



NanoPhotometer[®] **N50-Go/C40-Go**

User Manual

Version 4.2.2

Software Version 4.2.14900



The end user of the NanoPhotometer® product (“End User”) hereby takes full responsibility for safe storage and backup of all files and/or data that may be created, saved on or transferred from the device. End User acknowledges that it is possible that data and/or files may be lost or damaged, and further acknowledges and agrees that it has sole responsibility to maintain all appropriate backup of files and data. By using the NanoPhotometer® device, End User hereby agrees to these terms, and agrees that Implen shall not be held liable for any loss, deletion or damage of any data or files for any reason, including any damages attributable thereto.

Telephone support is available using one of the following phone numbers from your geographic region:

North and South America

Phone: +1 818 748 6400

Fax: +1 818 449-0416

Email: info@implen-go.com

Website: www.implen-go.com

Implen, Inc.

Unit 104

31194 La Baya Drive

Westlake Village, CA 91362

USA

Windows and Excel are trademarks of Microsoft Corporation Redmond, WA
macOS is a trademark of Apple Inc. Cupertino, CA



Declaration of conformity for the NanoPhotometer® (C40-Go/N50-Go)

This is to certify that the Implen NanoPhotometer® conforms to the requirements of the following directives:

2014/35/EU	Low Voltage Equipment Safety Directive
2014/30/EU	Electromagnetic compatibility (EMC) directive
IEC 60529	Protection class IP20
2011/65/EU	Restrictions on the use of certain Hazardous Substances in Electrical and Electronic Equipment (ROHS)
2012/19/EU	EC Directive on Waste Electrical and Electronic Equipment (WEEE) 2003/108/EC & 2008/34/EC. By ensuring this product is disposed of correctly, you will help prevent potential negative consequences for the environment and human health, which could otherwise be caused by inappropriate waste handling of this product.
FCC 47 CFR Part15 §15.107 and §15.109	
EN 301 489-1 V1.9.2	Radio and ancillary equipment for portable use (portable equipment); EUT Operating frequency range: 2.4 – 2.4835 GHz
EN 300 328 V1.8.1	Electromagnetic compatibility and Radio spectrum Matters (ERM); Wideband transmission systems; Data transmission equipment operating in the 2,4 GHz ISM band and using wide band modulation techniques; Harmonized EN covering the essential requirements of article 3.2 of the R&TTE Directive
EN 301 489-17 V2.2.1	Electromagnetic compatibility and Radio Spectrum Matters (ERM)

Standards to which conformity is declared, where relevant, are as follows:

IEC/EN 61010-1:2012	Safety requirements for electrical equipment for measurement, control, and laboratory use. General requirements.
EN61326-1:2013	Electromagnetic compatibility- generic emission standard electrical equipment for measurement, control, and laboratory use.

Signed:



Dr. Thomas Sahiri
Managing Director
Implen GmbH

Contents

1. NANOPHOTOMETER® AT A GLANCE	8
NANOPHOTOMETER® OVERVIEW	8
NANOPHOTOMETER® N50-Go.....	9
NANOPHOTOMETER® C40-Go.....	10
INSTRUMENT REAR PANEL	11
INSTRUMENT BOTTOM VIEW	11
ACCESSORIES	12
STANDARD ACCESSORIES.....	12
OPTIONAL ACCESSORIES.....	12
CONNECTIVITY.....	15
NANOPHOTOMETER® SPECIFICATIONS	17
2. GETTING STARTED.....	19
SPECTROPHOTOMETER INSTALLATION	19
SAFETY INFORMATION	19
UNPACKING AND POSITIONING	20
SOFTWARE INSTALLATION.....	21
NPOS OVERVIEW.....	21
REQUIREMENTS AND COMPATIBILITY.....	21
INSTALLING SOFTWARE ON COMPUTER	21
INSTALLING NANOPHOTOMETER® APP ON TABLET OR SMARTPHONE.....	24
FIRST STEPS AND CONFIGURATION WIZARD	24
PRINTER INSTALLATION	24
3. NANOPHOTOMETER® BASICS.....	25
APPLICATIONS OVERVIEW	25
ICONS	27
BUTTONS.....	28
MEASUREMENT SCREENS	28
METHODS	28
DATA PROCESSING DIALOGS	30
PRINT 30	
SAVE 31	
DELETE.....	32
BASIC OPERATION.....	34
NANOVOLUME MEASUREMENT BASICS (N50-Go).....	34
CUVETTE MEASUREMENT BASICS (C40-Go).....	35
SAMPLE HANDLING TIPS.....	36
SOLVENT COMPATIBILITY (N50-Go).....	37
DATA TRANSFER.....	38
4. NANOPHOTOMETER® APPLICATIONS	39

NUCLEIC ACIDS	39
<i>METHOD OVERVIEW</i>	39
<i>MEASUREMENT PROTOCOL</i>	39
<i>CALCULATIONS</i>	42
PROTEIN UV	47
<i>METHOD OVERVIEW</i>	47
<i>MEASUREMENT PROTOCOL SINGLE SAMPLE</i>	48
<i>CALCULATIONS</i>	51
PROTEIN ASSAYS	54
<i>METHOD OVERVIEW</i>	54
<i>MEASUREMENT PROTOCOL</i>	55
<i>CALCULATIONS</i>	58
KINETICS	58
<i>METHOD OVERVIEW</i>	58
<i>MEASUREMENT PROTOCOL</i>	59
<i>CALCULATIONS</i>	60
OD600	61
<i>METHOD OVERVIEW</i>	61
<i>MEASUREMENT PROTOCOL</i>	62
<i>CALCULATIONS</i>	64
MORE APPS	65
MORE APPS: WAVELENGTH	65
<i>METHOD OVERVIEW</i>	65
<i>MEASUREMENT PROTOCOL</i>	65
<i>CALCULATIONS</i>	67
MORE APPS: WAVESCAN	68
<i>METHOD OVERVIEW</i>	68
<i>MEASUREMENT PROTOCOL</i>	68
<i>CALCULATIONS</i>	70
MORE APPS: ABSORBANCE RATIO	71
<i>METHOD OVERVIEW</i>	71
<i>MEASUREMENT PROTOCOL</i>	71
<i>CALCULATIONS</i>	73
MORE APPS: CONCENTRATION	74
<i>METHOD OVERVIEW</i>	74
<i>MEASUREMENTS PROTOCOL</i>	74
<i>CALCULATIONS</i>	77
MORE APPS: STANDARD CURVE	77
<i>METHOD OVERVIEW</i>	77
<i>MEASUREMENTS PROTOCOL</i>	77
<i>CALCULATIONS</i>	80
CUSTOM APPS	80
STORED RESULTS	81

STORED METHODS	82
5. PREFERENCES	83
GENERAL	83
DATE AND TIME	83
DISPLAY	83
ABOUT	84
STORAGE	84
ILLUMINATION SAMPLE WINDOW	84
DYES 84	
WARNING MESSAGES	86
BLANK CONTROL	86
SAMPLE QUALITY CONTROL	86
NETWORK	87
NETWORK SETTINGS	87
WLAN SETTINGS	88
FILE SERVER ACCESS	89
NETWORK FOLDER	90
PRINTER	91
NETWORK PRINTER	91
REPORT CONFIGURATION	92
6. TROUBLESHOOTING	93
SELF-CALIBRATION TEST	93
MESSAGES	93
IMPORTANT WARNING MESSAGES:	93
IMPORTANT ALERT MESSAGES:	94
SYSTEM FREEZE	96
7. ASSISTANCE	97
SUPPORT	97
REPORT PROBLEM	97
SOFTWARE MAINTENANCE	98
UPGRADE TO N50-TOUCH	98
NPOS UPDATE	98
CREATE LOG FILE	99
FACTORY RESET	99
CREATE BACKUP	99
LICENSE INFORMATION	99
END-USER LICENSE AGREEMENT (EULA)	99
TRADEMARKS	99
CONTACT IMPLEN	100
8. MAINTENANCE	101

MAINTENANCE FREE TECHNOLOGY.....	101
REPLACEMENT PARTS	101
CLEANING AND GENERAL CARE	102
9. WARRANTY.....	103
10. ALPHABETICAL APPENDIX.....	104

1. NANOPHOTOMETER® AT A GLANCE

NANOPHOTOMETER® OVERVIEW

The Implen NanoPhotometer® Go is a simple to use UV/Visible spectrophotometer with a CCD array detector with options ranging from NanoVolume to standard cuvette.

The N50-Go is a NanoVolume spectrophotometer with limited applications like Nucleic Acids and Protein UV. There is an upgrade option to full application access. The C40-Go is a standard cuvette solution.

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® N50-Go

Off Switch

7" Color Touchscreen

Illuminated Sample Window



Front USB Port

NANOPHOTOMETER® C40-Go

Off Switch

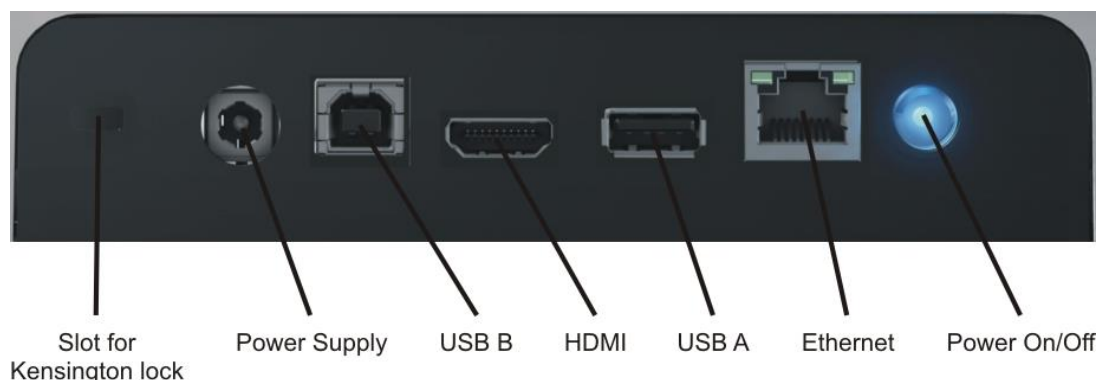
7" Color Touchscreen

Temperature Controlled
Cuvette Holder



Front USB Port

INSTRUMENT REAR PANEL



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



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 21 Software Installation).

- **NanoPhotometer® Power Adapter**



Power adapter for the NanoPhotometer®.

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-Go)**



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

▪ Standard Solution (N50-Go)



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.

▪ DiluCell™ (C40-Go)



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.

▪ 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 reader 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):

#2 dsDNA
Sample 1
6628.5 ng/ul
2017-01-30; 11:00:36

#2
Sample

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
Concentration	2015-11-27; 15-51-48
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
Dilution	2015-11-27; 15-52-18
Concentration	15.0
A230 (10 mm)	533.55
A260 (10 mm)	4.726
A280 (10 mm)	10.72
A320 (10 mm)	7.615
A260/A280	0.049
A260/A230	1.410
	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
Concentration	2015-11-27; 15-51-48
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
Dilution	2015-11-27; 15-52-18
Concentration	15.0
A230 (10 mm)	533.55
A260 (10 mm)	4.726
A280 (10 mm)	10.72
A320 (10 mm)	7.615
A260/A280	0.049
A260/A230	1.410
	2.282
Result	1 / 2
	2015-11-27 15:56:00

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.

▪ 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.

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. 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 computers within close proximity. This Ethernet connection can be used for data transfer from the NanoPhotometer® to a computer or to control the NanoPhotometer® via a Windows computer. Data can be saved actively on a defined network folder (see page 90 Network Folder) or saved on the NanoPhotometer® file server. To access the file server/directory of the NanoPhotometer® from a Windows computer via LAN enter the serial number or IP (\\serial number / \\IP) of the NanoPhotometer® in the Windows-Explorer and confirm with the enter key. For Mac OS X open the "Connect to Server" dialog in the "Go" menu of the Mac OS X Finder. Enter the instrument serial number or the active NanoPhotometer® IP address in the server address field to connect.

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 data transfer and 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 Windows computer, tablets or smartphones.

