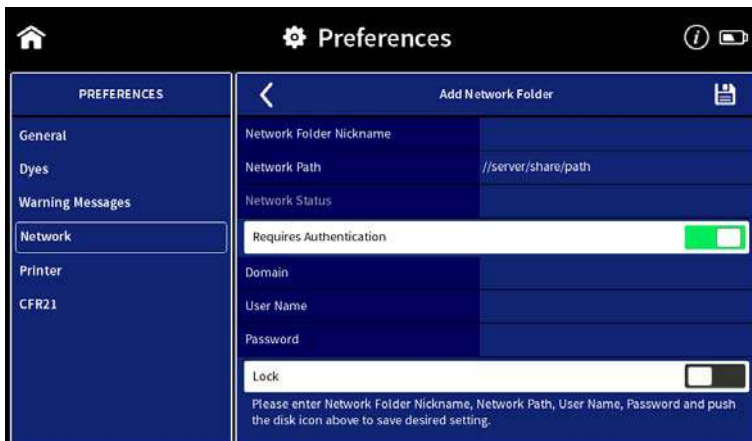
Note: Allowed characters for user name are: A...Z a...z 0...9 _ -
Do not use Blank character.

NETWORK FOLDER

The network folder preferences allow creating network folder for saving data directly from the NanoPhotometer® to a network folder.

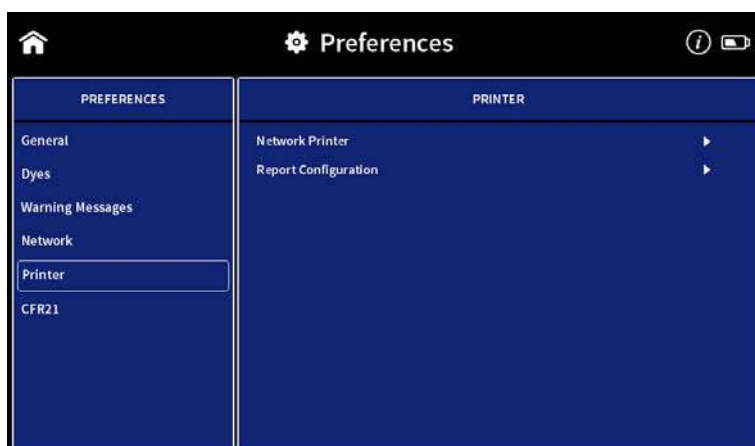


To add a network folder push the + icon and enter network folder nickname and network path of the network folder. If the local network requires authentication enter user name and password for login and if necessary the domain. To save the Network folder push the disk icon (📁).



PRINTER

Selecting Printer in the Preferences menu opens a window to the right of the preferences menu with the following options: Network Printer and Report Configuration.



NETWORK PRINTER

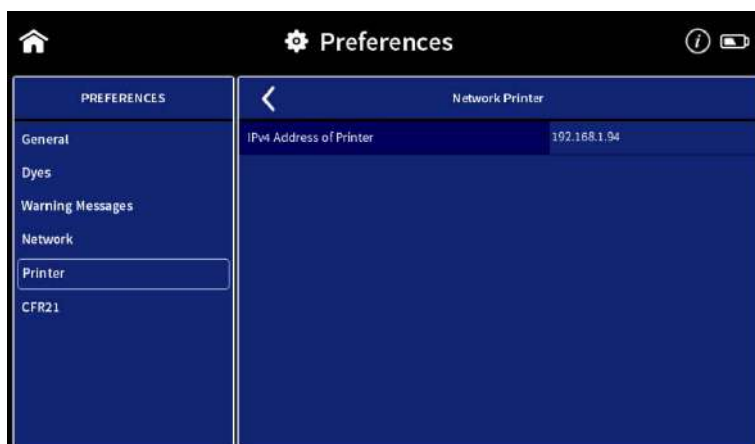
It is possible to print via network (Ethernet) or Airprint® / IPP compatible (supporting PDF format) printers in the network.

