

Together. All the way™

LASERSHOT M™ NAVILASE® USER MANUAL

Recommended use of Laser Systems
February 2023 US Edition, version 6

Vitrolife 

© 2020 Vitrolife GmbH. All rights reserved.

Distribution and reprinting of this document, use and communication of its contents is not permitted without written authorisation from Vitrolife GmbH. This user manual covers both the usage of the static LaserShot M and the NaviLase. Sections referring to dynamic laser system NaviLase are explicitly intended for users of this system.

You may copy this for internal use only, not for publishing.

The Vitrolife logotype is a trademark of Vitrolife Sweden AB, registered in Europe, the U.S. and other countries.

Vitrolife Sweden AB
Box 9080
SE-400 92 Göteborg
Sweden
Tel: +46-31-721 80 00



Vitrolife GmbH
Roedersteinstr. 6
84034 Landshut
Germany
Tel: +49 (0)871 4306570

Indications for Use of the LaserShot M and NaviLase device

For use in assisted reproduction procedures to ablate or thin the zona pellucida of an embryo to facilitate assisted hatching or recovery of cells for pre-implantation genetic diagnosis (blastomeres). The device can also be used on blastocyst stage embryos for biopsy of trophectoderm cells for pre-implantation diagnosis procedures, and blastocyst collapse prior to vitrification procedures.

R_x Only

Caution: Federal law restricts this device to sale by or on the order of a physician or a practitioner trained in its use

Contraindications LaserShot M and NaviLase device

At present there are no known cell-specific contraindications, i.e. there are no morphological or other cell indicators, that are contraindications for using the LaserShot M and NaviLase device. Contraindications on the patient side regarding Assisted Hatching or biopsy of embryos are left to the judgement of the physician and are related to the patient or the number of embryos available. The LaserShot M and NaviLase device does not have any influence on treatment related contraindications.

Side effects LaserShot M and NaviLase device

When using the laser systems not according to their intended use, there is a risk of partial damage in the cytoplasm due to heat and degeneration of the cell treated. This is especially the case when the laser beam is directly focussed or applied to a cell. When using the NaviLase device in multi-pulse mode and intentional or unintentional movement of the embryo treated during the application of the laser beam, there is a chance that the laser interacts with non-defined areas of the cells and damage to the cells. Laser energy which is absorbed by medium causes a temperature increase. When releasing twenty laser pulses at 10 ms pulse length using a 150 mW laser beam, a calculated energy of 30 mJ is transferred to the medium. In an isolated media droplet of 20 µl volume this amount of energy, evenly spread over the volume, would cause a temperature increase of 0.36°C.

CONTENTS

Definitions	6	Video Page with Laser Targeting Feature	38
Warnings	6	Calibration of the Hole Size Predictor	40
Precautions	10	The Full Screen Mode: LaserShot M and dynamic operation of NaviLase	46
Electromagnetic Compatibility (EMC)	13	Quick File Page for Rapid and Temporary Storage of Images	54
Electromagnetic Immunity	14	The Quick File Toolbar	55
Symbol Glossary	16	Compare Images Page	55
Part I: Introduction	20	Image Page with Measurement Feature	55
Introduction	20	Measurement Toolbar	56
Key Features of the LaserShot M / NaviLase System	20	Storage Wizard for Associating Images with Patients	58
Working with LaserShot M / NaviLase and EyeWare	20	The Database Page for Managing Data Sets	61
Working Principle	21	The Database Toolbar	62
Manipulation of the Zona Pellucida using LaserShot M / NaviLase	22	Report Page for Printing Examination Results	63
Indications for Use of LaserShot M and NaviLase	23	The Report Page Toolbar	64
Setting up LaserShot M / NaviLase System Components	26	Getting Started	65
System Components	27	Starting Eyeware Software	65
Part II: Working with LaserShot M / NaviLase	32	Laser Aiming Verification Procedure	65
Control of the Laser by EyeWare Software	32	Relationship between irradiation time and opening size	67
Installation Requirements	34	How to determine the “default pulse length setting” and verify the calibration of the Hole Size Predictor	67
System Components	34	Important Notes on Laser Irradiation Time	68
Setting up EyeWare Software	35	Variation of the Laser Drilling Position and Strength	70
Main concept and workflow	36		
Structure of EyeWare	37		

CONTENTS

Closing Eyeware Software	70
Part III: Additional Information	71
Advanced Image Handling Functions	71
Open Image and Save Image Dialog	71
Program Settings	73
Camera Settings	75
Generating Support Request Data	76
Maintenance	77
Reprocessing instructions Octax	
LaserShot M, Octax NaviLase	77
Troubleshooting Guide	81
Decommissioning of	
LaserShot M / NaviLase	83
Customer Service	83
Part IV: Quick Guide	84
Laser Aiming Verification Procedure	84
Laser Hole Size Predictor	
Adjustment	85
Working Principle LaserShot M	
- NaviLase	86
Taking Snapshots	87
Part IV: Appendix	88
Target Pointer	88
Laser Module Specifications	94
Related Products	95
Contact and support	rear side

DEFINITIONS



WARNING!

This symbol denotes important information regarding the correct treatment of cells and the proper use of the laser. Please read all warnings carefully before treating any embryos to ensure safe application and optimal results.



CAUTION!

This symbol denotes important cautions. Please read all precautions before treating any embryos to ensure safe and optimal results.



IMPORTANT!

This symbol denotes important additional information regarding the laser device and treatment of cells.

WARNINGS



ABOUT THIS MANUAL

The procedures described in this manual concern a particular device installed by Vitrolife GmbH authorized personnel at a designated location. The LaserShot M or NaviLase devices must be operated by trained personnel according to the instructions contained in this user manual.



LASER

The laser of the LaserShot M and the NaviLase system are classified as a class 1M laser. Class 1M lasers emit in the wavelength range from 302,5 nm to 4000 nm.

Laser Radiation, do not view directly with optical instruments.



EYE SAFETY OF THE OPERATOR

Eye safety of the operator is guaranteed under normal operation of the LaserShot M and NaviLase, and in a situation when user removable parts may be missing. However, do not disassemble or uninstall the LaserShot M or NaviLase system and watch the beam employing optics. Any installation / deinstallation of hard- and software, respectively, is strictly reserved to trained and certified service personnel authorized by Vitrolife GmbH.



WARNING

To avoid the risk of electric shock, this equipment must only be connected to a supply mains with protective earth.



INTEGRITY OF THE ELECTRO-OPTICAL SYSTEM

Maintenance of microscope components, wrong handling of the microscope or strong displacement of the electro-optical system, e. g. by mechanical shock can result in an incorrect position of the beam splitter system, the magnifier lens, the camera, and the turret, respectively. As a result of any of the above, aiming of the laser may no longer correspond to the crosshair position displayed at the video image and damage to embryos may occur if the laser is used in this condition. After maloperation of the microscope, repeat the *Laser aiming verification procedure* or contact *Technical Service*.



INCORRECT LASER AIMING

Failure to follow the laser aiming verification procedure could result in wrongly located openings and therefore may damage the treated embryo.



MULTIPLE OR SMALL OPENINGS

Only a single opening should be made in the zona pellucida. Multiple openings or openings that are too small may prevent embryo hatching and/or lead to abnormal embryo development.



THERMAL LENSING

Thermal lensing is a defocusing of the laser beam as it passes through and heats the medium. Thermal lensing that can lead to laser treatment outside of the targeted area, thereby increasing the risk of negative thermal or mutagenic effects on the embryo. To minimize the occurrence of thermal lensing, we recommend that the pulse length be kept below 10 ms for assisted hatching zona drilling or for trophectoderm cell biopsy.



DEVELOPMENT STAGE

Laser assisted hatching should only be performed on 4-8 cell embryos. Effects of laser assisted hatching on embryos of later developmental stages (> 8 cell stage) are not known.



WARNING

Only use the 25x laser lens when using LaserShot M or NaviLase. Use of other objectives for laser treatments may damage the embryo.



LONG-TERM FOLLOW UP

To date there are no known reports showing a greater occurrence rate of major or minor defects in children born after laser assisted hatching of embryos. Long-term follow-up data on children derived from embryos treated by laser assisted hatching does not yet exist. A follow-up study of 134 such babies found no increase in the major congenital malformations, chromosomal aberrations or minor congenital malformations between the laser assisted hatching treated group and all deliveries at their hospital. (Kanyo, K., Konc, J. "A follow-up study of children

born after diode laser assisted hatching.” European Journal of Obstetrics and Gynecology. 110: 176-180 (2003)).



INSTALLATION AND MAINTENANCE

Installation and repair of the LaserShot M or NaviLase shall only be carried out by a person certified by Vitrolife. The LaserShot M must remain on the microscope and at the location where it was installed. If a LaserShot M or NaviLase is disconnected and/or moved without supervision by a person certified by Vitrolife the LaserShot M or NaviLase will no longer be approved for clinical use and the warranty may be voided.

If the LaserShot M , NaviLase or parts of it are modified, appropriate inspection and testing must be conducted by a Vitrolife certified person to ensure continued safe use.