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	5 – 7,500 ng/μl
Detection Range BSA	0.15 – 217 mg/ml
Sample Volume	0.3 – 2 μl
Photometric Range (10 mm equivalent)	0.1 – 150 A
Path length	0.67 and 0.07 mm
Dilution Factor	15 and 140

Cuvette Performance

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-Go: 200 – 900 nm N50-Go: 200 – 650 nm
Measure Time For Full Scan Range	2.5 – 4.0 seconds
Wavelength Reproducibility	C40-Go: ± 0.2 nm N50-Go: ± 1 nm
Wavelength Accuracy	C40-Go: ± 0.75 nm N50-Go: ± 1.5 nm
Bandwidth	C40-Go: 1.8 nm N50-Go: 5 nm
Absorbance Reproducibility	C40-Go (cuvette): < 0.002 A @ 0 - 0.3 A @ 280 nm CV < 1% @ 0.3 – 2.0 A @ 280 nm N50-Go (Lid 15): < 0.004 A (0.67 mm path) @ 280 nm CV < 1% @ 0,3 - 1,5 A @ 280 nm
Absorbance Accuracy	< 1.75 % @ 0.7 A @ 280 nm of the reading
Stray Light	C40-Go: < 0.5% @ 240 nm using NaI N50-Go: < 2% @ 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-Go, N50-Go: 32 GB
Software Compatibility	Windows 7, 8, 10 (32 & 64 bit) OS X, 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, 50/60 Hz, 90 W, 18/19 VDC
Display	1024 x 600 pixels; touchscreen glove compatible
Certifications	CE, IEC 61010-1:2012 and EN 61326-1:2013
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

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

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

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

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. 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®.

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 101 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

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; 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 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, 50-60 Hz power supply system.

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. The software 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 7 / Windows 8 / Windows 10 (32 & 64 bit)

Mac: OS X

Tablets (minimum requirements):

iPad: iOS12.4

Android (quad core 1.2 GHz with 1 GB RAM): Android version 8.0

Smartphones (minimum requirements):

iPhone: iOS12.4

Android (quad core 1.2 GHz with 1 GB RAM): Android version 8.0

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.

INSTALLING SOFTWARE ON COMPUTER

The NanoPhotometer® software can be installed on compatible PC and Mac computer systems. Various operating systems and PC 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.

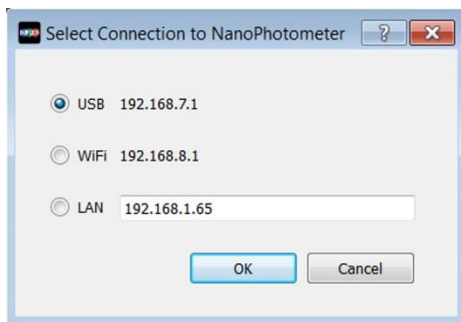
Note: If a previous version of the NPOS software is already installed on the PC, remove the USB cable and uninstall the NPOS software before installing the new software version.

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 user on Windows computer

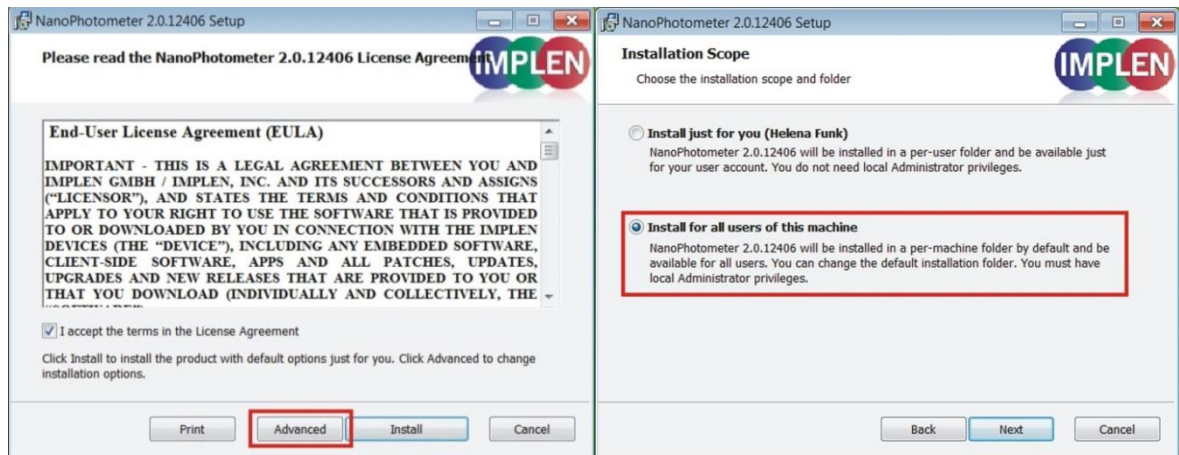
1. Check the installed version of the NanoPhotometer® firmware (Preferences/About) and update it to the latest version if necessary, before starting the installation/update of the NPOS software on your computer.
2. Start the NPOS installation file (NanoPhotometer.msi) and follow the installation routine. Full administration rights are required for the installation. If you have insufficient privileges, installation may fail. If in doubt consult your PC administrator.
3. 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.



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.

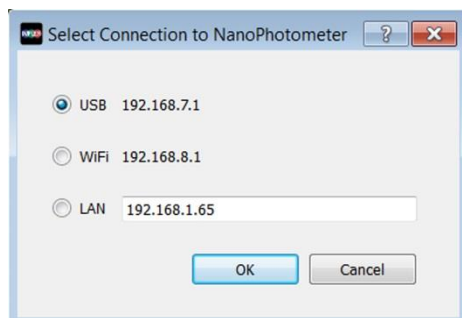
▪ NPOS installation for multi-user on Windows computer

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 (NanoPhotometer.msi) and follow the installation routine.
3. Choose on the License Agreement dialog the option “Advanced” and on the following dialog “Install for all users of this machine”



Full administration rights are required for the installation. If you have insufficient privileges, installation may fail. If in doubt consult your PC administrator.

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**: Implenuer). For a network connection, connect the NanoPhotometer® to the local network via an Ethernet cable or WiFi 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. 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 to the NanoPhotometer®.
SSID: Serial number, **password:** Implenuser
3. Open the NanoPhotometer® App
4. When connected via WiFi HotSpot 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 14 Printer)

For network printers:

1. Assure either LAN or WiFi network connection
2. Set printer IP in preferences (page 91 Network Printer)
3. Printer is available in methods for printing

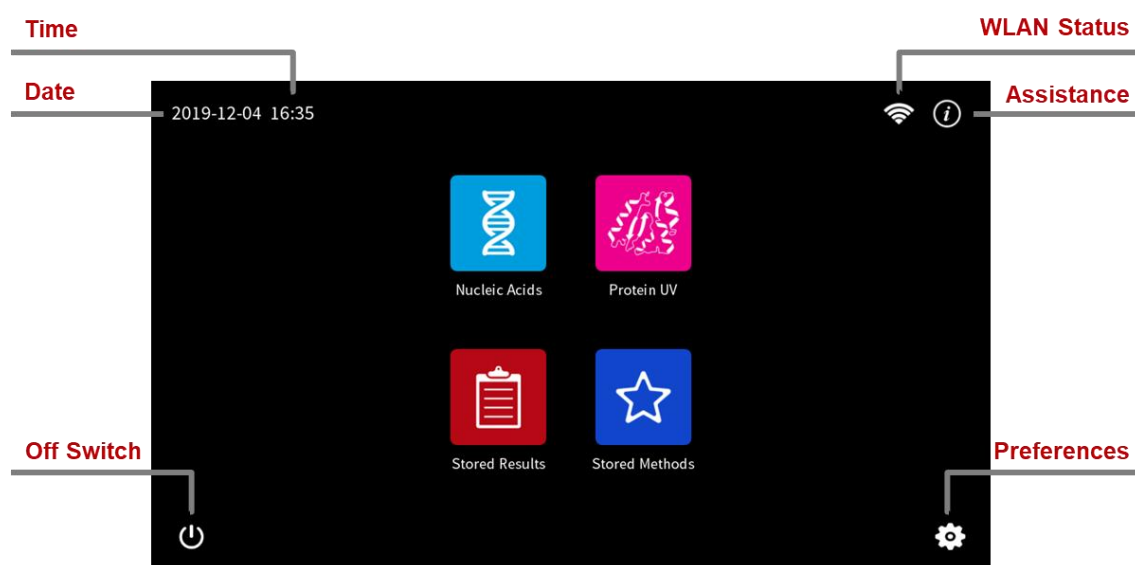
Note: Check printer compatibility (page 14 Printer)

3. NANOPHOTOMETER® BASICS

The NanoPhotometer® Go product line offers a solution for NanoVolume (N50-Go) and standard cuvette (C40-Go) applications. NanoVolume applications start with a minimum sample volume of 0.3 µl. 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.

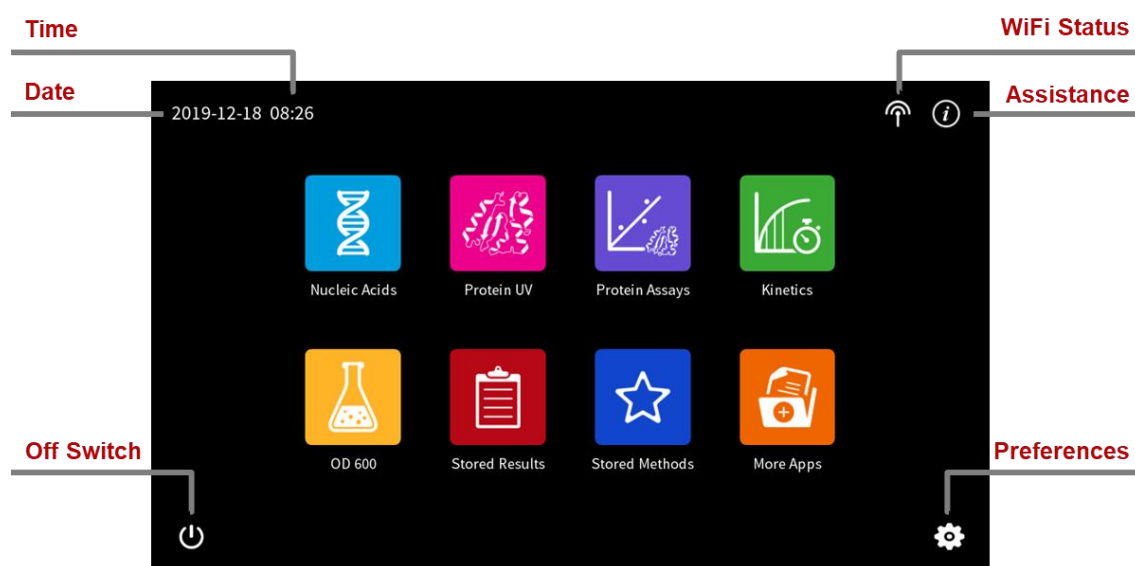
APPLICATIONS OVERVIEW

The NanoPhotometer® N50-Go comes with two pre-programmed applications, Nucleic Acids and Protein UV. To select a method, tap the corresponding icon and the method opens immediately.















There is an upgrade option for the N50-Go available. The upgraded N50-Go is a N50 Touch with access to all applications.

Homescreen C40-Go:



The following methods are available for the upgraded N50-Go and the C40-Go.

*not available for N50-Go

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) 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-Go: 200 – 650 nm) for absorbance measurements
 Wavescan*	Define desired full scan range anywhere between 200 - 900 nm (N50-Go: 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
	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 the 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
	Parameter	Opens parameter window
	Results	Opens result window
	Graph	Opens graph result window
	Table	Open/shows results in table format
	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 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.



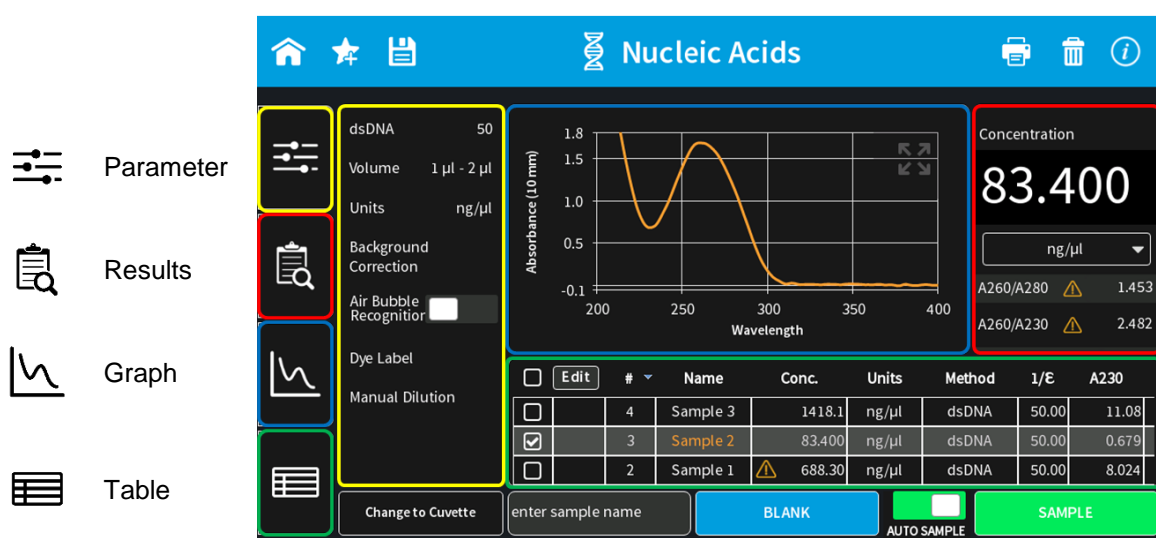
The auto sample button is disabled by default. When switched on sample measurements are started automatically as soon as the lid arm is closed. Auto sample function is only available for sample and not for blank measurements.