Note: IPP version 2.2 is required and some printer configuration settings might need to be changed in order to allow communication with the NanoPhotometer®.

Enter the IP of the network printer to the input window.

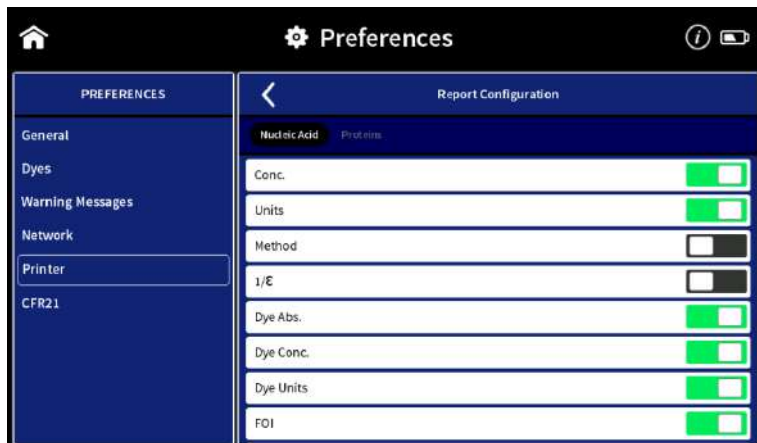
Note: For printing on network printer the NanoPhotometer® needs to be connected to the network either via Ethernet (LAN cable) or WiFi network.

Note: If a printer is directly connected to the NanoPhotometer® via USB, this printer will have the highest priority and will be the printer used by default when selecting Print on NanoPhotometer®. In order to print utilizing a printer on the network, please disconnect the installed USB printer.



REPORT CONFIGURATION

In report configuration it is possible to define the table columns of PDF files and A4 printouts. This feature is available for the methods Nucleic Acids and Protein UV. To toggle between the nucleic acid and protein list push on the Nucleic Acid/Protein buttons in the header.



CFR21

The CFR21 Software is an optional software tool ideal for GxP laboratories, which require proper electronic record keeping. It includes user management, access control, electronic signatures, data integrity, security, and audit trail functionality.

In the CFR21 Preferences it is possible to enable the optional CFR21 Software. This is only possible with a license key. For further details please contact the Implen Support team or refer to the CFR21 software user manual.

6. TROUBLESHOOTING

INITIALIZATION TEST

The NanoPhotometer® initialization test is performed automatically every time the instrument is powered on. If the instrument passes the initialization test the home screen is shown. If the instrument does not pass the test, a message window will appear explaining the reason for the failed test along with the recommended solution. If the ok button is selected, the window is closed and the home screen will be shown. Initialization failed will be displayed on top of all method screens. If the initialization test fails please contact the Implen Support Team.

MESSAGES

The software gives three different kind of messages: confirmation (green), warning (yellow) and alert (red)

Confirmation messages are shown e.g. after the initialization test has passed successfully or if files or folders are copied or moved successfully. These messages are self-explaining and not listed in the user manual.

IMPORTANT WARNING MESSAGES:

Message Text	Explanation/Solution
Air bubble, lint residue or bad sample. Please reapply sample.	Air bubble recognition is on and has detected either an air bubble, lint residue or a bad sample like e.g. a turbid sample. Check sample, clean the sample window on pedestal and mirror in the lid arm thoroughly and reapply sample carefully. To avoid air bubbles apply sample by reverse pipetting.
Another control device is currently connected.	Message is shown on a control device when another control device is currently connected with an open measurement session. Please close method on control device.
Battery is low. Please connect to power!	This message pops up twice. Between the first and second warning message, there is at least one hour battery power left for use of the NanoPhotometer® under standard conditions. After the second warning the instrument will automatically begin shutting down within 10 to 15 seconds.
Concentration too high. Change to lid10/lid50/lid100/lid250	The concentration of the sample used is too high for submicroliter cell measurements with the utilized lid. Change to the recommended lid. A new blank is recommended when changing the dilution lid.
Concentration too high. Dilute sample.	The concentration of the sample is too high for submicroliter cell measurements using Lid 250 with the C40. Sample concentration is above the detection limit of the instrument. Dilute sample manually to measure within the detection range of the instrument.