A preventative maintenance of the laser is recommended every 12-18 months to ensure optimal performance of the laser.



ELECTROMAGNETIC COMPATIBILITY

The LaserShot M and Navilase have been tested and found to comply with the limits for medical devices to the IEC 60601-1-2:2007 / EN 60601-1-2:2007 for electromagnetic compatibility. These limits are designed to provide reasonable protection against harmful interference in a typical medical installation.

This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, or if connected to material not certified by Vitrolife, may cause harmful interference to other devices in the vicinity. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to other devices, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving device.
- Increase the separation between the equipment.
- Connect the equipment into an outlet on a circuit different from that to which the other device(s) are connected.

Consult the manufacturer, their representative or dealer for help.

WARNING: The use of accessories and cables other than those supplied by Vitrolife, may result in increased emissions or decreased immunity of the ME equipment or ME system.

WARNING: Portable RF communications equipment (including peripherals such as antenna cables and external antennas) should be used no closer than 30 cm (12 inches) to any part of the LaserShot M , including cables specified by the manufacturer. Otherwise, degradation of the performance of this equipment could result.



CONNECTION TO EXTERNAL EQUIPMENT

To guarantee basic safety and compliance with relevant EC standard (i.e. EN 60601-1 – Part 1 for medical electrical equipment) and essential performance, this equipment must only be connected to computer equipment certified by Vitrolife, and connection must only be made using certified cables.



LIMITED WARRANTY

Vitrolife warrants the LaserShot M or NaviLase to be free from defects in materials and workmanship for a period of two (2) year from the day of shipping.

The limited warranty shall terminate immediately if installation, maintenance, repair or relocation of the time-lapse incubator is carried out by other than Vitrolife certified personnel.

- The limited warranty shall not apply to damage resulting from:
- Failure to perform routine maintenance in accordance with this User Manual;
- Accident, abuse, misuse, or misapplication of the device;
- Use and operation that does not comply with instructions provided in the User Manual;
- Normal wear and tear.



CONNECTORS

Do not disconnect the USB cable connector unless instructed to do so by qualified support personnel.



WARNING

The recipient end user of the LaserShot M / NaviLase systems should not unpack and install it upon receipt. Unpacking, installation, setup and end-user training of LaserShot M / NaviLase systems must be carried out by appropriately qualified technical staff authorized by Vitrolife GmbH.



TE MODE: RESTRICTIONS IN USE

The TE mode must only be used by experienced users trained in doing trophectoderm biopsies. The laser puls(es) can assist the release of mechanically stretched intracellular bonds between trophectoderm cells for biopsy. The TE mode must never be applied to the zona pellucida.



REPEATED LASER SHOTS

Repeated laser shots to the same position of the embryo could result in an increased risk of embryo damage. In case of risk of applying repeated laser shots to the same position of the embryo the laser action can be immediately stalled by pressing the emergency stop button.



INFLUENCE OF TEMPERATURE ON DRILL OPENING SIZE

When using a heated stage during zona manipulation, make sure that it has been set to the correct temperature. Inappropriate temperature settings will lead to unexpected drill hole sizes. Lower temperatures result in smaller openings, while higher temperatures cause openings of excessive sizes that may lead to embryo damage.



WARNING

Never use abrasive disinfectants! Only use disinfectant based on quaternary ammonium compound tested for use in IVF laboratories for reprocessing.



WARNING

Do not use any automated method for cleaning.

PRECAUTIONS



INFLUENCE OF Z-POSITION OF THE EMBRYO ON DRILL OPENING SIZE

Inappropriate Z-positioning will result in smaller drill holes and in reduced laser beam quality. It is recommended to keep the cell near the bottom of the culture dish during laser treatment.



CAUTION!

Federal law restricts this device to sale by or on the order of a physician or a practitioner trained in its use.



CAUTION!

To minimize the risk of damage to the embryos, administer as few laser pulses as possible at the lowest energy levels possible to achieve the prescribed effect.

**CAUTION!**

Direct the laser beam towards a section of the zona pellucida where the adjacent perivitelline space is widest or next to an area of fragmentation.

**CAUTION!**

A holding pipette should be used during laser treatment to minimize the risk of embryo movement.

**CAUTION!**

In case that the target indicator did not match with the center of the drilled opening in one or more cases, stop using NaviLase in dynamic mode. Contact customer service and provide the snapshots of the deviation(s). Only use NaviLase in verified home position until the error will be fixed.

**CAUTION!**

Check that the central LED dot of the Target Pointer is superimposed with the digital laser target overlay on the screen and verify the selected hole size before using the Target Pointer for laser aiming.

**CAUTION!**

See chapter "Reprocessing instructions" for reprocessing procedures for laser lens, the lateral surfaces of the mirror block, and the USB camera mounted at the camera port of the microscope. All other laser components should only be cleaned by service engineers certified by Vitrolife GmbH.

**CAUTION!**

Never remove any of the laser components from the microscope for reprocessing! Never change their position or orientation or apply pressure!

**CAUTION!**

Never spray any of the laser components with disinfectants or any other reprocessing agent! Never use abrasive disinfectants!

**CAUTION!**

Before starting with the reprocessing procedure, make sure all embryos are back to the incubators and the lab is well ventilated. The cleaning agent is volatile!

**CAUTION!**

Dispose all cleaning tissues outside the IVF laboratory! The cleaning agent is volatile!

**CAUTION!**

Cover the laser module in the back of the microscope, the USB camera and cables, respectively, with surgical drape to protect from contamination.

**CAUTION!**

Never remove any of the laser components from the microscope for the reprocessing procedure! Never change their position or orientation! Never apply pressure!

**CAUTION!**

Never spray any of the laser components with disinfectant!

**CAUTION!**

before starting with the reprocessing procedure, make sure all embryos are back to the incubators!

**CAUTION!**

Do not remove or rinse disinfectant before drying.

ELECTROMAGNETIC COMPATIBILITY (EMC)

The below table contains the applicable information required for CISPR11 systems:

Guidance and manufacturer's declaration – Electromagnetic emissions

LaserShot M and NaviLase laser systems is intended for use in the electromagnetic environment specified below. The customer or the user of LaserShot M and NaviLase laser systems should assure that it is used in such an environment

Emissions test	Compliance	Electromagnetic environment - guidance
RF emissions EN/CISPR 11	Group 1	LaserShot M and NaviLase laser systems use RF energy only for their internal function. Therefore, their RF emissions are very low and not likely to cause any interference in nearby electronic equipment.
RF emissions EN/CISPR 11	Class B	LaserShot M and NaviLase laser systems are suitable for use in all establishments, including domestic establishments and those directly connected to the public low-voltage power supply network that supplies building used for domestic purposes.
Harmonic emissions IEC 61000-3-2	Class A	
Voltage fluctuations (flicker emissions)	Complies	

Electromagnetic Immunity

Guidance and manufacturer's declaration – Electromagnetic immunity

LaserShot M and NaviLase laser systems is intended for use in the electromagnetic environment specified below. The customer or the user of LaserShot M and NaviLase laser systems should assure that the used in such an environment

Immunity test	IEC 60601 Test level	Compliance level	Electromagnetic environment - guidance
Electrostatic discharge (ESD) IEC 61000-4-2	± 4 kV contact ± 2 kV, ± 4 kV, ± 8 kV air	± 4 kV contact ± 2 kV, ± 4 kV, ± 8 kV air	Floors should be wood, concrete or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30%
Electrical fast transient /burst IEC 61000-4-4	+/- 2kV for power supply lines +/- 1 kV for input/output lines	+/- 2kV for power supply lines +/- 1 kV for input/output line	Mains power quality should be that of a typical commercial or hospital environment
Surges IEC 61000-4-5	± 0.5 kV, ± 1 kV Line-to-line @ 0°, 90, 180°, 270°	± 0.5 kV, ± 1 kV Line-to-line @ 0°, 90, 180°, 270°	Mains power quality should be that of a typical commercial or hospital environment
Voltage dips, short interruptions and voltage variations on power supply input lines IEC 61000-4-11	EUT: Reduction of input voltage to 80% for 0.5 cycles @ 0° and 180°, 1, 5, 10, 25, 50, 250 cycles	Result: PASS: No change in operation. The system remained safe.	Mains power quality should be that of a typical commercial or hospital environment If the user of LaserShot M and NaviLase laser systems requires continued operation during power mains interruptions, it is recommended that the incubator be powered from an uninterruptible power supply or battery. No degradation of the essential performance was observed and EUT remains safe during the test.
	Reduction of input voltage to 70 % for for 0.5 cycles @ 0° and 180°, 1, 5, 10, 25, 50, 250 cycles	PASS: No change in operation. The system remained safe.	
	Reduction of input voltage to 70 % for for 0.5 cycles @ 0° and 180°, 1, 5, 10, 25, 50, 250 cycles	PASS: No change in operation. The system remained safe	
	Reduction of input voltage to 0% for 0.5 cycles @ 0° and 180°, 1, 5 cycles	PASS: System is allowed to turn off as long as it establishes normal operation after the test	
	Reduction of input voltage to 0% for 10, 25, 50, 250 cycles	PASS: System is allowed to turn off as long as it establishes normal operation after the test	
Power frequency (50/60 Hz) Magnetic field IEC 61000-4-8	30 A/m	10 A/m PASS: No change in operation. The system remained safe	Power frequency magnetic fields should be at levels characteristic of a typical commercial or hospital environment.