MEASUREMENT SCREENS

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 default measurement screen includes the parameter area as open. 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® 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 show results of further graphs by changing the sample in the sample name dropdown.

Sample Name	L10
Wavelength	260
Absorbance	0.207

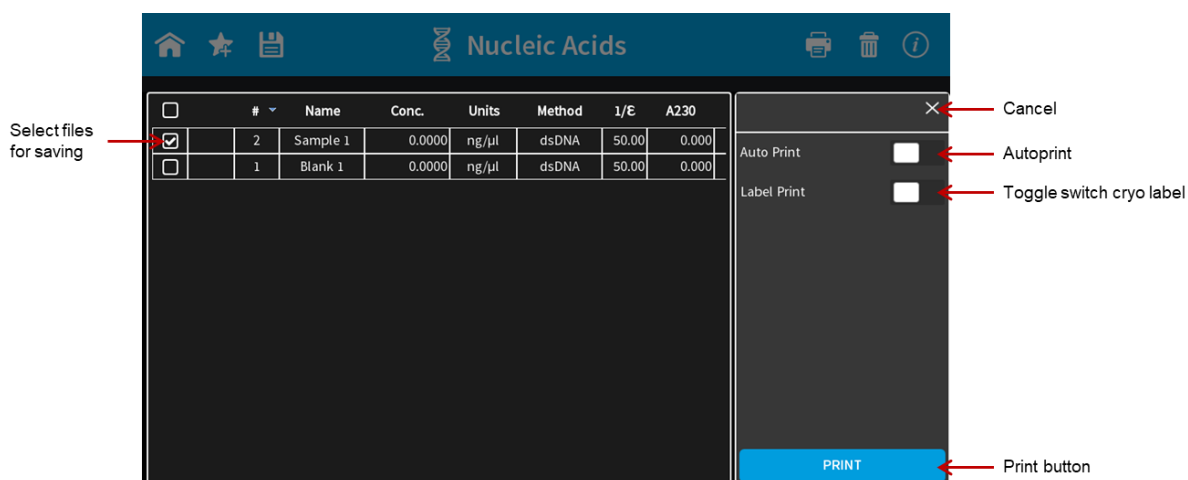
DATA PROCESSING DIALOGS

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 will always print to a DYMO or HP printer, which is directly connected to the NanoPhotometer® via USB cable. If no USB printer is available the print command will print to a network printer configured in the preferences of the NanoPhotometer®. All ticked samples will be 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 installed 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 of the NanoPhotometer®.

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.

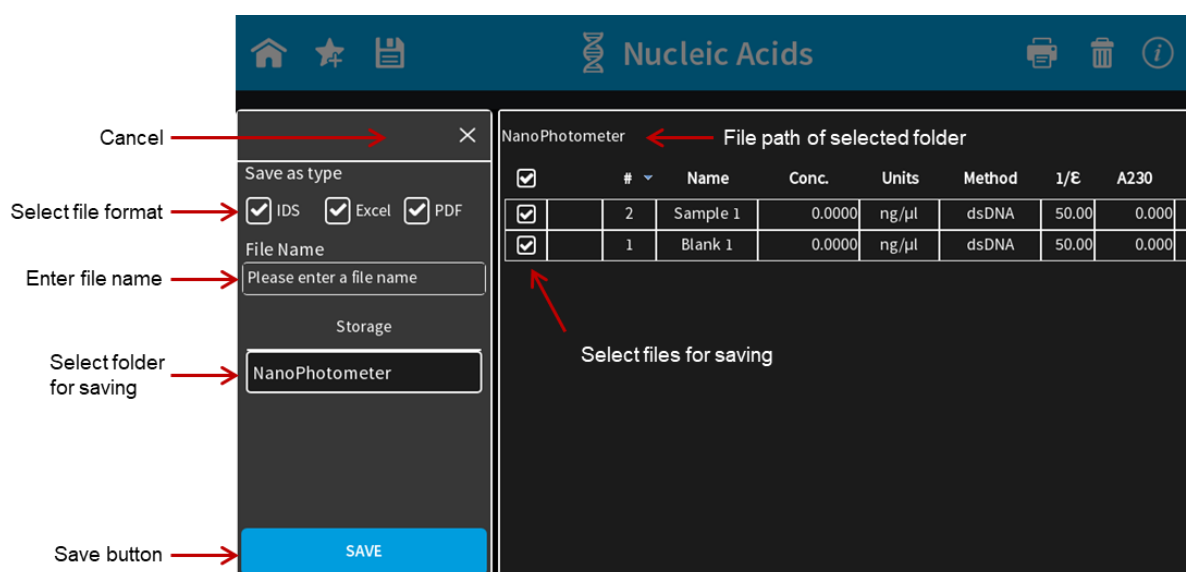
Note: The auto print function is not available for printing via control device (local computer/network printer).

▪ 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).

SAVE

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



As 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 can only be saved on the NanoPhotometer® and 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 do data backups on a regularly basis to a hard drive of a computer or network. In the rarely case that the micro SD card crashes data loss cannot be ruled out.

Implen Document Source File

Implen has a custom file format referred to as an Implén Document Source (IDS) file. This is a hardcopy file to which no changes can be made. This file type contains all information including raw data, results, values, and parameters. IDS files can only be opened with the Implén software.

Excel File

Measurement data can be saved as Excel file. This file format is compatible with Microsoft Excel software versions 2007/2010/2013 and can be edited. This file type contains all information including raw data, results, and parameters. The saved file contains only the selected samples at the time the file was saved.

PDF File

Measurement data can be saved as PDF file. The saved file contains only the selected samples at the time the file was saved. It is possible to configure the table columns in preferences (page 92 Report Configuration).

▪ 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 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® right after each reading. These backup copies can be found in the Autosave folder of the NanoPhotometer® (Stored Results/NanoPhotometer/Autosave) for up to eight days. Files contain the base name Backup, the method name, and a time/date stamp.

After eight days the autosave files are automatically moved to an autosave archive folder. The autosave archive folder can only be accessed via the file server. Data in the autosave archive folder are not deleted automatically.

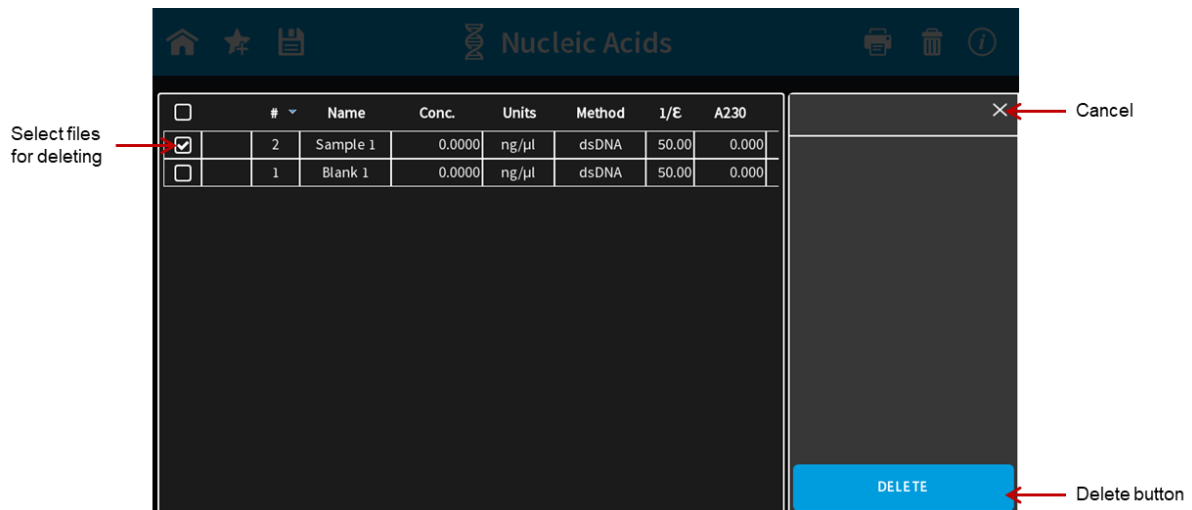
DELETE

Selecting the delete icon () opens a full screen overlay dialog window to delete data. 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 and confirming with delete, deletes the selected data.

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

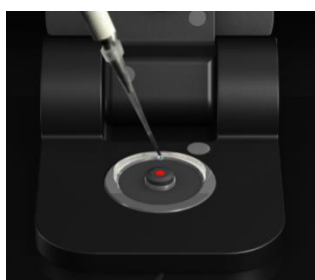


BASIC OPERATION

The NanoPhotometer® Go product line offers a complete solution for NanoVolume (N50-Go) and standard cuvette (C40-Go) applications. NanoVolume applications start with a minimum sample volume of 0.3 µl. 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.

NANO VOLUME MEASUREMENT BASICS (N50-Go)

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. 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

4. Lower the lid arm and initiate a blank measurement with the blank button
5. 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 37 solvent compatibility). If unsure please contact support@implen.de for detailed information about your specific reagent/solvent.

6. 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.

7. 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 µl adjusts the path length automatically. The parameter setting Volume 0.3 µl measures only the 0.07 mm path length for higher concentrations (dsDNA > 420 ng/µl / 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.

CUVETTE MEASUREMENT BASICS (C40-Go)

The NanoPhotometer® is compatible with standard cuvettes having an 8.5 mm center height. The light path is indicated with two white arrows.



1. Select a method depending on your sample and set the parameters for the measurement.
2. Add a blank solution to a cuvette and ensure that the filling volume is sufficient to allow light path to pass through the solution.
3. Insert cuvette into the cell holder.
4. Initiate a blank measurement with the blank button. Upon completion of measurement remove the cuvette.
5. Add sample to a cuvette and ensure that the sample volume is sufficient to allow light to pass through the sample.
6. 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.
7. Initiate a sample measurement with the sample button. Upon completion of measurement remove the cuvette.
8. Apply further samples.

SAMPLE HANDLING TIPS

▪ NanoVolume Methods (N50-Go)



- The sample window on pedestal is illuminated (N50-Go) 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 (N50-Go dsDNA > 420 ng/ μl and BSA > 12.6 mg/ml). For automatic path length setting at least 1 μl is needed.
- The maximum volume that can be used for NanoVolume samples is 2.0 μl (N50-Go).
- 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.

▪ Cuvette Methods (C40-Go)



- The cuvette holder is compatible with standard 10 mm path length quartz, glass and plastic cuvettes with an center 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-Go)

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-Go at room temperature:

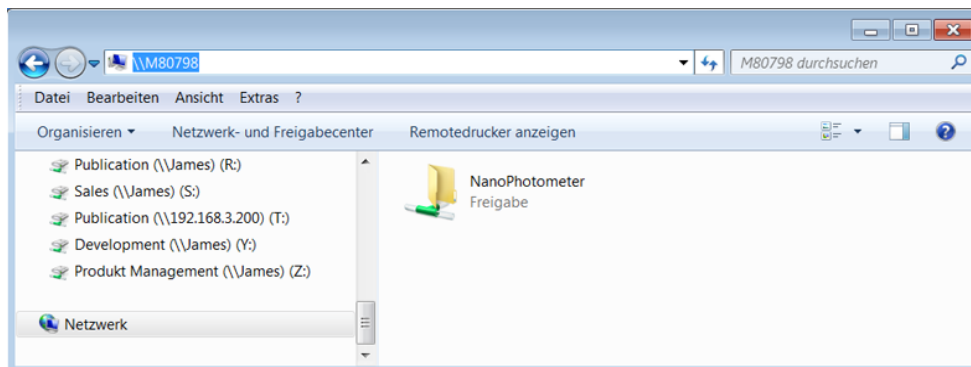
- Acetone ($\leq 5\%$)
- Acetonitrile
- Benzene
- Butanol
- Carbon tetrachloride
- Chloroform
- Ethanol
- Ether
- HEPES
- Hexane
- Isopropanol
- MES
- Methanol
- Methylene chloride
- MOPS
- Phenol ($\leq 1\%$)
- N-propanol
- Toluene
- Phosphate containing buffers
- PBS (pH 4-10)
- Citrate
- Borate
- 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

All data saved on the NanoPhotometer® can easily be accessed from and transferred to a computer within the lab through LAN, USB cable or a password secured WiFi connection.

To access/transfer data from the NanoPhotometer® network drive via LAN connection, the serial number or the NanoPhotometer® IP needs to be entered in the address bar of the Windows Explorer (e.g. \\M80798\ or \\Assigned IP Address\).