<p>Concentration too low.</p>	<p>The concentration of the sample used is too low for submicroliter cell measurements using Lid 5 with the C40. Sample is below the detection limit of the instrument. Only cuvette measurement may be possible</p>
<p>Concentration too low. Change to lid5/lid10/lid50/lid100</p>	<p>The concentration of the sample used is too low for submicroliter cell measurements with the used lid. Change to the recommended lid. A new blank is recommended when changing the dilution lid.</p>
<p>High absorbance at 250-280nm, 280-340nm, 340-400nm, 400-475nm, 475-550nm, 550-625nm, 625-700nm. Bad Blank or insufficient cleaning.</p>	<p>The warning message is shown when a blank measurement (NanoVolume) detects a significant absorbance a potential area of interest. The warning message shows the wavelength range where the absorbance is appearing. Two things can cause high absorbance in a blank measurement: either the blank solution/buffer has an absorbance in this wavelength range or the sample window on pedestal and the mirror in the lid arm was not cleaned properly after the last reading. Clean the sample window on pedestal and the mirror in the lid arm thoroughly and check the blank solution for absorbance (Use water for blank and measure the blank buffer as sample).</p>
<p>Instrument connected to control device! Do you really want to intercept?</p>	<p>This message is shown on the NanoPhotometer® when a method is open on another control device. Push on OK and the measurements session is interrupted and the data are saved in the auto save folder.</p>
<p>Lint residue or bad sample. Please reapply sample.</p>	<p>Displayed when Air Bubble Recognition is off. Software has detected either a lint residue or a bad sample like e.g. a turbid sample. Check sample, clean the sample window on pedestal and mirror in the lid arm thoroughly and reapply sample carefully.</p>
<p>Maximum absorbance level at specified wavelength reached. Calculations may lead to low/wrong results.</p>	<p>The concentration of the sample used is too high and exceeds the specified absorbance range. Maximum absorbance is 330 A (10 mm path) for NanoVolume and 2.65 A for cuvette measurements. Dilute sample and measure again.</p>
<p>Maximum level exceeded.</p>	<p>This message is shown when for example, too many dyes or wavelength are added with the Add + button. It is possible to add up to 20 dyes or wavelengths.</p>
<p>Measurement currently in progress. Please close method on device.</p>	<p>Displayed when there is an open measurement session on the NanoPhotometer® and the user is trying to connect via a control device (phone, tablet or computer). In order to move forward with connection of control device, please close method on NanoPhotometer®.</p>
<p>No printer present.</p>	<p>Printer connection is lost. Please check printer connection. If it is still not working go back to home screen reconnect the printer and wait for 30 seconds. If the message still appears please create a log file and send it to Implen customer support.</p>

<p>Sample concentration too low for 0.3 µl - change volume and parameter settings to 1 µl</p>	<p>The measurement parameters for 0.3 µl NanoVolume samples read only the 0.07 mm path length (dilution 140). The utilized sample concentration is too low. Please use 1 µl of the sample and change the volume setting to 1-2 µl. Minimum concentrations for the 0.07 mm path length for dsDNA 420 ng/µl and for BSA 12.6 mg/ml.</p>
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IMPORTANT ALERT MESSAGES:

<p>Cuvette port blocked - please check.</p>	<p>The automatic sliding cover of the NanoPhotometer® NP80 could not close properly. Please check if the cuvette port is empty. If message still appears please contact Implen Customer Support.</p>
<p>ERROR: Lid open - please close lid.</p>	<p>Lid arm is open during initialization test. Close lid arm and confirm with OK.</p>
<p>Firmware not found - NPOS.bin must be in root folder of USB flash drive</p>	<p>Make sure that the sample name is NPOS.bin without any additions or extensions like from multiple downloads, otherwise rename file to NPOS.bin exactly. NPOS.bin file must be in the root folder of the USB flash drive. Make sure that the NPOS.bin file for installation has a higher software version than the installed one. NPOS software is not downward compatible.</p>
<p>Firmware not found - NPOSX.bin must be in root folder of USB flash drive</p>	<p>NPOSX.bin file must be in the root folder of the USB flash drive. If necessary unzip the downloaded installation file. The file name should not have any additions or extensions like from multiple download. Do NOT rename a NPOS.bin to NPOSX.bin. Make sure that the NPOS.bin file for installation has a higher software version than the installed one. NPOS software is not downward compatible.</p>
<p>Initialization failed – problem with optical path. Please contact Implen customer support.</p>	<p>Check if the cuvette port is empty and/or clean the sample window on pedestal and the mirror in the lid arm with 70% EtOH and distilled water then reboot the NanoPhotometer®. If the message still appears please create a log file and send it to Implen Customer Support.</p>
<p>Initialization failed 51/52/53/54/55. Please contact Implen customer support.</p>	<p>Possible hardware failure. Please create a log file and send it to Implen Customer Support.</p>
<p>Initialization failed. Please contact Implen customer support.</p>	<p>Clean the sample window on pedestal and the mirror in the lid arm with 70% EtOH and distilled water and then reboot the NanoPhotometer®. If the message still appears please create a log file and send it to Implen Customer Support.</p>
<p>Light path blocked – please remove cuvettes from port.</p>	<p>Make sure that the cuvette port is empty during initialization test. Restart the NanoPhotometer®</p>

	with the cuvette port empty. If the message still appears please create a log file and send it to Implen Customer Support.
NanoVolume head not clean! Please clean sample windows thoroughly.	Clean the sample window on pedestal and the mirror in the lid arm with 70% EtOH and distilled water and then reboot the NanoPhotometer®. If the message still appears please create a log file and send it to Implen Customer Support.
No connection possible	Connection is lost between control devices and NanoPhotometer®. Check the connection. If the message still appears please create a log file and send it to Implen Customer Support.
Server Not Available	Internal software communication problem during an open measurement session. Reboot the NanoPhotometer®. If the message still appears please create a log file and send it to Implen Customer Support.
Server Side Failure	Internal software communication problem during an open measurement session. Reboot the NanoPhotometer®. If the message still appears please create a log file and send it to Implen Customer Support.
Software update failed. Please contact Implen customer support.	Make sure that the sample name is NPOS.bin without any additions or extensions like from multiple downloads, otherwise rename file to be NPOS.bin exactly. NPOS.bin file must be in the root folder of the USB flash drive. Try the update a second time, if it still fails please create a log file and send it to Implen Customer Support.
Stored method/result was created for unavailable mode NanoVolume/Cuvette/Submicroliter Cell!	Message appears if stored results or a stored method made on another NanoPhotometer® type is opened with an unavailable mode like Cuvette or NanoVolume e.g. data or methods made in cuvette mode and opened on a N60 (NanoVolume only).

SYSTEM FREEZE

In case of harsh ESD impulses it could happen that the device does freeze or switch-off. Please wait first about 30 sec to see if the system recovers automatically itself. If there is no reaction please press the power key at the instrument rear 4 sec to switch-off and then again shortly to restart the system.

7. ASSISTANCE

The Assistance menu includes: support, report a problem (only available for tablet and computer versions), user manual, software maintenance, diagnostics and legal as functions to help with any technical issues or questions that may arise with the NanoPhotometer®.

Note: Assistance is not available on smartphones with screen sizes less than 7 inches.

SUPPORT

Selecting support on the left side assistance menu will show the available options for contacting Implen.