The below two tables contain the applicable information required for a system other than those specified for use only in a shielded location and for systems that are not life supporting.

Guidance and manufacturer's declaration – Electromagnetic immunity

LaserShot M and NaviLase laser systems is intended for use in the electromagnetic environment specified below. The costumer or the user of LaserShot M and NaviLase laser systems should assure that it is used in such an environment

Immunity test	IEC 60601 Test level	Compliance level	Electromagnetic environment - guidance
Conducted RF IEC 61000-4-6	3 V(EMK) 150 kHz to 80 MHz	3 V(EMK) 150 kHz to 80 MHz	No degradation of the essential performance was observed and EUT remains safe during the test in normal operational mode and in alarm mode. Portable and mobile RF communications equipment should be used no closer to any part of LaserShot M and NaviLase laser systems, including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter.
Radiated RF IEC 61000 4-3	3 V/m 80 MHz to 3 GHz @ 0, 90, 180 and 270° horizontal and vertical polarization	3 V/m 80 MHz to 3 GHz @ 0, 90, 180 and 270° horizontal and vertical polarization	<p>Recommended separation distance:</p> <p>150 kHz to 80 MHz: $1.2\sqrt{P}$</p> <p>80 MHz to 800 MHz: $1.2\sqrt{P}$</p> <p>800 MHz to 3.0 GHz: $2.3\sqrt{P}$</p> <p>Where P is maximum power rating of the transmitter in Watts (W) according to the transmitter manufacturer and d is the recommended separation distance in metres (m). Field strengths from fixed RF transmitters, as determined by an electromagnetic site survey² should be less than the compliance level in each frequency range^b.</p>







NOTE 1 At 80 MHz and 800 MHz, the higher frequency range applies.




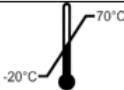
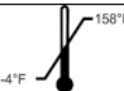

NOTE 2 These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.





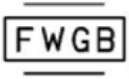

2 Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which the LaserShot M and NaviLase laser systems incubator is used exceeds the applicable RF compliance level above, the LaserShot M and NaviLase laser systems incubator should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as reorienting or relocating the incubator.



b Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 3 V/m.

SYMBOL GLOSSARY

Symbol	Description	Reference number symbol	Titel of standard	SDO	Designation number of standard
	Medical device manufacturer	ISO 7000-3082	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Medical device manufacturer Date of manufacture, Year-month	ISO 7000-3082	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Date of manufacture	ISO 7000-2497	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Serial number	ISO 7000-2498	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Catalogue number	ISO 7000-2493	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Consult instructions for use	ISO 7000-1641	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1

	Declaration of conformity according to 93/42/EEC The identification number of the Notified Body is 0123			EC	European Medical Device Directive 93/42/EEC, Annex II amended by Directive 2007/47/EC under the supervision of Notified Body No 0123
	Declaration of conformity according to 93/42/EEC			EC	European Medical Device Directive 93/42/EEC, Annex II amended by Directive 2007/47/EC
Rx only	Statement of prescription Caution: Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner		21CFR801.109	FDA	21CFR801.109 (b)(1)
	Caution, laser radiation Class 1M laser product		Safety of laser products - Part 1: Equipment classification, and requirements [Including: Technical Corrigendum 1 (2008), Interpretation Sheet 1 (2007), Interpretation Sheet 2 (2007)]	IEC	EN 60825-1, Edition 2.0, 2007-03
	Temperature limit, °C	ISO 7000-0632	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Temperature limit, °F	ISO 7000-0632	Medical devices - Symbols to be used with medical devices labels, labeling, and information to be supplied - Part 1: General requirements	ISO	EN ISO 15223-1:2016, Third Edition, Table 1
	Indicates instruction for disposal of goods according to WEEE directive				European directive 2012/19/EU on waste electrical and electronic equipment (WEEE), Annex IX

	Indicates instruction for disposal of goods according to WEEE directive				WEEE directive
	Caution, risk of electric shock	IEC 60417-6042	C1:2009/(R)2012 and A2:2010/(R)2012 (Consolidated Text) Medical electrical equipment - Part 1: General requirements for basic safety and essential performance (IEC 60601-1:2005, MOD)	IEC	IEC 60601-1 Edition 3.1, table D.2 (IEC 60601-1: 2005 And AMD1 :2012)
	UL – Underwriters Laboratories approval mark. Valid in the USA and Canada	UL E143128			
	Approval mark of SIQ Certifies that the devices is complaint to EN 60601-1: 2006 And A11 :2011 And A12:2014 (IEC 60601-1:2005 + A1 :2012; EN 60601-1-2:2007 IEC 60601-1-2:2007)				
	Trademark of the manufacturer of laser power supply	Registered trademark of the commercial register			
	Class II equipment	IEC 60417-5172	C1:2009/(R)2012 and A2:2010/(R)2012 (Consolidated Text) Medical electrical equipment - Part 1: General requirements for basic safety and essential performance (IEC 60601-1:2005, MOD)	IEC	IEC 60601-1 Edition 3.1, table D.1 (IEC 60601-1: 2005 And AMD1 :2012)
IP40	IP codes	ANSI-IEC 60529 Degrees of protection provided by enclosures (IP Code) Table D.3	C1:2009/(R)2012 and A2:2010/(R)2012 (Consolidated Text) Medical electrical equipment - Part 1: General requirements for basic safety and essential performance (IEC 60601-1:2005, MOD)	IEC	IEC 60601-1 Edition 3.1 (IEC 60601-1: 2005 And AMD1 :2012)

ta 40	Rated – ambient temperature	IEC 61558-1 clause 8.11	C1:2009/(R)2012 and A2:2010/(R)2012 (Consolidated Text) Medical electrical equipment - Part 1: General requirements for basic safety and essential performance (IEC 60601-1:2005, MOD)	IEC	IEC 60601-1 Edition 3.1 (IEC 60601-1: 2005 And AMD1: 2012)
	Direct current	IEC 60417-5031	C1:2009/(R)2012 and A2:2010/(R)2012 (Consolidated Text) Medical electrical equipment - Part 1: General requirements for basic safety and essential performance (IEC 60601-1:2005, MOD)	IEC	IEC 60601-1 Edition 3.1, Table D.1 (IEC 60601-1: 2005 And AMD1: 2012)
	Indoor use only	IEC 60417-5957	Graphical symbols for use on equipment	IEC	60417:2002 DB IEC 61558-1:2017 29.09.2017

PART I: INTRODUCTION

This chapter provides an outline of the key features and applications of the LaserShot M/Navilase system

Introduction

The laser systems for microsurgery utilized in the field of assisted reproductive technology (ART). The laser systems can be used to manipulate the zona pellucida of embryos in order to assist hatching and to extract cells for subsequent genetic analysis.

Key Features of the LaserShot M / Navilase System

The LaserShot M / Navilase system is based on an infrared laser diode emitting at a wavelength of 1,48 μm which is coupled to an inverted microscope. The laser beam is directed along the microscope optical axis. The spatial arrangement of lenses and mirrors within the laser unit allows focusing of the laser beam to the image plane of the microscope objective. In addition to LaserShot M, Navilase includes motion elements which allow the controlled movement of the laser beam to any position within the working field which is visible from the camera image. The microscope should be equipped with a heating stage to ensure optimal conditions for embryos. The infrared 1,48 μm wavelength emitted by the laser diode is not known to be mutagenic and thus, it is ideally suited for the use in non-contact procedures in ART.