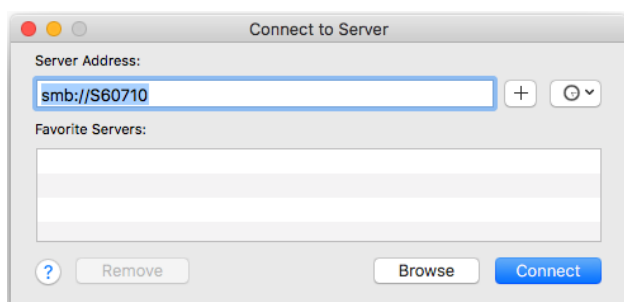


Using a USB cable connection \\192.168.7.1\ will provide access and for password secured WiFi connections \\192.168.8.1\ will need to be entered in the address bar to show the NanoPhotometer® drive within Windows File Explorer.

For Mac OS X open the "Connect to Server" dialog in the "Go" menu of the Mac OS X Finder.



For LAN connection enter the instrument serial number or the active NanoPhotometer® IP address in the server address field to connect. For USB cable or WiFi connection use 192.168.7.1 or 192.168.8.1, respectively.



4. NANOPHOTOMETER® APPLICATIONS


The NanoPhotometer® Go comes with pre-programmed applications. For the N50-Go are only the Nucleic Acid und Protein UV method available. There is an N50 upgrade option available for full applicational access. Any application method can be selected by tapping the icon once or clicking on the icon (computer based software).



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 click on the alert icon shows additional information about the inconstancy.

MEASUREMENT PROTOCOL

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



2. To change the nucleic acid type click 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 43).

- 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.

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

3. For NanoVolume application (N50-Go):

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 (C40-Go):

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

4. Select the Units in which the concentration should be calculated. Options are ng/µl, µg/µl and µg/ml.

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

5. If it is desired to heat the sample to 37°C use the toggle switch to turn on

Heat To 37°C	<input type="checkbox"/>
--------------	--------------------------

cell holder heater. When the cuvette holder has reached 37°C the toggle color changes to green.

Note: Only available for cuvette applications (C40-Go).

- The background correction is enabled at 320 nm as a 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

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	320 nm

- Air bubble recognition is disabled as a 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.

Air Bubble Recognition	<input type="checkbox"/>
------------------------	--------------------------

- 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.

dsDNA	50	Dye Correction	<input checked="" type="checkbox"/>
Pathlength	10 mm	<input type="checkbox"/> AlexaFluor350	<input type="checkbox"/> AlexaFluor647
Heat To 37°C	<input checked="" type="checkbox"/>	<input type="checkbox"/> AlexaFluor488	<input type="checkbox"/> AlexaFluor660
Units	ng/µl	<input type="checkbox"/> AlexaFluor532	<input type="checkbox"/> AlexaFluor680
Background Correction	<input checked="" type="checkbox"/>	<input type="checkbox"/> AlexaFluor546	<input type="checkbox"/> Cy3
Dye Label		<input type="checkbox"/> AlexaFluor555	<input type="checkbox"/> Cy3_5
Manual Dilution		<input type="checkbox"/> AlexaFluor568	<input type="checkbox"/> Cy5
		<input type="checkbox"/> AlexaFluor594	<input type="checkbox"/> Cy5_5
		<input type="checkbox"/> Oyster500	<input type="checkbox"/> Oyster550
		<input type="checkbox"/> Oyster556	<input type="checkbox"/> Oyster645
		<input type="checkbox"/> Oyster650	<input type="checkbox"/> Oyster656
		<input type="checkbox"/> TexasRed	

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

dsDNA	50	Dilution Factor	1
Pathlength	10 mm	Calculate Dilution Factor	<input type="checkbox"/>
Heat To 37°C	<input type="checkbox"/>		
Units	ng/μl		
Background Correction	<input type="checkbox"/>		
Dye Label			
Manual Dilution			

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.

BLANK

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.

SAMPLE

CALCULATIONS

▪ Nucleic Acid Concentration

To determine the concentration of nucleic acids in solution, the absorbance as measured at the wavelength of 260 nm is used. 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 option.

Without background correction:

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

With background correction:

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

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
ϵ_{nuc}	Extinction coefficient/nucleic acid factor in ng*cm/ μl

Table 1. Nucleic acids extinction coefficients (ϵ_{nuc})

Type	ϵ_{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 45). The concentration of a dye-labeled nucleic acid is calculated with or without background/dye correction as follows:

With background and with dye correction:

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

With background and without dye correction:

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

Without background and with dye correction:

$$C = [A_{260} - cf_{\text{dye}} * A_{\text{max, dye}}] * \epsilon_{\text{nuc}} * \text{D}$$

Without background and without dye correction:

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

C	Concentration in ng/ μl
A_{260}	Absorbance at 260 nm (10 mm path)
A_{BKG}	Absorbance at selected background wavelength (10 mm path)
$A_{\text{max, dye}}$	Absorbance value at the absorbance maximum of the dye (10 mm path)
ϵ_{nuc}	Extinction coefficient/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 45). The dye concentration is calculated with or without background correction as follows:

With background correction:

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

Without background correction:

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

C	Concentration in ng/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)
ε _{dye}	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:

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

With background correction without dye correction:

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

Without background correction and with dye correction:

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

Without background correction and without dye correction:


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

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, dye}	Absorbance value at the absorbance maximum of the dye (10 mm path)
ε _{dye}	Extinction coefficient of dye in M ⁻¹ *cm ⁻¹
ε _{nuc}	Extinction coefficient/nucleic acid factor in ng*cm/μl
cf _{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)	Dye-dependent ext. coeff. ε _{dye} in M ⁻¹ *cm ⁻¹	Dye-dependent correction factor at 260 nm cf _{dye}
N50-Go/C40-Go	Alexa Fluor 350	345	18,400	0.25
N50-Go/C40-Go	Alexa Fluor 488	492	62,000	0.30
N50-Go/C40-Go	Alexa Fluor 532	525	82,300	0.24
N50-Go/C40-Go	Alexa Fluor 546	555	104,000	0.21
N50-Go/C40-Go	Alexa Fluor 555	555	150,000	0.04
N50-Go/C40-Go	Alexa Fluor 568	576	93,000	0.45
N50-Go/C40-Go	Alexa Fluor 594	588	80,400	0.43
C40-Go	Alexa Fluor 647	650	239,000	0.00
C40-Go	Alexa Fluor 660	660	107,000	0.00
C40-Go	Alexa Fluor 680	680	164,000	0.00
N50-Go/C40-Go	Cy3	550	150,000	0.08
N50-Go/C40-Go	Cy3.5	581	150,000	0.08
C40-Go	Cy5	649	250,000	0.05
C40-Go	Cy5.5	675	250,000	0.05
N50-Go/C40-Go	Oyster-500	503	78,000	0.29
N50-Go/C40-Go	Oyster-550	553	150,000	0.05
N50-Go/C40-Go	Oyster-556	560	155,000	0.03
C40-Go	Oyster-645	649	220,000	0.05
C40-Go	Oyster-650	653	200,000	0.04
C40-Go	Oyster-656	660	200,000	0.04
N50-Go/C40-Go	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 click 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 51). 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 Mol. Ext. Coefficient or Ext. Coefficient protein option. For correct calculation, it is necessary to supply either: a) the mass extinction coefficient (ϵ in $\text{l/g}\cdot\text{cm}$); or b) the molar extinction coefficient (ϵ_M in $\text{M}^{-1}\cdot\text{cm}^{-1}$), and the molecular weight of the protein expressed in molar mass units (g/mol).

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 51). 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 click 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 the protein type click 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, Molar Extinction Coefficient, and Extinction Coefficient.

- For Mol. Ext. Coefficient enter Molecular Weight (default 66,400 g/mol) and Mol. Ext. Coefficient (default 44,289 M⁻¹*cm⁻¹)
- For Ext. Coefficient enter Ext. Coefficient (default 0.69 l/g*cm)

BSA	1.5		
Volume	1 µl - 2 µl	SA Mouse	1.49
Wavelength	280 nm	IgG Mouse	0.71
Units	mg/ml	IgE Human	0.65
Background Correction	<input checked="" type="checkbox"/>	Lysozyme	0.38
Air Bubble Recognition	<input type="checkbox"/>	OD1	1
Dye Label		Mol. Ext. Coeff.	Please enter MW (g/mol)
Manual Dilution		Ext. Coeff.	Please enter Ext. Coeff. (l/g*cm)

3. **For NanoVolume application (N50-Go):**

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 change; 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 (C40-Go):

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

4. The background correction is enabled at 320 nm as a 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

BSA	1.5	Background Correction	<input checked="" type="checkbox"/>
Volume	1 µl - 2 µl	Background Correction at 320 nm	
Wavelength	280 nm	Background Correction at 340 nm	
Units	mg/ml	Background Correction at	320 nm
Background Correction			

5. Air bubble recognition is disabled as a 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.

Air Bubble Recognition	<input type="checkbox"/>
------------------------	--------------------------

6. 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 (C40-Go).

Heat To 37°C	<input type="checkbox"/>
--------------	--------------------------

7. 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.

BSA	1.5	Dye Correction <input checked="" type="checkbox"/>			
Pathlength	10 mm	<input type="checkbox"/> AlexaFluor350	<input type="checkbox"/> AlexaFluor594	<input type="checkbox"/> dfa	<input type="checkbox"/> pHrodoRed
Heat To 37°C	<input type="checkbox"/>	<input type="checkbox"/> AlexaFluor405	<input type="checkbox"/> AlexaFluor647	<input type="checkbox"/> DyLight488	<input type="checkbox"/> rPE
Wavelength	280 nm	<input type="checkbox"/> AlexaFluor488	<input type="checkbox"/> AlexaFluor680	<input type="checkbox"/> DyLight649	<input type="checkbox"/> TexasRed
Units	mg/ml	<input type="checkbox"/> AlexaFluor532	<input type="checkbox"/> AlexaFluor790	<input type="checkbox"/> FITC	<input type="checkbox"/> tret
Background Correction	<input checked="" type="checkbox"/>	<input type="checkbox"/> AlexaFluor546	<input type="checkbox"/> asdf	<input type="checkbox"/> PacificBlue	
Dye Label		<input type="checkbox"/> AlexaFluor555	<input type="checkbox"/> Cy3	<input type="checkbox"/> PacificOrange	
Manual Dilution		<input type="checkbox"/> AlexaFluor568	<input type="checkbox"/> Cy5	<input type="checkbox"/> pHrodoGreen	

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

BSA	1.5	Dilution Factor	1
Pathlength	10 mm	Calculate Dilution Factor	<input type="checkbox"/>
Heat To 37°C	<input type="checkbox"/>		
Wavelength	280 nm		
Units	mg/ml		
Background Correction	<input checked="" type="checkbox"/>		
Dye Label			
Manual Dilution			

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 the preferences.

BLANK

10. 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.



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

SAMPLE

CALCULATIONS

■ Protein UV280 Concentration

The protein concentration in the Protein UV method is calculated with the absorbance value of the sample at 280 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:

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

Without background correction:

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

C	Concentration (mg/ml)
A ₂₈₀	Absorbance at 280 nm (10 mm path)
A _{BKG}	Absorbance at selected background wavelength (10 mm path)
ε _{prot}	Extinction coefficient/protein factor in g*cm/l
D	Dilution factor

Table 3. Protein extinction coefficients (ε_{prot})

Type	ε _{prot} [g*cm/l]	Ext. Coeff. [l/g*cm]	Mol. Ext. Coeff. [M ⁻¹ *cm ⁻¹]	MW [g/mol]
BSA	1.50	0.667	44,289	66,400
SA Mouse	1.49	0.670	44,220	66,000
SA Human	1.72	0.582	40,370	69,365
IgG Mouse	0.71	1.400	224,000	160,000
IgG Human	0.74	1.360	204,000	150,000
IgE Human	0.65	1.530	290,700	190,000
Lysozyme	0.38	2.640	37,984	14,388
OD1	1.00	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 53). The dye concentration is calculated with or without background/dye correction for are as follows:

With background and with dye correction:

$$C = [A_{280} - A_{BKG} - cf_{\text{dye}} * (A_{\text{max, dye}} - A_{BKG})] * \epsilon_{\text{prot}} * D$$

With background and without dye correction:

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

Without background and with dye correction:

$$C = (A_{280} - cf_{\text{dye}} * A_{\text{max, dye}}) * \epsilon_{\text{prot}} * D$$

Without background and without dye correction:

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

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)
ε _{prot}	Extinction coefficient/protein factor in g*cm/l
cf _{dye}	Dye-dependent correction factor at 280 nm
D	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 53). The dye concentration is calculated with or without background correction for are as follows:

With background correction:

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

Without background correction:

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

C	Concentration
A _{max, dye}	Absorbance at the max dye absorbance value (10 mm path)
A _{BKG}	Absorbance at selected background wavelength (10 mm path)
ε _{dye}	Extinction coefficient of dye in M ⁻¹ *cm ⁻¹
D	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:

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

With background and w/o dye correction:

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

W/o background and with dye correction:

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

W/o background and w/o dye correction:

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


DOL	Degree of labeling/dye per protein ratio
$A_{\text{max, dye}}$	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)
ϵ_{dye}	Extinction coefficient of dye in $\text{M}^{-1} * \text{cm}^{-1}$
ϵ_{prot}	Extinction coefficient/protein factor in $\text{g} * \text{cm} / \text{l}$
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)	Dye-dependent ext. coeff. ϵ_{dye} in $\text{M}^{-1} * \text{cm}^{-1}$	Dye-dependent correction factor at 280 nm cf_{dye}
N50-Go/C40-Go	Alexa Fluor 350	346	19,000	0.19
N50-Go/C40-Go	Alexa Fluor 405	401	34,500	0.70
N50-Go/C40-Go	Alexa Fluor 488	494	71,000	0.11
N50-Go/C40-Go	Alexa Fluor 532	530	81,000	0.09
N50-Go/C40-Go	Alexa Fluor 546	554	104,000	0.12
N50-Go/C40-Go	Alexa Fluor 555	555	150,000	0.08
N50-Go/C40-Go	Alexa Fluor 568	577	91,300	0.46
N50-Go/C40-Go	Alexa Fluor 594	590	73,000	0.56
C40-Go	Alexa Fluor 647	650	239,000	0.03
C40-Go	Alexa Fluor 680	679	184,000	0.05
C40-Go	Alexa Fluor 790	785	260,000	0.08
N50-Go/C40-Go	Cy3	550	150,000	0.05
C40-Go	Cy5	649	250,000	0.05
C40-Go	DyLight 649	654	250,000	0.04
N50-Go/C40-Go	DyLight 488	493	70,000	0.15
N50-Go/C40-Go	FITC	495	68,000	0.30
N50-Go/C40-Go	Pacific Blue	409	30,000	0.20
N50-Go/C40-Go	Pacific Orange	397	24,500	0.60

N50-Go/C40-Go	pHrodo Green	505	75,000	0.20
N50-Go/C40-Go	pHrodo Red	560	65,000	0.12
N50-Go/C40-Go	r-PE	566	200,000	0.18
N50-Go/C40-Go	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 click 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

Available only for C40-Go, N50-Go needs upgrade for activation of 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.

▪ 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

1. Select protein assays icon from home screen.



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

Options are: BCA Assay, Biuret Assay, 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

3. **For Cuvette Application (C40-Go):**

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

For NanoVolume Application (only with N50-Go Upgrade):

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.

4. 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 (C40-Go).

5. The baseline correction is disabled as default. It is possible to choose between different wavelengths for the baseline correction.

Options are: 377 nm, 604 nm, 650 nm, 770 nm and 823 nm Option to enter any wavelength between 200 nm and 900 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 750 nm

6. 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
CurveFit	

7. Select Unit

Bradford		mg/ml	mg/l
Dilution	15	µg/ml	U/l
Baseline Correction		µg/µl	%
Curve Fit		µg/l	ppm
Units	mg/ml	pmol/µl	conc.
Concentration		mmol/l	ng/µl
Replicates	None	µmol/l	
		g/l	

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

Bradford		Add Concentration +		Conc. 1	0	Conc. 2	0
Dilution	15						
Baseline Correction							
Curve Fit							
Units	mg/ml						
Concentration							

9. Select Replicates none, 2 or 3

Bradford		None
Dilution	15	2
Baseline Correction		3
Curve Fit		
Units	mg/ml	
Concentration		
Replicates	None	

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 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.



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

Available only for C40-Go, N50-Go needs upgrade for activation of 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. **For Cuvette Application (C40-Go):**

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
Wavelength	340 nm	Pathlength	2 mm
Time settings		Pathlength	5 mm
		Pathlength	10 mm

For NanoVolume Application (only with N50-Go Upgrade):

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).

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



Note: Only available for cuvette applications (C40-Go).

4. 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.



5. 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.

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

BLANK

- 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.

SAMPLE

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

Available only for C40-Go, N50-Go needs upgrade for activation of 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 62). 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.

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 default. 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.

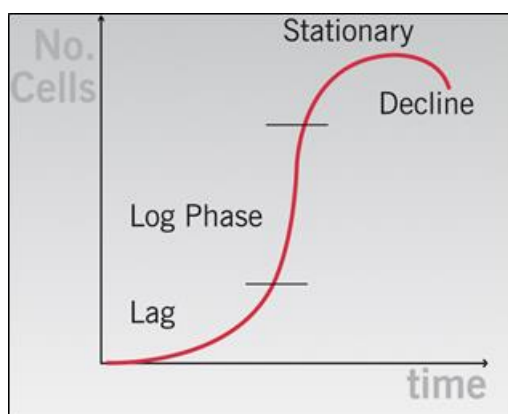


Figure 1 Bacterial growth curve

MEASUREMENT PROTOCOL

1. Select the OD600 icon on the home screen



2. For **Cuvette Application (C40-Go)**:

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
Wavelength	600 nm	Pathlength	2 mm
cells/ml	Off	Pathlength	5 mm
		Pathlength	10 mm

For **NanoVolume Application (only with N50-Go Upgrade)**:

Select the dilution depending on the sample concentration

Dilution	15	Dilution 15 / 0.67 mm path	1 - 2 µl sample volume
Wavelength	600 nm	Dilution 140 / 0.07 mm path	0.3 - 2 µl sample volume

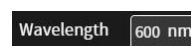
Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm)

3. 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 (C40-Go).

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



application.

5. Toggle switch cells/ml is disabled as default. Enable cells/ml to get the cells/ml calculated. Enter the cell specific factor and multiplier (e.g. 1 OD600 = 5×10^8 cells/ml)

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

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

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

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

Pathlength	10 mm	Smoothing	<input checked="" type="checkbox"/>
Heat To 37°C	<input type="checkbox"/>	Smoothing 1	
Wavelength	<input type="text" value="600 nm"/>	Smoothing 2	
cells/ml	On	Smoothing 3	
Correction			
Smoothing			

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

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

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

BLANK

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

SAMPLE

CALCULATIONS

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

OD ₆₀₀	Optical density at 600 nm
A ₆₀₀	Absorbance at 600 nm (10 mm path)
D	Dilution factor
cf	Correction factor for spectrophotometer

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

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



MORE APPS

Available only for C40-Go, N50-Go needs upgrade for activation of 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® Go. The applications featured in this menu include: wavelength, concentration, wavescan, absorbance/ratio, standard curve and custom applications.




MORE APPS: WAVELENGTH

Available only for C40-Go, N50-Go needs upgrade for activation of 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.

MEASUREMENT PROTOCOL

1. Select the More Apps icon  from the home screen and the Wavelength icon from the More Apps screen.
2. **For cuvette application (C40-Go):**
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 10 mm	Pathlength	1 mm
Heat To 37°C <input type="checkbox"/>	Pathlength	2 mm
Wavelength	Pathlength	5 mm
	Pathlength	10 mm

For NanoVolume application (only with N50-Go Upgrade):

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

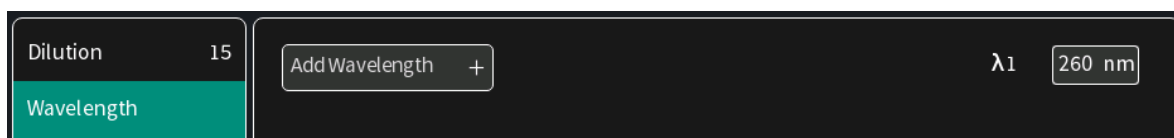
Note: There is no automatic path length setting in this method. Select either a virtual dilution of 15 (path length 0.67 mm) or of 140 (path length 0.07 mm).

3. 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.

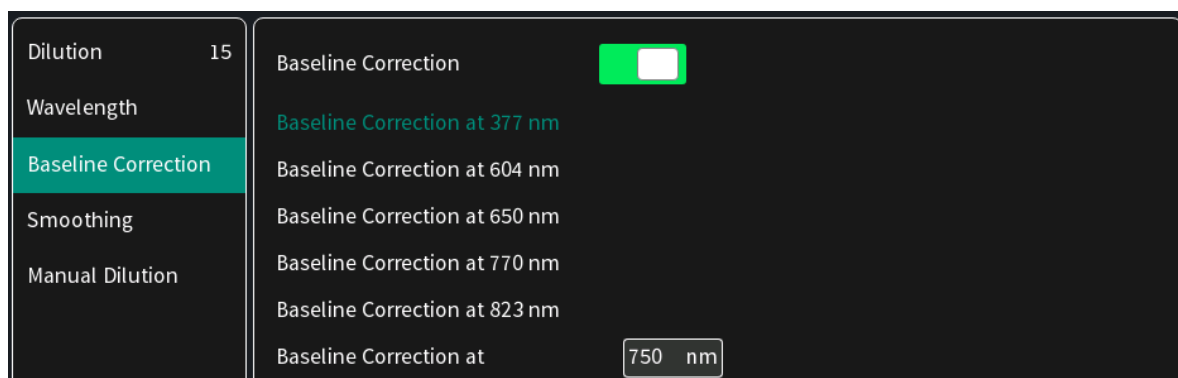


Note: Only available for cuvette applications (C40-Go).

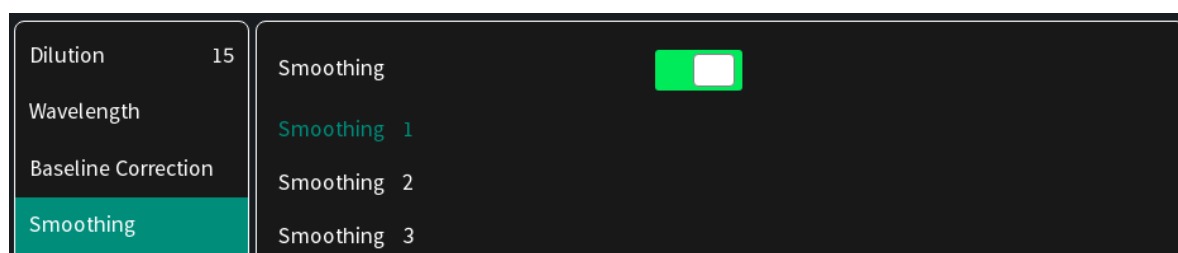
4. Enter desired wavelength (λ) to be measured. It is possible to measure up to 20 wavelengths simultaneously. More wavelength (λ) options can be added by selecting the Add Wavelength button. Added wavelength can be deleted with ⊗



5. Baseline correction is set to off as 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 (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 set/calculate a dilution factor for manual diluted samples.

Dilution	15	Dilution Factor	<input type="text" value="1"/>
Wavelength		Calculate Dilution Factor	<input type="checkbox"/>
Baseline Correction			
Smoothing			
Manual Dilution			

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

BLANK

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

9. 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.



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

SAMPLE

CALCULATIONS

▪ 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):

$$T = \frac{I}{I_0}$$

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




MORE APPS: WAVESCAN

Available only for C40-Go, N50-Go needs upgrade for activation of 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 (C40-Go) or from 200-650 nm (N50-Go/Upgrade necessary).

MEASUREMENT PROTOCOL

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



2. **For cuvette application (C40-Go):**

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

For NanoVolume application (only with N50-Go Upgrade):

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

3. 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 (C40-Go).

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

Dilution	15	Start Wavelength (nm)	200 nm
Wavelength Range		End Wavelength (nm)	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-Go: 200–650 nm).

5. Baseline correction is set to off as 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 (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	750 nm

6. 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	

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

Dilution	15	Dilution Factor	1
Wavelength Range		Calculate Dilution Factor	<input type="checkbox"/>
Baseline Correction			
Smoothing			
Manual Dilution			

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

BLANK

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

preferences.

9. 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.



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



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 clicking on the graph. The peak can then be added to the results by tapping on the Add Peak button in the pop-up window.

Add peak	
Sample Name	Sample22
Wavelength	259
Absorbance	0.771




MORE APPS: ABSORBANCE RATIO

Available only for C40-Go, N50-Go needs upgrade for activation of 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. **For cuvette application (C40-Go):**

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

For NanoVolume application (only with N50-Touch Upgrade):

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).

3. 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 (C40-Go).

4. 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 \otimes .

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

5. Baseline correction is set to off as 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 (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

6. 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	

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

Dilution	15	Dilution Factor	1
Ratio		Calculate Dilution Factor	<input type="checkbox"/>
Baseline Correction			
Smoothing			
Manual Dilution			

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

BLANK

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

9. 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.



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



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

$\lambda_1 =$ Absorbance 1 corresponding absorbance value 1 selected normalized to 10 mm path

$\lambda_2 =$ Absorbance 2 corresponding absorbance value 2 selected normalized to 10 mm path




MORE APPS: CONCENTRATION

Available only for C40-Go, N50-Go needs upgrade for activation of 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.