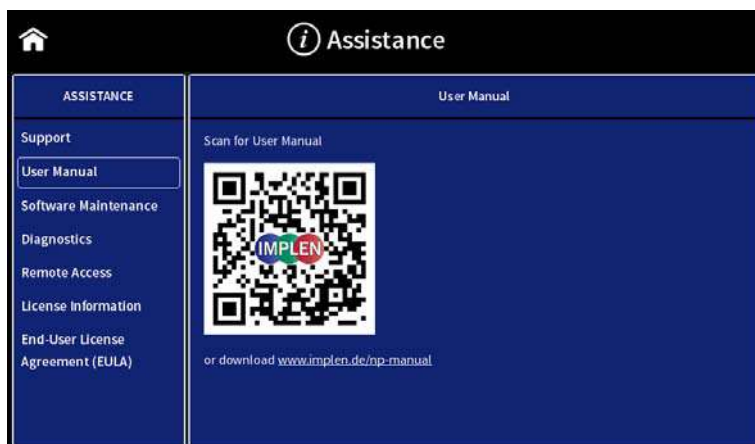
REPORT PROBLEM

The function to report a problem is only available for computer and tablet versions. Selection of Report Problem in the assistance menu, a form with the following information is shown on the right side: first name, last name, phone number, email, and country. A dropdown menu provides the option to select the type of problem and includes the following choices: error message, software, firmware, measurements, and other. It is possible to enter a question or comment at the end of the form. Once the form is completed and the send button is selected a message will be sent directly to Implen and the appropriate support person will contact the end user as soon as possible to provide further support.

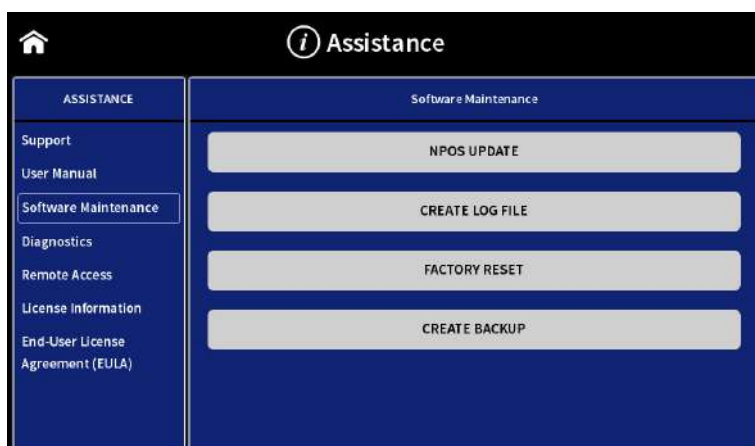
Note: The report a problem function is only available for computer and tablet versions.

USER MANUAL

The actual user manual can be downloaded using the QR code or download link.



SOFTWARE MAINTENANCE



NPOS UPDATE

Download the firmware update file (zip folder) from the Implen homepage: www.implen.de/downloads/ and unzip the file into the root folder of a USB flash drive.

Note: Do not change the file names of the unzipped installation files.

Note: Save all data on the NanoPhotometer® before updating.

Note: Make sure that the NanoPhotometer® is connected to power and that the power connection is not interrupted during the update.

It is recommended to do the update via the built-in touchscreen. If the touchscreen is not available it is also possible to do the update via computer or tablet. In this case it is necessary to relaunch the NanoPhotometer® software after the reboot of the NanoPhotometer®. For tablets the WiFi connection needs to be reconnected.

Note: Always update the firmware of the NanoPhotometer® first and then update the client software (PC/Mac, smartphone, tablet).

Update procedure:

1. Unzip the installation file to a USB flash drive into the root folder
2. Insert in the USB flash drive to the USB port of the NanoPhotometer®
3. Select Assistance/Software Maintenance
4. Push on “Update” and wait until the NanoPhotometer® reboots

Note: Once you have updated the firmware of the NanoPhotometer®, please update the client software (PC/Mac, tablet, smartphone).