Working with LaserShot M/ Navilase and EyeWare

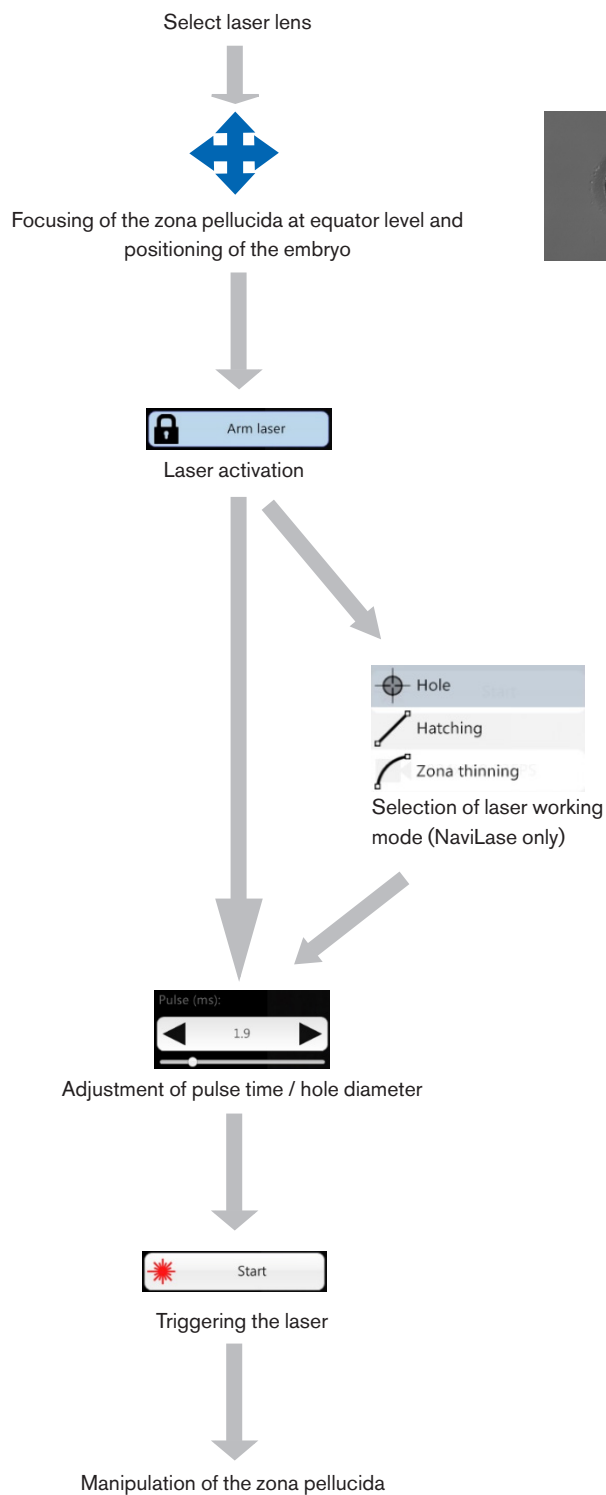
The LaserShot M / Navilase system provides an advanced laser technology for ART featuring digital control and digital video / image processing combined with high optical and electro-mechanical quality. Controlled by the EyeWare imaging software the LaserShot M / Navilase system can be intuitively used for the daily routine.

Acquired by a high resolution digital camera a live video stream of the cells is displayed at the computer monitor. The video image is overlaid by an computer generated crosshair, which marks the aiming position of the laser beam. A laser pulse is triggered either by mouse or optionally by foot switch. The irradiation time of the laser is set within the user interface of the EyeWare software.

With EyeWare not only microscopic devices, microscopic imaging and measurements can easily be managed, but also documentation has been made very convenient. With the database module patient data and snapshots are stored and administrated. Connection to an external database allows importing and exporting of datasets. A predefined report with

any set of examination results can be printed after only a few mouse clicks. Datasets may be exported to PDF-files for e-mail forwarding and to RTF-files or to CSV-files for further text or spreadsheet program processing, respectively.

Working Principle

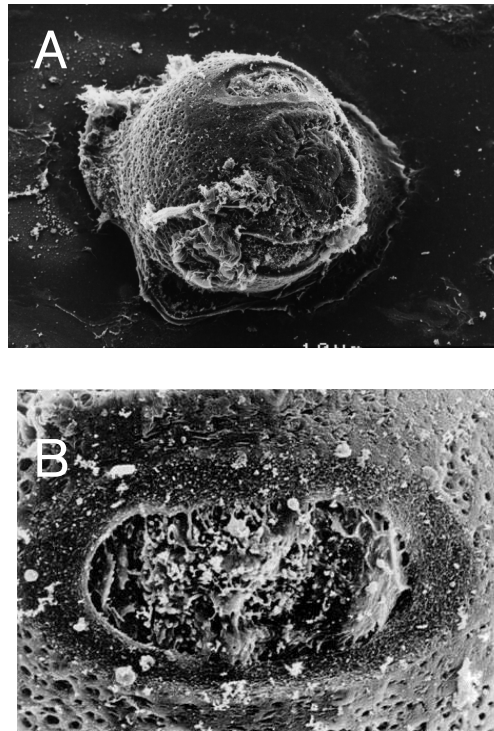


Manipulation of the Zona Pellucida using LaserShot M / NaviLase

The laser beam generated by the LaserShot M / NaviLase causes a tangential thinning or opening of the zona pellucida of embryos by a highly localized photo thermal process which lyses the glycoprotein matrix.

Thereby, trench-like openings with even walls are generated (Fig. 1) which appear circularly shaped in a two-dimensional view. The size of the opening can be adapted to the respective procedure simply by varying the irradiation time of the laser. The reproducibility of the drilling effect is very high.

The LaserShot M / NaviLase system uses a laser that has no known potential mutagenicity as compared to, e. g. UV laser procedures. Furthermore, with the laser having a relatively low power in focus (100 mW - 250 mW) fundamental safety studies have been conducted. Until now no adverse effect of the described laser procedures has been documented. A follow-up study on 134 children born after laser assisted hatching (LAH) was performed and revealed no increase in the major congenital malformation rate, no increase in chromosomal aberrations and no difference in the minor congenital malformation rate.



Photos courtesy CHUV, Lausanne, Switzerland

Fig. 1: Scanning electron micrographs of a laser treated murine zygote at low magnification (A) and at higher magnification (B).

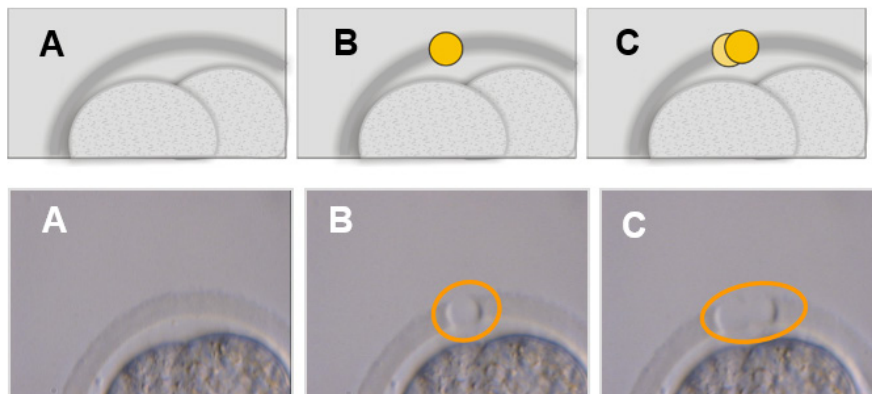
Indications for Use of LaserShot M and NaviLase

Assisted hatching (AH)

The aim of AH is to locally weaken the zona pellucida by creating a trench along the optical axis of the laser beam (see. Fig. 1, p.17) which appears as a hole when seen through an inverted microscope.

To minimize the risk of damage to blastomeres, users should administer as few laser pulses as possible at the shortest pulse lengths possible to achieve zona drilling or thinning effects. Only a single opening should be made in the zona pellucida. Multiple openings or those that are too small might prevent the embryo from hatching or lead to abnormal development. The laser beam should be directed towards a section of the zona pellucida where the adjacent perivitelline space is widest.

For AH, the drilled hole size should be approximately 1.5 times of the thickness of the zona pellucida. A minimal invasive strategy is to create an opening by means of 2 holes with a diameter of 20 μm , i.e. slightly larger than the zona thickness (typically 16-18 μm in human embryos). An overlap of approximately 50% will generate the desired hole size resulting in the formation of an oval shaped opening. (see figure C below.) Due to the 50% overlap, this approach is robust with respect to small variations in the actual zona thickness. Depending on the setup and zona pellucida hardness the pulse times to generate this hole size will vary a little but should be in the range of 0.4-4 ms.



Pictures: University of Bonn

Alternatively, the outer layers of the zona pellucida may be ablated largely and across a wider area, but without breaching it. This process is commonly referred to as zona thinning. An area of 25-40% of the circumference of the zona pellucida should be thinned by contiguous laser shots generating holes of 15-20 μm in diameter with maximum 50% overlap. Depending on the setup and zona pellucida hardness the pulse times to generate this hole size will vary a little but should be in the range of 0.4-4 ms. The overlapping laser shots should be positioned in a way to ablate about 50-70% of the initial zona pellucida thickness.

Note: AH is not recommended for routine use in all ART patients.

Blastomere biopsy (cleavage stage)

The aim of cleavage stage biopsy is to retrieve 1 or 2 blastomeres from a day three embryo for genetic analysis.

A pulse time should be selected to create an opening of about 20 μm . The embryo should be rotated and blastomere(s) selected for biopsy should be positioned using a holding capillary. The embryo should be held close to the bottom of the dish to maximize laser efficiency.

An oval shaped opening should be drilled using two or three overlapping laser pulses to open the zona pellucida for easy access of the single blastomere selected for biopsy. If two blastomeres were selected for biopsy, the opening should be made in between the two cells. Depending on the setup, pulse times will vary and should be in the range of 0.4-4 ms.

Trophectoderm (TE) cell biopsy

The aim of TE biopsy is to retrieve 2-10 TE cells as samples for genetic analysis. TE cells are separated from a blastocyst stage embryo without causing damage to the inner cell mass (ICM).

Recall technical details on TE mode of NaviLase (pp.50) or LaserShot M (pp. 51) before following the steps below.