MEASUREMENTS PROTOCOL

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



2. **For cuvette application (C40-Go):**

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
Wavelength	260 nm	Pathlength	2 mm
Factor / Ext. Coeff.		Pathlength	5 mm
		Pathlength	10 mm

For NanoVolume application (only with N50-Go Upgrade):

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).

3. 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 (C40-Go).

4. 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.



5. Enter a factor for concentration calculation.

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

6. 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	

7. Baseline correction is set to off as 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 (N50: 650 nm)

Dilution	15	Baseline Correction	<input checked="" type="checkbox"/>
Wavelength	260 nm	Baseline Correction at 377 nm	
Factor / Ext. Coeff.		Baseline Correction at 604 nm	
Units	mg/ml	Baseline Correction at 650 nm	
Baseline Correction		Baseline Correction at 770 nm	
Smoothing		Baseline Correction at 823 nm	
		Baseline Correction at	750 nm

8. 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	260 nm	Smoothing 1	
Factor / Ext. Coeff.		Smoothing 2	
Units	mg/ml	Smoothing 3	
Baseline Correction			
Smoothing			

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

Dilution	15	Dilution Factor	1
Wavelength	260 nm	Calculate Dilution Factor	<input type="checkbox"/>
Factor / Ext. Coeff.			
Units	mg/ml		
Baseline Correction			
Smoothing			
Manual Dilution			

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.



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

Available only for C40-Go, N50-Go needs upgrade for activation of 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.

MEASUREMENTS PROTOCOL

1. Select the More Apps icon  from the home screen and the Standard Curve icon from the More Apps screen.



2. **For cuvette application (C40-Go):**

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

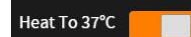
3. **For NanoVolume application (only with N50-Go upgrade):**

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).

4. 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 (and C40-Go).

5. Baseline correction is set to off as 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 (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

6. 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		

7. Select Unit

Dilution	15	mg/ml	mg/l
Wavelength	260 nm	µg/ml	U/l
Baseline Correction		µg/µl	%
Curve Fit		µg/l	ppm
Units	mg/ml	pmol/µl	conc.
Concentration		mmol/l	ng/µl
Replicates	None	µmol/l	
		g/l	

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

Dilution	15	Add Concentration +		Conc. 1	0	Conc. 2	0
Wavelength	260 nm						
Baseline Correction							
Curve Fit							
Units	mg/ml						
Concentration							

- Select Replicates none, 2 or 3

Dilution	15	None
Wavelength	260 nm	2
Baseline Correction		3
Curve Fit		
Units	mg/ml	
Concentration		
Replicates	None	

- 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.

BLANK

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

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

A green rectangular button with the word "SAMPLE" in white capital letters.

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.



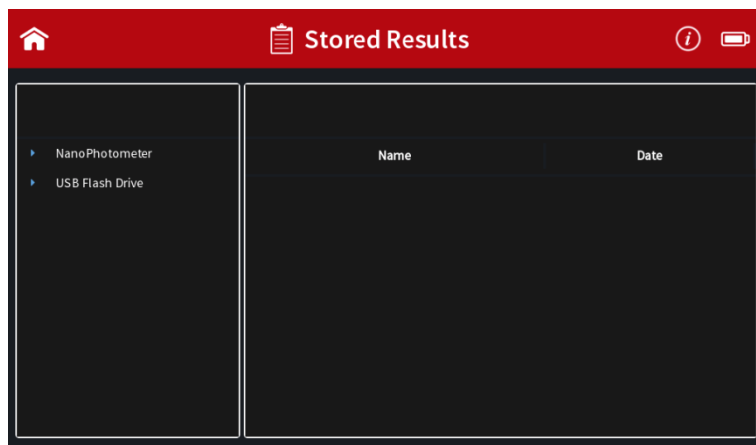
CUSTOM APPS

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. A click on a storage folder shows on the left side the subfolders of that storage folder and on the right side the individual files. 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 clicking on the  icon. It is also possible to delete, rename, move or copy files by clicking 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.

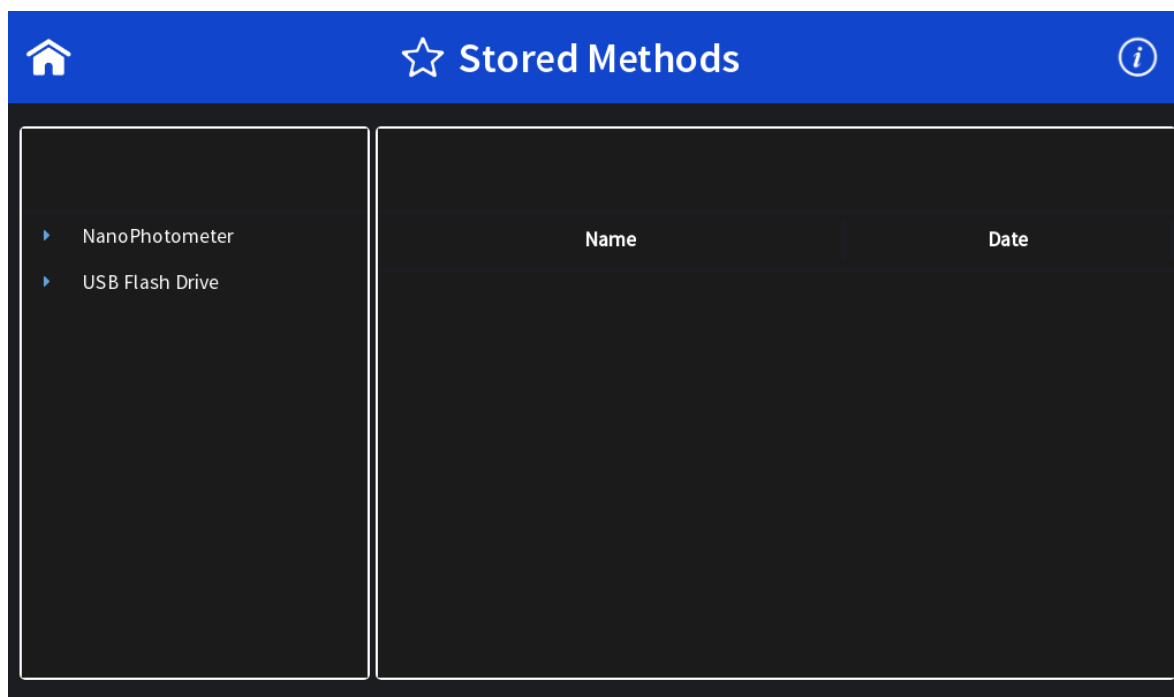
Note: Control device is only available on computer, tablets and smartphones not on the NanoPhotometer® version of the software.

For data transfer via Ethernet or WiFi see page 38 Data Transfer.





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®, Network and/or USB flash drive. A click on a storage folder shows on the left side the subfolders of the storage folder and on the right side the method files. 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 clicking on **+**. Folders can be deleted, renamed, moved or copied by clicking on the  icon. It is also possible to delete, rename, move or copy methods by clicking on the  icon.

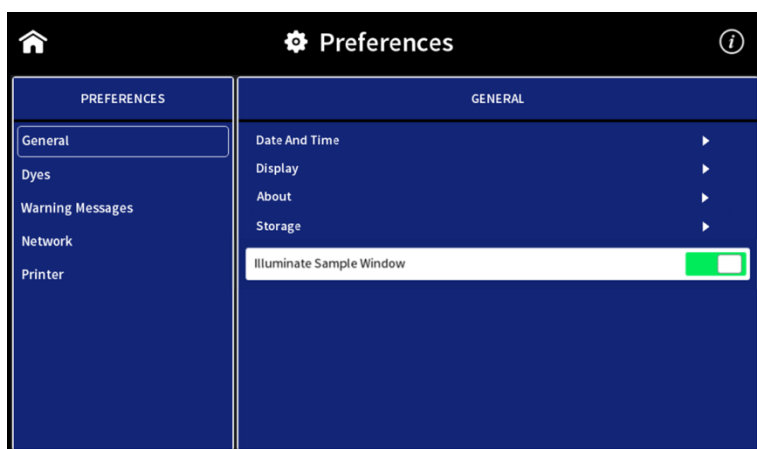
5. PREFERENCES

System preferences can be set by selecting preferences  on the home screen. The preferences menu includes: General, Dyes, Warning Messages, Network and Printer. The menu item which is selected will have its preference options listed in the window to 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 and Illumination Sample Window (N50-Go).

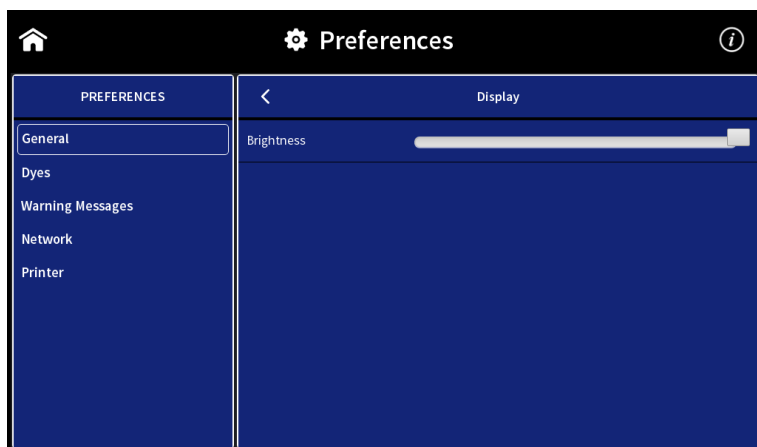


DATE AND TIME

Within the Date and Time it is possible to set the actual date and time. Change the date and/or time by clicking on the date/time field. The selected time is shown below the date and time field. To change the time it is necessary to reboot the NanoPhotometer® by tapping on the Set and Reboot button.

DISPLAY

Brightness: adjustment of the built-in screen brightness



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 Self-test.

STORAGE

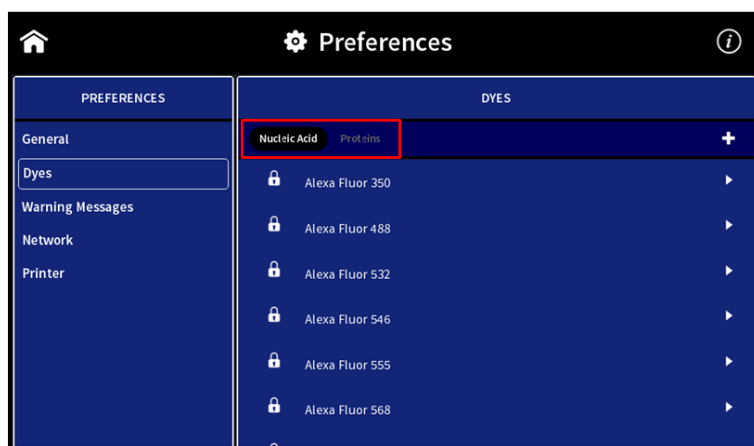
Shows the total storage capacity and free space of the internal NanoPhotometer® storage.

ILLUMINATION SAMPLE WINDOW

Toggle switch to switch on/off the illumination of the sample window (for N50-Go only)

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 click 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} ($\text{M}^{-1} \cdot \text{cm}^{-1}$), 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).

The screenshot shows the 'Preferences' screen with a sidebar on the left containing 'General', 'Dyes', 'Warning Messages', 'Network', and 'Printer'. The 'Dyes' section is selected, showing a list of dyes. The 'Cy3' dye is highlighted, and its details are shown in a table below. The 'Show' toggle is turned on.

Dye Name	Cy3
Abs. max. dye (nm)	560
Dye-dependent ext. coeff. ($M^{-1} \cdot cm^{-1}$)	150000
Dye-dependent corr. factor at 260 nm	0.08

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} ($M^{-1} \cdot cm^{-1}$), 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.

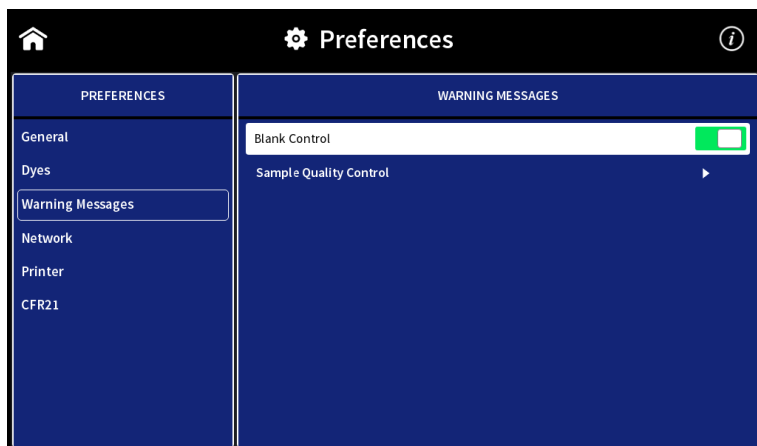
The screenshot shows the 'Add Label' window for adding a new dye. The 'Show' toggle is turned on. The fields for 'Dye Name', 'Abs. max. dye (nm)', 'Dye-dependent ext. coeff. ($M^{-1} \cdot cm^{-1}$)', and 'Dye-dependent corr. factor at 260 nm' are empty. The 'Lock' toggle is turned off.