CREATE LOG FILE

To create a log file insert a USB flash drive to the USB port of the NanoPhotometer®. Push on “Create Log File”. The log (NPOS.log) file will be saved on the root folder of the USB flash drive.

FACTORY RESET

There is an option to reset the instrument to factory settings. By selecting the reset button a window will open that says “Reset the NanoPhotometer®?” Selecting the cancel button will close the window without changing the settings and selecting reset will open a window that will ask again “Reset the NanoPhotometer® to factory settings? All data, stored methods and settings will be lost.” If it is confirmed the factory settings will be restored.

Note: All stored methods, settings and data on the NanoPhotometer® will be deleted if the reset option is executed.

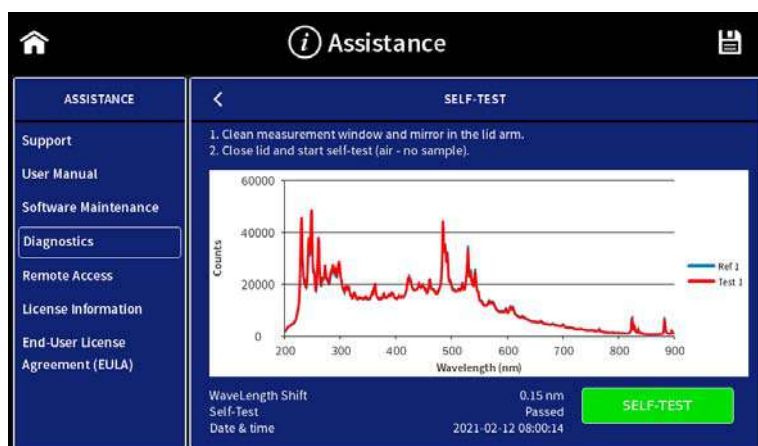
CREATE BACKUP

Option to create backup file to recover data and settings of the NanoPhotometer® at a later date. To create a backup file insert a USB flash drive to the USB port of the NanoPhotometer®. Push on “Create Backup”. The backup (NPOS.bak) file will be saved into the root folder of the USB flash drive.

Recovery of the NanoPhotometer® is only possible via the Implen support (remote access). In the case a NanoPhotometer® recovery is necessary please get in touch with the Implen support team (support@implen.de).

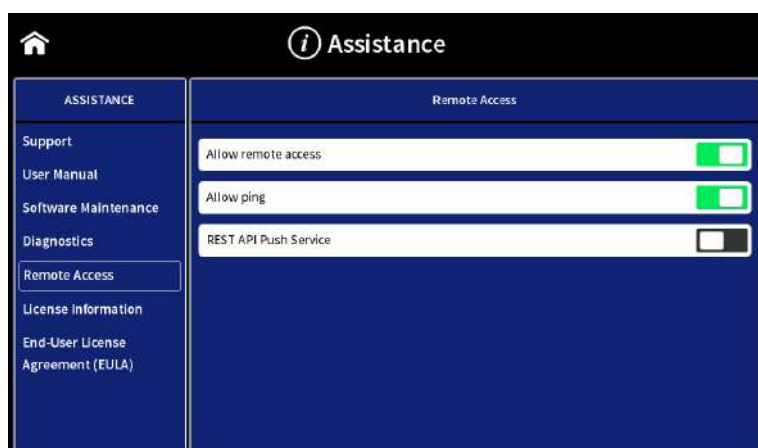
DIAGNOSTICS

Diagnostics offers the possibility of performing the self-test without rebooting the NanoPhotometer®. Make sure that the measurement head is clean and no sample is applied and push the self-test button to start the self-test. As result reference and signal counts and the wavelength shift are shown. If the self-test is fails, please contact the Implen support (support@implen.de).