Step 1: opening the zona pellucida

15 to 20 hours prior to biopsy, AH is performed making a small hole or channel (approx. 5 μm wide) in the zona pellucida of the embryo using 1-3 laser pulses. A pulse length in the range of 0.5-1.5 ms should be sufficient to create a hole of this width. The opening should be placed at the opposite side of the ICM. Typically, 5-7 TE cells will have formed a hernia, protruding from the hole, at the time point of biopsy. This step is optional but will facilitate the biopsy procedure.

Step 2: biopsy of TE cells

Under the inverted microscope, a holding pipette is used to position and firmly fix the blastocyst in a way that the herniating TE cells are facing the biopsy pipette. The blastocyst should be held close to the bottom of the dish to maximize laser efficiency. The biopsy pipette should have an inner diameter of 20-30 μm .

The laser should be switched to TE mode and a pulse duration of 0.3-3 ms should be selected.

For biopsy, 2-10 of the herniated TE cells should be aspirated into the biopsy pipette. Subsequently, the aspirated TE cells should be gently pulled away from the blastocyst to stretch and expose the intracellular junctions and minimize cell damage. Subsequently, two or three laser pulses are applied to the intracellular junctions of the cells to be separated. Pulling gently will completely break the intracellular junctions and the separated cells should be aspirated into the biopsy capillary carefully. They are finally placed at some distance from the embryo for subsequent transfer into a tube for genetic analysis.

If cells are to be biopsied from cryopreserved blastocysts, laser application should be performed after thawing, as soon as the ICM can be located and before the blastocyst has fully re-expanded. In a first step, the laser is used in single pulse (Hole) mode to create a hole of 15-20 μm opposite from the ICM. The pulse time to create this opening should be in the range of 0.4-4 ms. Thawed blastocysts will have TE cells herniating out of the zona pellucida after 2 to 3 hours of re-expansion.

To perform the actual biopsy of TE cells, follow the procedures described above in Step 2.

Blastocyst collapsing

Blastocyst collapsing is a common procedure to remove the fluid inside the blastocoel in order to improve the efficiency of cryo-protectants and increase the survival rate during vitrification and warming.

The procedure is similar to that performed in Step 1 of trophectoderm biopsy (p. 24) ie making a small hole (5 -10 μm wide) in the zona pellucida of the embryo at the opposite side of the ICM. Subsequently, select the single pulse mode (Hole) of the laser. For blastocyst collapsing, the laser should be positioned to target the junction between two TE cells that are located at the opposite side of the ICM. The blastocyst collapse should be induced by applying one (and only one) single laser pulse of 0.5 -1.5 ms. Full collapse of the TE layer may take up to 10 minutes after applying the laser pulse. Occasionally, an embryo will not collapse even after time back in the incubator, but it will collapse during the vitrification procedure.

Collapsing may be performed on full, expanded, hatching and completely hatched blastocysts.



DAILY ROUTINE

For maximal confidence, we recommend to confirm positioning and aiming of the crosshair and the correct opening size of the drilling on a daily basis before treating any patient embryo (please refer to the section Adjustment and Control of Laser Impact).



TECHNICAL SUPPORT

If you need assistance at any time, please contact Technical Service. For contact details please refer to the section Customer Service.

Setting up LaserShot M / NaviLase

The recipient end user of the device should not install the device upon receipt. Unpacking, installation, setup and end-user training of LaserShot M / NaviLase systems must be carried out by appropriately qualified technical staff authorized by Vitrolife GmbH.

Use the attached power cable to connect the product.
Install a power outlet within reach of operator. In the event anything abnormal happens, unplug the power cable to cut off the product's supply.



EYE SAFETY OF THE OPERATOR

Eye safety of the operator is guaranteed under normal operation of the LaserShot M / NaviLase, and in a situation when user removable parts may be missing. However, do not disassemble or uninstall the LaserShot M / NaviLase system. Any installation/ deinstallation of hard- and software, respectively, is strictly reserved to trained and certified service personnel authorized by Vitrolife GmbH.



INTEGRITY OF THE ELECTRO-OPTICAL SYSTEM

Maintenance of microscope components, wrong handling of the microscope or strong displacement of the electro-optical system, e. g. by mechanical shock can result in an incorrect position of the beam splitter system, the magnifier lens, the camera, and the turret, respectively. As a result of any of the above, aiming of the laser may no longer correspond to the crosshair position displayed at the video image and damage to embryos may occur if the laser is used in this condition.



LASER

The laser of the LaserShot M / NaviLase system is classified as a class 1M laser. Class 1M lasers emit in the wavelength range from 302,5 nm to 4000 nm and are safe under normal operation, but may be hazardous if the user employs optics within the beam.



TECHNICAL SUPPORT

If you need assistance at any time, please contact Technical Service. For contact details please refer to the section Customer Service.

System Components

Figure 2 shows the LaserShot M / NaviLase system mounted to an inverted "Olympus" microscope, consisting of:

- the NaviLase Motion Module (NaviLase only)
- the Laser Module
- the Mirror block
- the Laser Lens
- the Eye USB 2.0 camera

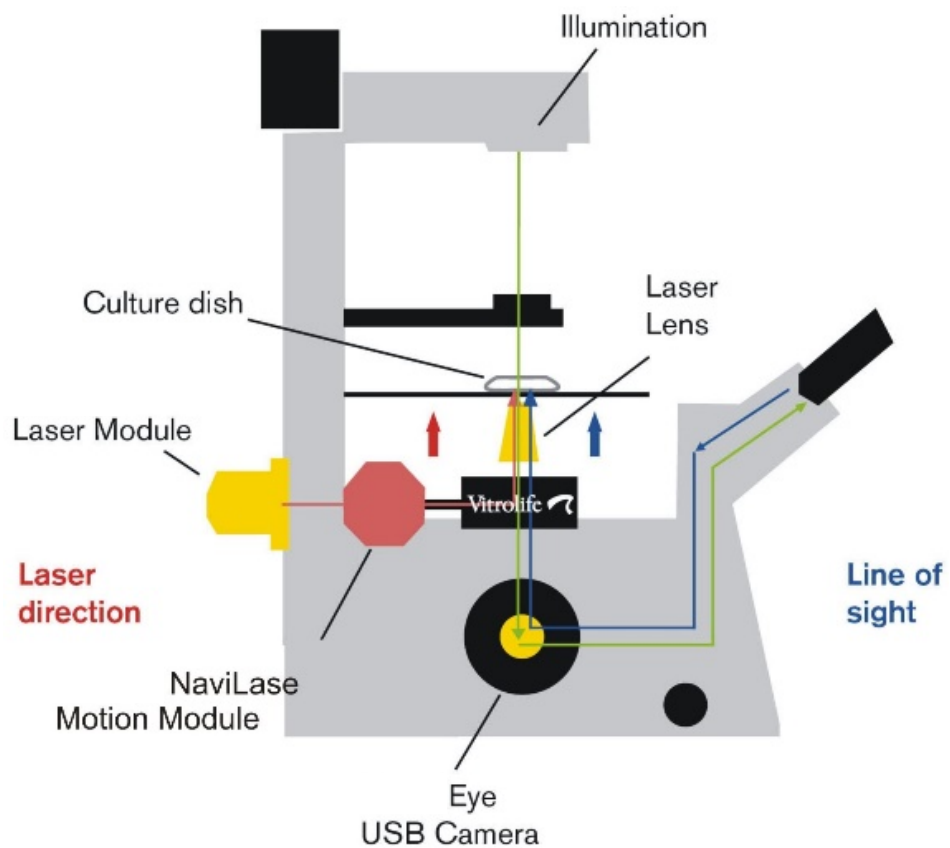


Fig. 2: Laser system components mounted onto an inverted microscope. Motion Module exclusively for NaviLase.

The following inverted microscopes are approved to be used with laser systems (this list raises no claim for completeness):

Olympus Corporation	IX 51	S8F-3
	IX 53	P1F
	IX 71	S8F-3, S1F-3
	IX 73	P1F, P2F
	IX 81	F-3
	IX 83	P1ZF, P2ZF
Leica Microsystems	DMI 3000	B and with fluorescence light path
	DMI 4000	B and with fluorescence light path
	DMI 6000	B and with fluorescence light path
	DMi8	with fluorescence light path
Nikon Corporation	Ti	S, U, E
	TE-2000	S, U, E
	Ti2	A, E
Carl Zeiss Microimaging	Axio Observer	A1, D1, Z1
	Axio Observer	3, 5, 7
	Axiovert	200

The LaserShot M and NaviLase can be supplied in different set-ups also covering Target Pointer as accessory.

Product name	REF
Target Pointer for laser systems	19310/4150
Breadboard for installing laser systems to	19310/0141
Olympus IX53/73/83	
Foot switch for releasing the laser	19310/1148US

A scheme of the complete LaserShot M / NaviLase system including the computer running the EyeWare software for visualizing the image from the Eye USB 2.0 digital camera and controlling the NaviLase by its motion control elements is depicted in below.