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 (N50-Go).



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.

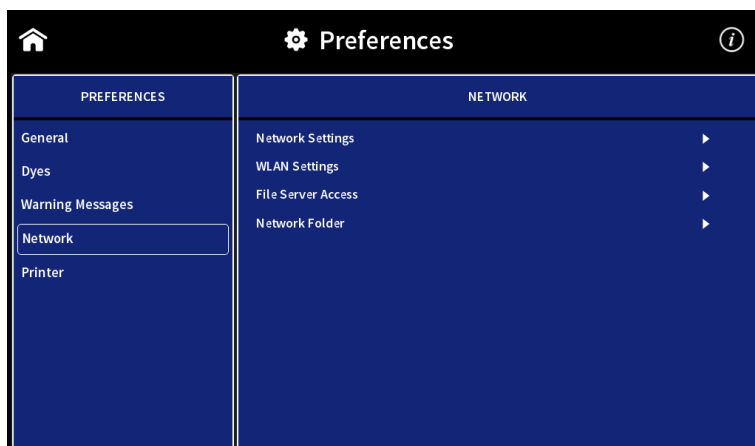
Default value for Protein UV ratio is:

260/280 ratio is 0.7 A.



NETWORK

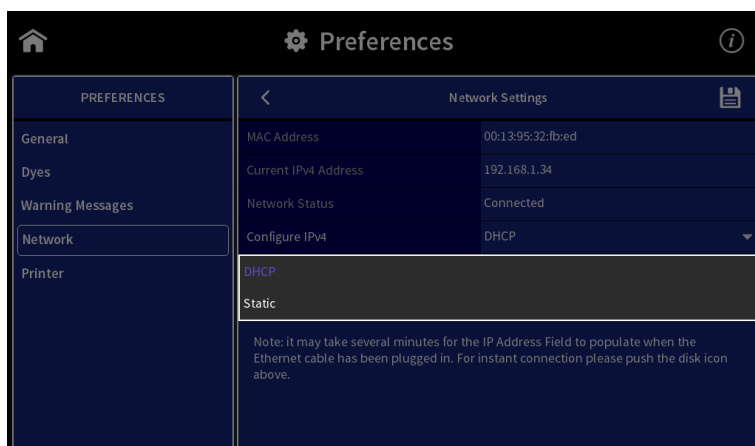
Selecting Network in the Preferences menu opens a window to the right of the preferences menu with the following options: Network Settings, WLAN Settings, File Server Access and Network Folder.



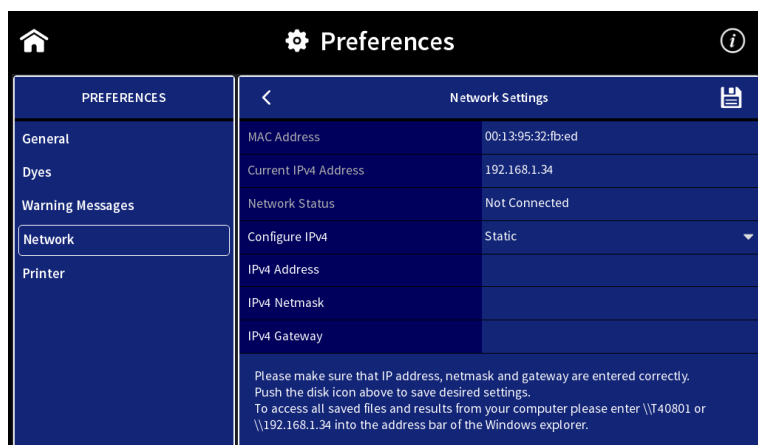
NETWORK SETTINGS

In Network Settings it is possible to choose between a Dynamic Host Configuration Protocol (DHCP) and a static network configuration. As default the DHCP protocol is selected.

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 (📀).

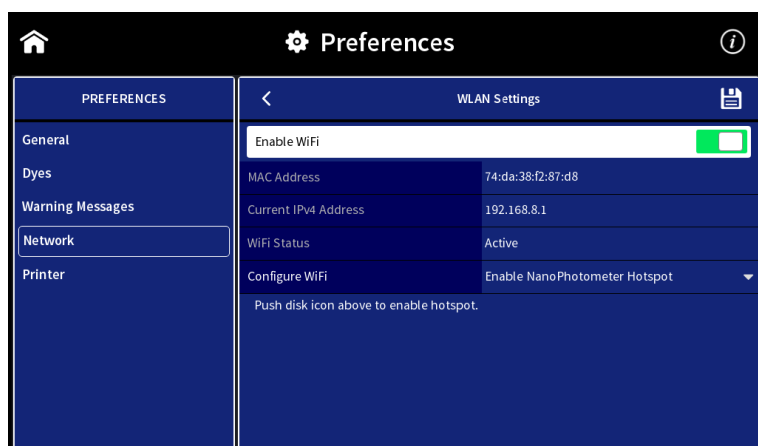


WLAN SETTINGS

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 click on the save icon.

The WiFi Hotspot is needed to connect a computer, an Android smartphone/tablet, an iPad or iPhone via WiFi to the NanoPhotometer®.

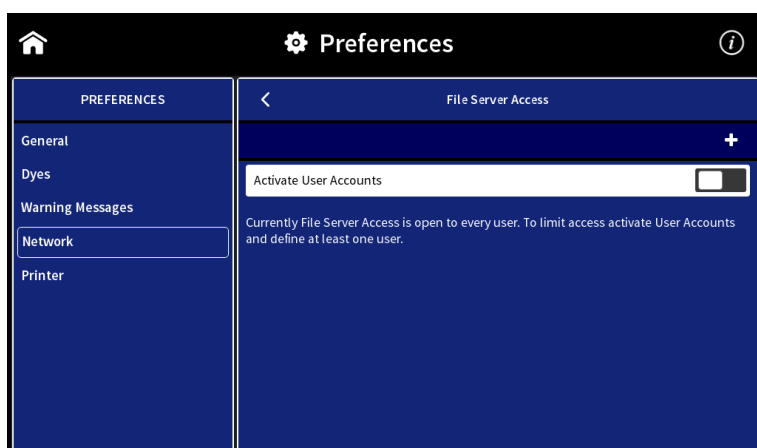


To set up a WiFi network select "Connect to WiFi Network" in the dropdown. Select an available WiFi network from the dropdown list or choose other for hidden networks and enter the password. Confirm with the disk icon (💾).

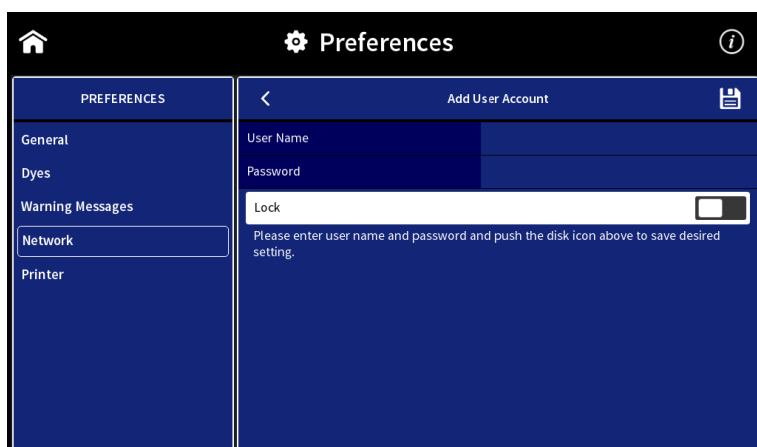


FILE SERVER ACCESS

The File Server Access preferences allow creating user accounts to limit the access to the NanoPhotometer® file server via network. Default setting is off and the file server access is open to every user. It is necessary to create at least one user account to activate the file server access option.



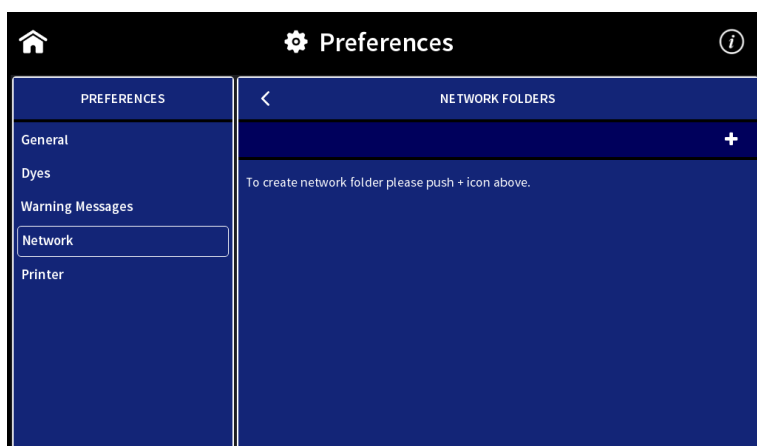
To create a user account click on the + button and enter a user name and password. Confirm with the disk icon (💾).



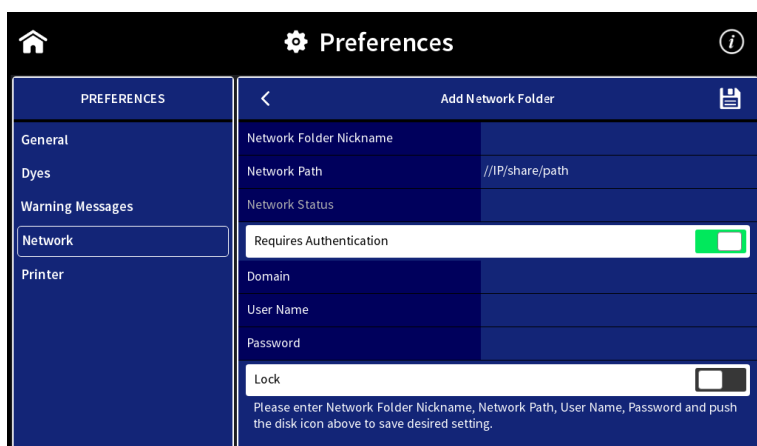
Note: Allowed characters for user name are: A...Z a...z 0...9 _ -
Do not use Blank character.

NETWORK FOLDER

The network folder preferences allow creating network folder for saving data directly from the NanoPhotometer® to a network folder.

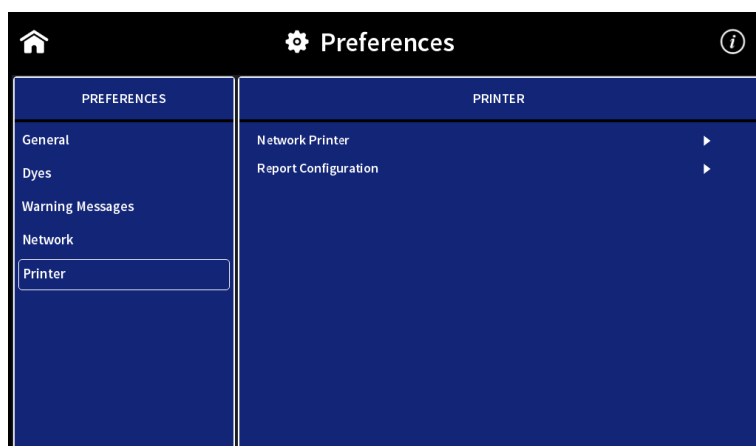


To add a network folder push the + icon and enter network folder nickname and network path of the network folder. If the local network requires authentication enter user name and password for login and if necessary the domain. To save the Network folder push the disk icon (💾).



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

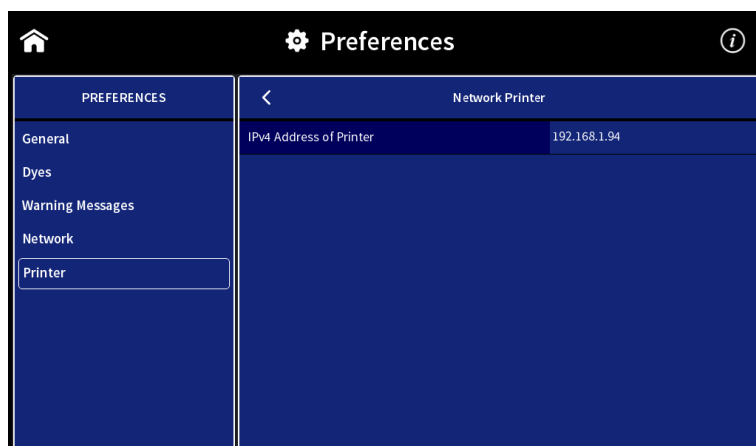
It is possible to print via network (Ethernet) or Airprint® / IPP compatible (supporting PDF format) printers in the network.