REMOTE ACCESS

Option to enable remote acces, ping and Rest API Push Services.



Remote access and ping are active after booting the NanoPhotometer® and are automatically switched off after 15 minutes. A renewed activation for remote access is possible for 30 minutes at a time. The activation of ping remains active until the next restart of the NanoPhotometer.

Rest API push service might be necessary for integration to LIMS systems. It is default off and can be switched on if necessary. It will stay active after switched on.

For further information contact the Implen support team (support@implen.de).

LICENSE INFORMATION

The NPOS software is Copyright of Implen and its affiliates. The NPOS software includes some open source software components under the licenses listed.

END-USER LICENSE AGREEMENT (EULA)

Shows the end-user license agreement.

TRADEMARKS

Windows is a trademark of Microsoft. Airprint, Mac OS, OS X, iOS, iPhone and iPad are trademarks of Apple. Android OS is a trademark of Google. Linux is a trademark of Linus Torvalds.

8. MAINTENANCE

MAINTENANCE FREE TECHNOLOGY

The NanoPhotometer® technology is maintenance free. Regular maintenance and calibration is not necessary.

For facilities that are working according to national as well as international guidelines and standards including: Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), or ISO9000-9004; the proper performance of the spectrophotometer has to be tested and proven on a regular basis with individually set intervals. Implen provides certified NanoPhotometer® secondary standards as an optional accessory. These NanoPhotometer® Didymium glass filters and standard solution sets are suitable for the control and documentation of the wavelength accuracy and the photometric accuracy of your system. IQ/OQ documentation is also available. Please contact your local Implen office or an authorized Implen partner for further information.

Note: Further information on the secondary standards can be downloaded from the Implen website in the download area - quality control (www.implen.de/downloads).

Support agreements that help to fulfill the demands of regulatory guidelines concerning GLP/GMP include: calibration certification using filters traceable to international standards (during production and quality control), certified engineers and calibrated test equipment, approved to ISO 9001 standard, automatic self-diagnostic calibration test during start of the NanoPhotometer®, result is documented in each data output file, and possibility to save a Implen Document Source (IDS) file (no data manipulation possible).

REPLACEMENT PARTS

▪ Lamp Replacement

The NanoPhotometer® is equipped with a xenon flash lamp with a lifetime of 10⁹ flashes (up to 10 years). This lamp should not need replacement for several years. In the unlikely event the lamp does need to be replaced, this should be done by the manufacturer or a certified service engineer from your supplier.

▪ Battery Replacement

The optional battery pack can only be assembled or replaced by the manufacturer or a certified service engineer from your supplier.

Note: Danger of explosion if battery is incorrectly replaced. Replacement only with the same or equivalent type recommended by the manufacturer: Replacement needs to be done by a certified service engineer

Note: Battery operation time is depending on display usage. Activating the screen saver video may reduce the battery operation time.

Note: Recharging of deep discharged batteries is not possible they need to be replaced by new ones.

- **Touchscreen Replacement**

The optional touchscreen can only be assembled or replaced by the manufacturer or a certified service engineer from your supplier.

CLEANING AND GENERAL CARE

Switch off the NanoPhotometer® and disconnect the power cord prior to external cleaning. Use a soft wet cloth or dry microfiber cloth to clean all external surfaces. A mild liquid detergent may be used to remove stubborn marks.

Approved disinfectant solutions include: Apesin disinfection spray (Tana Chemi GmbH), Incidin Liquid & Inciddin Foam (Ecolab), and Lysoformin Spezial (Lysoform Dr. Hans Roseman GmbH).

Note: Observe all necessary precautions if dealing with hazardous samples or solvents.

9. WARRANTY

Implen guarantees that the product supplied has been thoroughly tested to ensure that it meets its published specification. The warranty is as defined in our current terms and conditions and is only valid if the product has been used according to the instructions supplied. Implen or your supplier can accept no liability of loss or damage arising from the faulty or incorrect use of this product