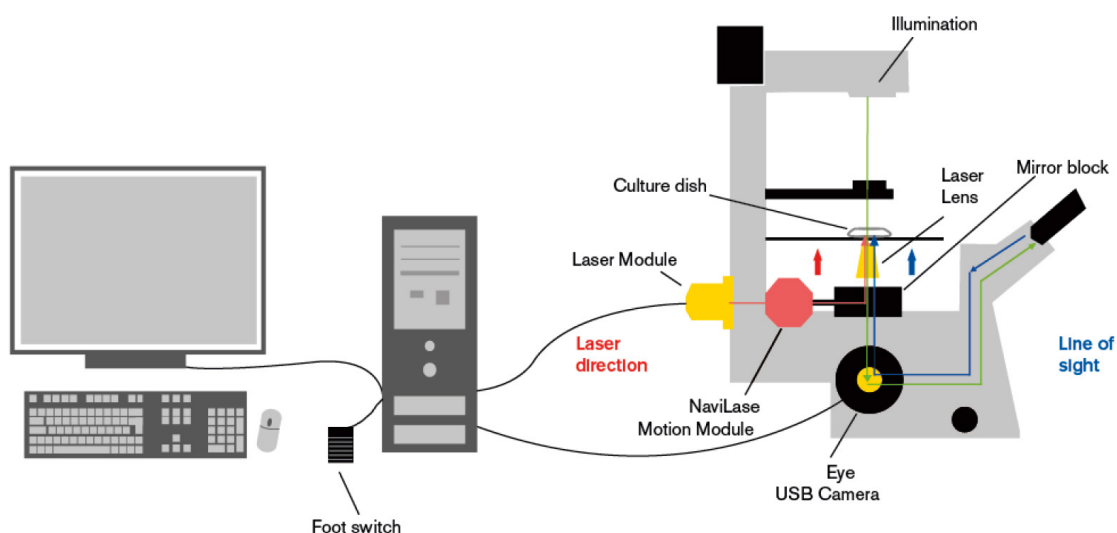


Fig. 3: Diagram of the complete LaserShot M / NaviLase system including computer, Eye USB USB 2.0 digital camera, Laser Lens and LaserShot M / NaviLase components



CONNECTORS

Do not disconnect the USB cable connector unless instructed to do so by qualified support personnel.

The Laser Systems

The schematic illustration in Figure 3 depicts the optical path of the visible light (green line) and the optical path of the LaserShot M / NaviLase laser beam (red line). Emitted from the laser module the beam can get diverted on demand by the NaviLase motion module. At the beam splitter located below the nosepiece the laser light is deflected from a horizontal to a vertical orientation and focused to the culture dish by the laser lens. Thus, the laser beam points perpendicularly upwards. The visible illumination light path proceeds perpendicularly downwards. After passing through the beam splitter of the laser, the visible light is diverted and intersected into two paths by microscope optics. Some part of the visible light gets directed towards the camera port whereas the remaining part of the light points towards the eyepiece. This design ensures the safe use of the laser even when looking through the eyepieces (blue line) whilst a laser pulse is released.

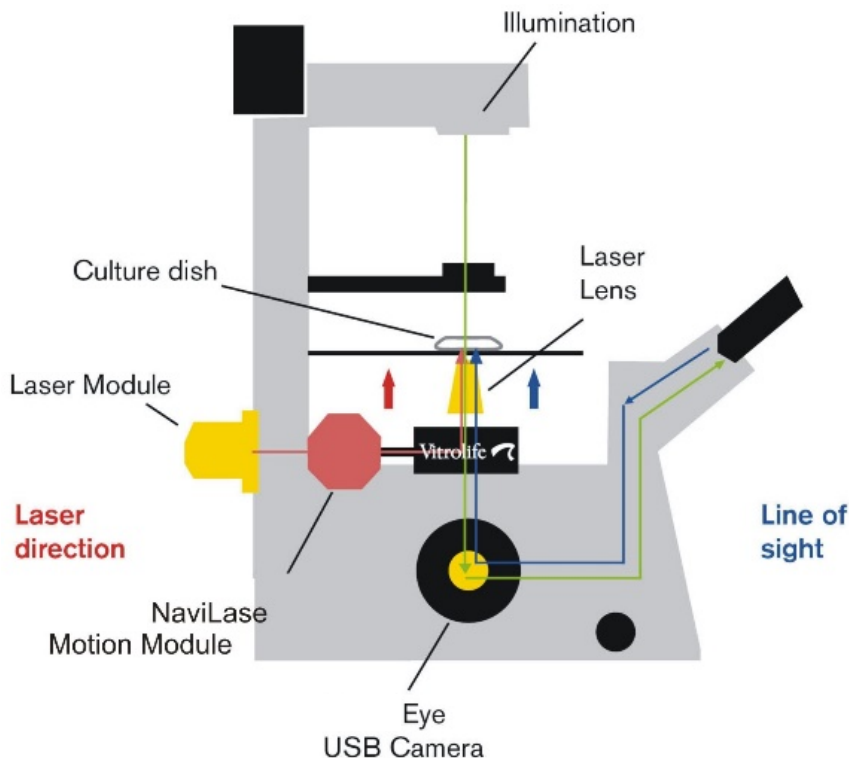


Fig. 4: Schematic view of an inverted microscope equipped with the LaserShot M / NaviLase system



EYE SAFETY OF THE OPERATOR

Eye safety of the operator is guaranteed under normal operation of the LaserShot M / NaviLase, and in a situation when user removable parts may be missing. However, do not disassemble or uninstall the LaserShot M / NaviLase system. Any installation / deinstallation of hard - and software, respectively, is strictly reserved to trained and certified service personnel authorized by Vitrolife GmbH.

On the laser module two status indicator LEDs and an USB connector are located, as shown in Figure 4. The status indicator LEDs provide information about the status of the device in addition to any messages/warnings displayed by EyeWare when controlling the LaserShot M / NaviLase system. The significance of the status indicator LEDs is as follows:

- (flashing) device USB initialization (during computer boot sequence, after device plugin)
- laser powered up, laser in standby position
- laser in unlocked position after activation through EyeWare

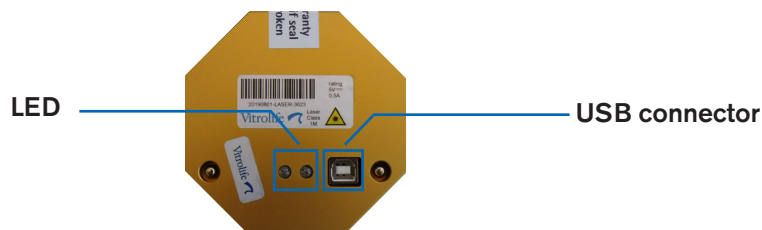


Fig. 5: laser module with detailed view of status LEDs and USB connector



LED

For normal operation, the status indicator LEDs do not provide any additional information. Please communicate the status of these LEDs when contacting Technical Support in case you are suspecting a problem with your LaserShot M / NaviLase system.

PART II: WORKING WITH LASERSHOT M / NAVILASE

This chapter provides basic information about operation and control of the LaserShot M and NaviLase device in conjunction with the EyeWare software.

Control of the Laser by EyeWare Software

The EyeWare software provides the user interface for targeting, controlling and activating laser pulses in LaserShot M and NaviLase system in combination with an Eye USB2 high-resolution camera (Fig. 6). Software applications also cover image acquisition and storage, measurements and documentation. EyeWare is a SQL server based multi-purpose imaging and archival software. The application field of EyeWare ranges from a stand-alone imaging station to a multioperational multi-user networked system. Offering specific adaptations for each user demand and application need, EyeWare is an ideal solution for clinics where image quality and authentic documentation are imperative.

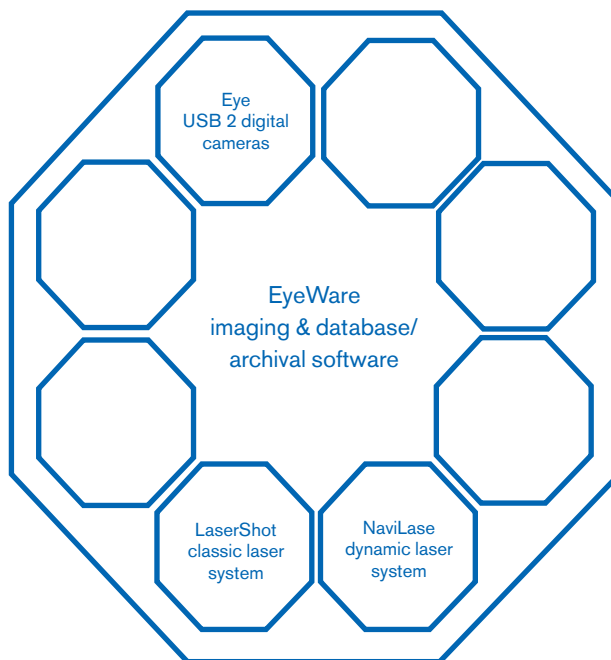


Fig. 6: EyeWare software platform for control and operation of devices

EyeWare is a SQL server based multi-purpose imaging and archival software. The application field of EyeWare ranges from a stand-alone imaging station to a multi-operational multi-user networked system. Offering specific adaptations for each user demand and application need, EyeWare is an ideal solution for clinics where image quality and authentic documentation are imperative.