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.

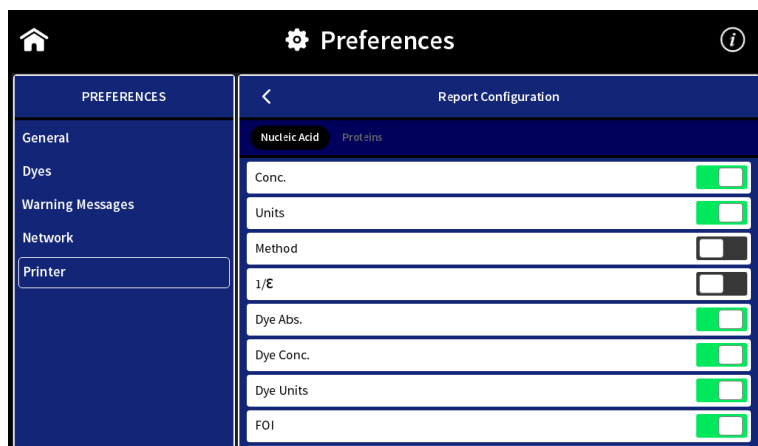
Note: For printing on network printer the NanoPhotometer® needs to be connected to the network either via Ethernet (LAN cable) or WiFi network.

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 click on the Nucleic Acid/Protein buttons in the header.



6. TROUBLESHOOTING

SELF-CALIBRATION TEST

The NanoPhotometer® self-calibration test is performed automatically every time the instrument is powered on. If the instrument passes the self-calibration 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 self-calibration 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.
High absorbance at 250-280nm, 280-340nm, 340-400nm, 400-475nm, 475-550nm, 550-625nm, 625-700nm. Bad Blank or insufficient cleaning.	The warning message is shown when a blank measurement (NanoVolume) detects an absorbance greater than 2.3 (10 mm path). 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).
Implen Folder is Empty	This message is shown on control devices like tablets and smartphones when no data has been saved in the control device folder. For Android tablets and smartphones this folder is always empty.

	Files are saved in the Download Folder of the Android tablet/smartphone directory.
Instrument connected to control device! Do you really want to intercept?	This message is shown on the NanoPhotometer® when a method is open on another control device. Click on OK and the measurements session is interrupted and the data are saved in the auto save folder.
Lint residue or bad sample. Please reapply sample.	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.
Maximum absorbance level at specified wavelength reached. Calculations may lead to low/wrong results.	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.
Maximum level exceeded.	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.
Measurement currently in progress. Please close method on device.	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®.
No printer present.	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.
Sample concentration too low for 0.3 µl - change volume and parameter settings to 1 µl	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.

IMPORTANT ALERT MESSAGES:

ERROR: Lid open - please close lid.	Lid arm is open during initialization test. Close lid arm and confirm with OK.
Firmware not found - NPOS.bin must be in root folder of USB flash drive	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.
Firmware not found - NPOSX.bin must be in root folder of USB flash drive	<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.</p> <p>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>
Initialization failed – problem with optical path. Please contact Implen customer support.	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.
Initialization failed 51/52/53/54/55. Please contact Implen customer support.	Possible hardware failure. Please create a log file and send it to Implen Customer Support.
Initialization failed. Please contact Implen customer support.	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.
Light path blocked – please remove cuvettes from port.	Make sure that the cuvette port is empty during initialization test. Restart the NanoPhotometer® 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.

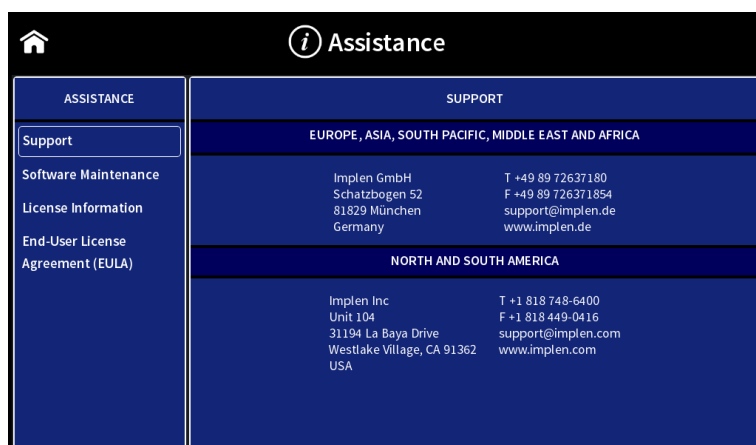
7. ASSISTANCE

The Assistance menu includes: support, report a problem (only available for tablet and computer versions), software maintenance 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.



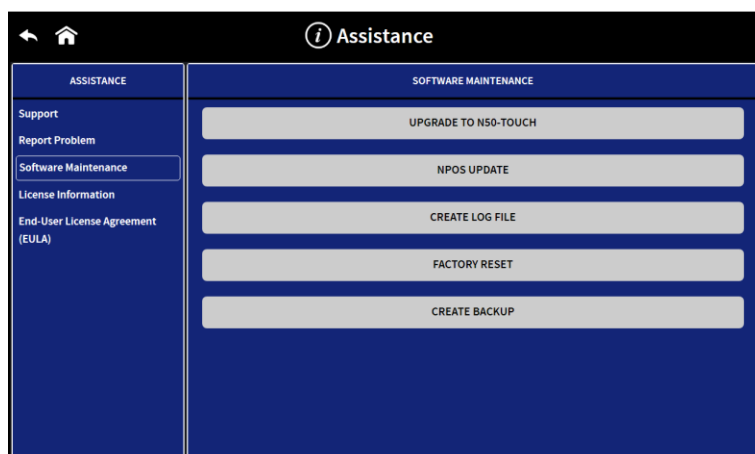
ASSISTANCE	SUPPORT
Support	EUROPE, ASIA, SOUTH PACIFIC, MIDDLE EAST AND AFRICA
Software Maintenance	Implen GmbH Schatzbogen 52 81829 München Germany T +49 89 72637180 F +49 89 726371854 support@implen.de www.implen.de
License Information	NORTH AND SOUTH AMERICA
End-User License Agreement (EULA)	Implen Inc Unit 104 31194 La Baya Drive Westlake Village, CA 91362 USA T +1 818 748-6400 F +1 818 449-0416 support@implen.com www.implen.com

REPORT PROBLEM

The function to report a problem is available on computer and tablet versions only. When report a problem is highlighted in the left side assistance menu, the right side will have a form to fill out with information including: first name, last name, phone number, email, and country. A dropdown menu provides the option to select the problem type and includes the following selections: error message, software, firmware, measurements, and other. It is also 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.

SOFTWARE MAINTENANCE



UPGRADE TO N50-TOUCH

To upgrade the N50-Go to a N50 Touch with full application access a license key is necessary.

Upgrade steps:

- Save the NPOS.lic (license file) into the root folder of a USB flash drive
- Insert the USB flash drive into the NanoPhotometer®
- Start the upgrade with the “upgrade to N50-Touch” button
- NanoPhotometer reboots. After the reboot the full application spectrum of a N50 Touch is available.

For further information about the upgrade licence key and quotation please get in contact with the Implen Sales Team (administration@implen-go.com).

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. Click 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®. Click 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®. Click 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).

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.

CONTACT IMPLEN

There is an option to contact Implen for instruments connected to the internet. For any support issues and questions please contact the Implen team directly:

North and South America

Implen, Inc.

Unit 104

31194 La Baya Drive

Westlake Village, CA 91362

USA

Phone +1 818 748-6400

Telefax +1 818 449-0416

Email: support@implen-go.com

Website www.implen-go.com

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.

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 10^9 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.

▪ 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 included in the conditions of supply is valid for 12 months and is valid only 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

10. ALPHABETICAL APPENDIX

A	EXCEL	32
ABSORBANCE RATIO		
Calculations	71	
Measurement Protocol	73	
ACCESSORIES	71	
Optional Accessories	12	
Standard Accessories	12	
AIR BUBBLE RECOGNITION	41, 49	
ALERT MESSAGE	94	
APPLICATIONS	25	
ASSISTANCE	97	
Maintenance	98	
Report Problem	97	
Support	97	
AUTO PRINT	30	
AUTOSAVE	32	
B		
BACKUP	99	
BARCODE READER	13	
BASIC OPERATION	34	
BCA ASSAY	55	
BIURET ASSAY	55	
BLANK CONTROL	8, 86	
BRADFORD ASSAY	54	
BUTTONS	28	
C		
CLEANING	102	
COMPATIBILITY	21	
Solvent	37	
CONCENTRATION	74	
Calculations	77	
Measurements Protocol	74	
CONNECTING CABLE	12	
CONTACT	100	
CRYO LABEL	13, 31	
CUSTOM APPS	80	
CUVETTE	35	
D		
DATA TRANSFER	38	
DATE AND TIME	83	
DELETE DATA	32	
DIDYMIUM GLASS FILTER	12	
DILUCELL	13	
DISPLAY	83	
DYMO PRINTER	13	
E		
EDIT	29	
	FIELD KIT	13
	FILE SERVER	89
	G	
	GRAPH AREA	29
	GRAPH OVERLAY	29
	H	
	HANDLING TIPS	
	Cuvette	36
	NanoVolume	36
	HDMI	16
	HP PRINTER	14
	I	
	ICONS	27
	IDS	32
	IDS FILES	32
	ILLUMINATION SAMPLE WINDOW	84
	IMPLEN DOCUMENT SOURCE	32
	INSTALLATION	
	Computer	21
	Multi-User	22
	Printer	24
	Single-User	22
	Smart Phone	24
	Software	21
	Spectrophotometer	19
	Tablet	24
	K	
	KINETICS	58
	Calculation	60
	Measurement Protocol	59
	L	
	LAMP	101
	LAN	15
	LOG FILE	99
	LOWRY ASSAY	55
	M	
	MAINTENANCE	101
	MEASUREMENT	
	Cuvette	35
	NanoVolume	34
	MESSAGE	93

METHODS		PROTEIN ASSAYS	54
Single Sample	28	PROTEIN UV	47
MORE APPS	65	Calculations	51
Absorbance Ratio	71	Measurements Protocol	48
Concentration	74		
Standard Curve	77	R	
Wavelength	65		
Wavescan	68	REAR PANEL	11
N		REPLACEMENT PARTS	101
NANOPHOTOMETER® C40-Go	10	Lamp	101
NANOPHOTOMETER® N50-Go	9	Touch Screen	101
NANOVOLUME	34	REPORT CONFIGURATION	92
NETWORK	87	RESET	99
NETWORK FOLDER	90	RESULTS AREA	29
NETWORK PRINTER	91		
NPOS	21	S	
NUCLEIC ACIDS	39	SAFETY INFORMATION	19
Calculations	42	SAMPLE CONTROL™	8, 39, 47
O		SAMPLE NAME	
OD600	61	Change	104
Calculations	64	SAMPLE QUALITY CONTROL	8, 86
Measurements Protocol	62	SAVE	31
ON/OFF SWITCH	9, 10, 11	SAVE DATA	31
P		SELF-CALIBRATION TEST	93
PARAMETER AREA	29	SIDE TAB BAR	28
PDF	32	SOFTWARE	
POSITIONING	20	Backup	99
POWER ADAPTER	12	Reset	99
POWER BUTTON	11	Update	98
PREFERENCES	83	SOLVENT COMPATIBILITY	37
Blank Control	86	SPECIFICATIONS	17
Dyes	84	STANDARD CURVE	77
File Server	89	Calculations	80
General	83	Measurements Protocol	77
Log File	99	STANDARD SOLUTION	13
Network	87	STORED METHODS	82
Network Folder	90	STORED RESULTS	81
Sample Quality Control	86	SYSTEM FREEZE	96
Warning Messages	86		
WLAN	88	T	
PRINT	30	TABLE AREA	29
Auto Print	30	TOUCH SCREEN	101
Cryo Label	31	TRADEMARKS	99
PRINTER	13, 14	TROUBLESHOOTING	93
DYMO	13	U	
HP	14	UNPACKING	20
Installation	24	UPDATE	98
Network	91	UPGRADE	98
Printouts	13, 14	USB	15
PROTEIN ASSAY		W	
Calculations	58	WARNING MESSAGE	93
Measurements Protocol	55	WARNING MESSAGES	86

WARRANTY	103	Calculations	70
WAVELENGTH	65	Measurement Protocol	68
Calculations	67	WiFi	16
Measurement Protocol	65	WLAN	88
WAVESCAN	68		