Installation Requirements

For proper operation of the EyeWare software, a computer system complying with the following minimum specifications is required:

- INTEL i5 or i7 CPU
- Intel Chipset (this is a must)
- 4 GB RAM minimum
- 500 GB hard disk minimum
- 6 USB 2.0 ports minimum
- Operating System Windows 10, 32 or 64 bit
- TFT screen with full HD resolution (1920x1080)

System Components

EyeWare software comes on a DVD or USB flash drive and only operates in conjunction with a corresponding USB dongle (Fig. 7).



Fig. 7: EyeWare software USB dongle

The EyeWare software provides the user interface for targeting, controlling and activating laser pulses. In the following sections, many different aspects of EyeWare are described with special focus on features which are relevant for laser control, image capture and processing.

Setting up EyeWare Software

As an integral part of the LaserShot M / NaviLase system, EyeWare software has to be installed in combination with the hardware of LaserShot M / NaviLase system itself.



WARNING

The recipient end user of the LaserShot M / NaviLase systems should not unpack and install it upon receipt. Unpacking, installation, setup and end-user training of LaserShot M / NaviLase systems must be carried out by appropriately qualified technical staff authorised by Vitrolife GmbH.

Calibration setup

Calibration of objectives and the intermediate magnification is done during the setup of the laser system. Verification or modification of the pre-set calibration can be done at any time. Any modification of the calibration settings require restart of EyeWare.

Open the “calibration setup” dialogue at the FILE menu and select brand of microscope at first.

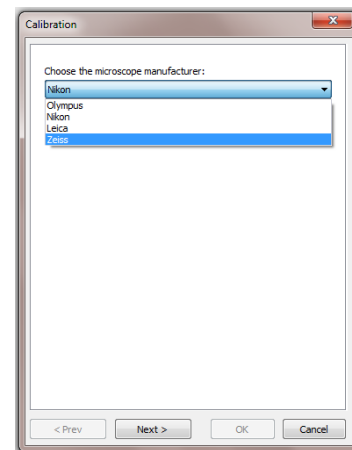


Fig. 8: EyeWare calibration setup dialogue

Specify whether or not the microscope is equipped with magnifier. If there is a magnifier define the magnification ratio.

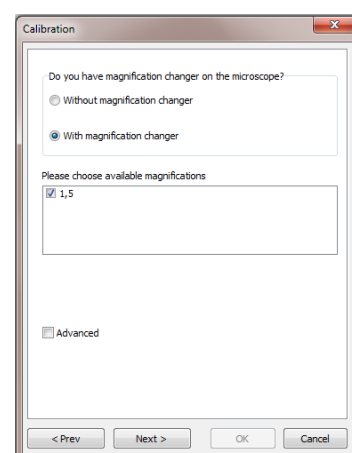


Fig. 9: EyeWare calibration setup dialogue

Specify manufacturer of the lens and its magnification for each individual nose head position. For any nose head position not hosting any lens uncheck the box "in use". After definition of all 6 positions is done, confirm the settings and restart EyeWare in order to apply the new settings.

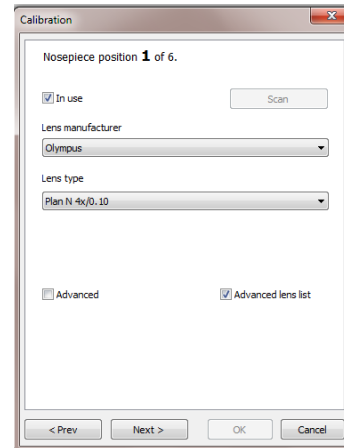


Fig. 10: EyeWare calibration setup dialogue

Main concept and workflow

The main concept of EyeWare is the transfer of data between different sections of the software. A typical workflow consists of the following steps:

1. Activate the live video display by accessing the **Video page**.
2. Find the target object at the live video display by adjusting the microscope.
3. Take a **Snapshot** by pressing the <F10> key. This automatically saves images to Quick File.
4. In order to select for the images which should be permanently stored, navigate to the **Quick File** page.
5. Double-clicking to the selected image opens the **Image page**.
6. Add some **Measurements**.
7. Return to the Video page. When changing the page, the **Storage Wizard** prompts to save data to the database. Complete the wizard in order to save data; then the Video page is displayed again.

Return to step 1 and repeat as often as necessary.

8. Finally, navigate to the **Database page** select patient and examination, switch to the **Report page**, preview and print the examination report.

In order to recall previously saved information, navigate to the Database page or to the Quick File Page.

All aspects **highlighted** are explained in more detail in the following.

Structure of EyeWare

The EyeWare software consists of several program sections each appearing as separate pages at the EyeWare main window. See Figure 11 for the different elements of the main window (menu bar, main toolbar, pages and status bar).

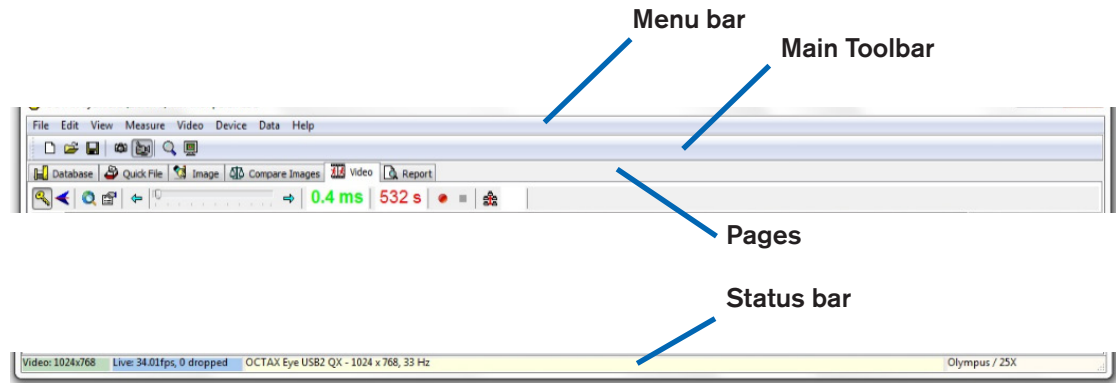


Fig. 11: Elements of the EyeWare main window

Menu Bar

The main menu of EyeWare is structured according to Windows standard, containing:

1. The File Menu: Navigate to Open / Save Image, Print, Change Report Template, Export, Send as Email Attachment, and Program Settings dialogs.
2. The Edit Menu: Contains standard Windows functions including Cut / Copy / Paste / Delete operations. Additionally, Insert Measure can be selected.
3. The View Menu: Activate / deactivate the display of Standard and Data Toolbars and Status Bar.
4. The Measure Menu: Navigate between Type of Measure Line and the Measurement Customisation dialog.
5. The Video Menu: This Menu is active when the Video page is in use. It allows navigating between Snapshots, Quick Snapshots, Full Screen display mode, video sources and Camera Setup.
6. The Device Menu: This menu allows managing the LaserShot M / NaviLase device or additional devices connected.
7. The Data Menu allows navigation and editing. Within the Database and Quick File page this function allows navigation in, and editing of the active category and images, respectively.
8. The Help Menu: Provides Information about the current version of EyeWare installed and allows generating and transmitting Support Request Data.

Main Toolbar

The main toolbar contains the most important commands, such as image file management, video snapshot and the standard clipboard buttons.

Pages

The software is organized into six pages, representing areas of the program. By changing between pages, certain actions are triggered.

1. The **Database** page: Management of data, containing patient data and examination records.
2. The **Quick File** page: Image storage for transient use in an image gallery.
3. The **Image page**: Processing of individual images including measurements and measurement labels. Any changes can be saved to the Database, the Quick File or export as Bitmap or JPEG file.
4. The **Compare Images** page: Possibility to compare up to 4 images
5. The **Video** page: Live video display, control of supported devices such as LaserShot M / NaviLase, optical calibration settings, and image gallery
6. The Report page: Preview of examination report, print report or save to PDF .

Status Bar

The status bar shows useful information about the status of the program, including the current video camera resolution, refresh rate, the number of measurements performed, current calibration and database information.

Video Page with Laser Targeting Feature

On the Video page, EyeWare displays a live video stream acquired by the Eye USB2.0 digital video camera. Depending on the use of the laser either in the classical 'static' mode or in the dynamic 'moveable' mode, two different appearances of the graphical user interface are available: the classical 'Windows'-like view and the full screen view.

The classical view of the Video page acts as a control interface for the LaserShot M system and the static mode of the NaviLase system. For laser targeting, control and activation, the Video page has to be opened. Figure 12 illustrates the essential elements of the Video page, including the Laser Toolbar and the Crosshair.

The Video page provides the basis for snapshots from the live video display. The status bar provides information about the camera resolution, the amount of images captured per second (fps) and the current calibration settings.

Appearance of the Crosshair

The video image can be overlaid by a Crosshair which indicates the target area of the laser beam. A combination of up to two crosshairs and three circles can be selected. The circles represent diameters of 10 μm to 40 μm of the microscopic image shown on the monitor. In order to activate or change the appearance of the Crosshair, select Video – Overlay – Crosshair. The preferred appearance can be selected from the drop-down menu (Fig. 13).

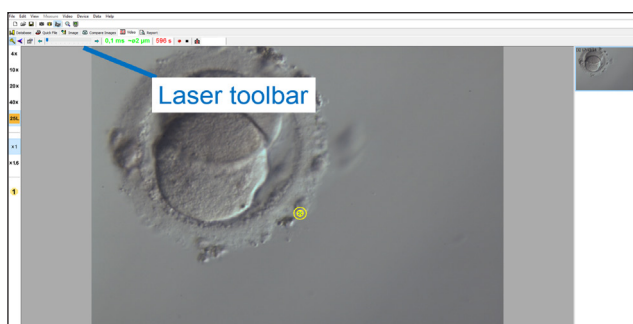


Fig. 12: EyeWare Video page with Laser Toolbar and laser targeting Crosshair

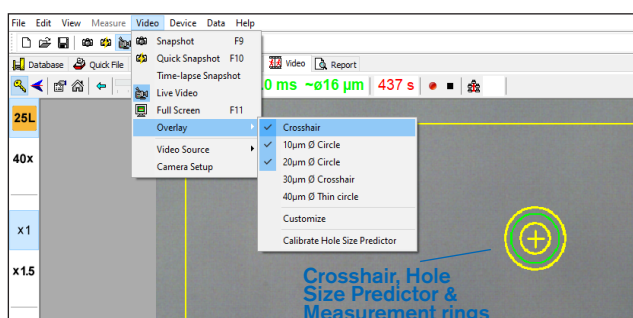


Fig. 13: Selection of the Crosshair mode at the Video pagewindow

Laser Hole Size Predictor

The Laser Hole Size Predictor is displayed as a green circle which is part of the electronic target overlay. The circle diameter of Laser Hole Size Predictor serves as a convenient means of reliably indicating the expected hole size in correlation to the selected pulse time. It interactively changes its diameter depending on the pulse time selected (Fig. 13). Vice versa the Laser Hole Size Predictor allows adjusting the pulse time in accordance to the required hole size.

Calibration of the Hole Size Predictor

After installation of the laser system or after introducing changes to the current microscope setup (e.g. heated microscope stage), the Hole Size Predictor has to be calibrated to reliably predict the hole size for the current microscope setup. The calibration procedure is based on the actual diameter of a hole which was drilled at defined settings (see in **bold** below).

The diameter of the Hole Size Predictor can be calibrated in a range between -70% and +200% in steps of 5% relative to the current value (Fig. 14).

Follow the steps below to calibrate the diameter of the Hole Size Predictor:

1. Select the live video page, full-screen mode (F11), in the EyeWare software.
2. Place a **warmed dish (37°C / 98,6°F)** containing a cell which will not be used for any further treatment on the **heated microscope stage**, e.g. an oocyte which failed to fertilize. The cell should be positioned at the **bottom of the dish**.
3. Locate the cell through the **laser lens** and focus on the zona pellucida.
4. Unlock the laser, using the corresponding software button.
5. Select "Hole" as working mode from the menu if using NaviLase.
6. Using the computer mouse and "drag & drop", direct the circle indicating the laser target to an area on the zona pellucida (NaviLase).
7. When using LaserShot M, align the zona pellucida with the laser target using the cross moving table of the microscope.
8. Using the ms scale, adjust the laser pulse length in a way that the diameter of the circle indicating the position and diameter of the laser holes corresponds to the zona pellucida thickness.
9. Release the laser by clicking the button or by pressing the footswitch while avoiding any vibrations of the microscope.
10. Observe the diameter of the drilled hole: if overlapping with the green circle of the Hole size predictor, no further action is needed.
11. In case the diameter of the drilled hole does not match with the diameter of the green circle, proceed with the next step for calibration:
12. Select Video - Overlay – Calibrate Hole Size Predictor from the menu and adjust the indicated hole size by clicking the arrows on the right side to overlap with the actual diameter of the drilled hole (Fig. 14).
13. Click ok to save the change.
14. To verify calibration, drill a new hole in another area of the zona pellucida and observe the diameter of the drilled hole and green circle.

The Hole Size Predictor is now calibrated to predict the diameter of drilled holes according to the selected pulse lengths (at 37°C / 98.6°F, laser lens, zona at the bottom of the dish, current microscope setup).

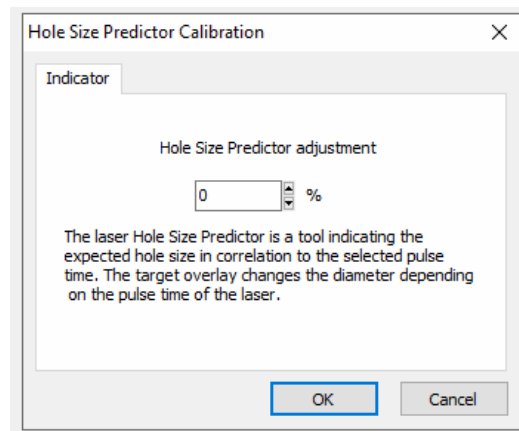


Fig. 14: Calibration of the Laser Hole Size Predictor at the Video page

INFLUENCE OF Z-POSITION OF THE EMBRYO ON DRILL OPENING SIZE



Inappropriate Z-positioning will result in smaller drill holes and in reduced laser beam quality. It is recommended to keep the cell near the bottom of the culture dish during laser treatment.

Alignment of the Crosshair

In order to match the position of the Crosshair with the center of drilled hole, select Video - Overlay – Customize from the menu and correct the position of the crosshair in XY-orientation by scrolling the sliders.

Alternatively, one slider may be highlighted by clicking and the Crosshair may be moved to the proper position by use of the cursors of the keyboard. This allows for a more precise control (Fig. 15). Another possibility to position the electronic target would be to move it to its exact position by keeping the <CTRL> key pressed whilst dragging it with the mouse.

Please note that the electronic Crosshairs can only be repositioned as long as the laser stays in its home position (NaviLase only).

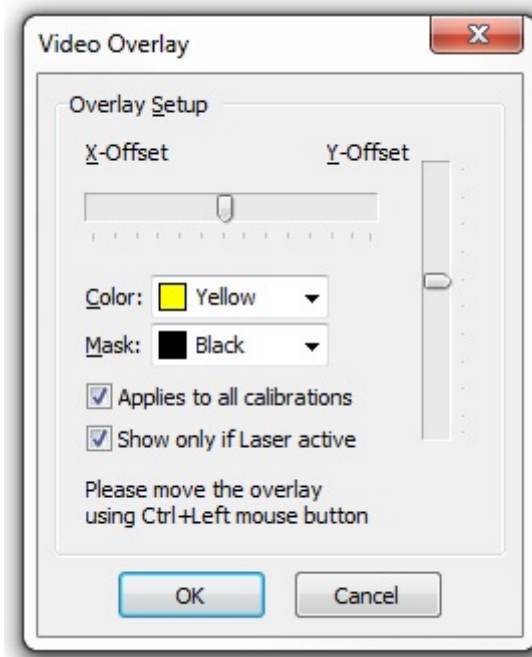


Fig. 15: Control of Crosshair position

The Video Toolbar



The Video toolbar provides the elements of the user interface for controlling the laser. Pressing the Key button turns the laser into active mode.



Clicking the Laser release button triggers a laser pulse. Alternatively the laser can be triggered by pressing the laser release button of the computer mouse or optionally by an external foot switch.



The homing function for NaviLase resets the position of the movable laser beam to the home position of the fixed laser. LaserShot M is invariably in home position.



The laser is secured by a timeout after 600 s of inactivity of operation and will switch automatically to an inactive mode.



The laser irradiation time can be adjusted with the slider. Additionally, both arrow icons next to the slider are available to increase or decrease the duration time in steps of 0,5 ms. A more precise adjustment of the laser duration time can be accomplished by using the arrow keys of the computer keyboard.



The current laser irradiation time is displayed in green color.



Whilst displaying a live video stream, pressing the video capture icon starts recording of a video sequence. There is no limitation in number and length of video files. After finishing the sequence by clicking the stop button the software starts the storage wizard. Video files will be saved and can be recalled from the Database page in the section Video clips.



Given that computer hosting EyeWare is connected to a local network (LAN) the broadcasting option of live images is supported. For this purpose a TCP/IP network connection between individual EyeWare stations is required. Whilst the software displays the live video stream, broadcasting can be activated by clicking on the "Network" icon. A successful launch will be displayed with the message "on air"



Information about the status of the laser is displayed in the Laser Properties dialog (Fig. 16), which can be activated by clicking the icon. The information is useful in case of contacting Technical Support.

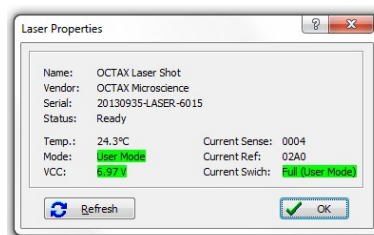



Fig. 16: Laser Properties dialog (Laser properties data shown are exemplary)


Triggering the Laser

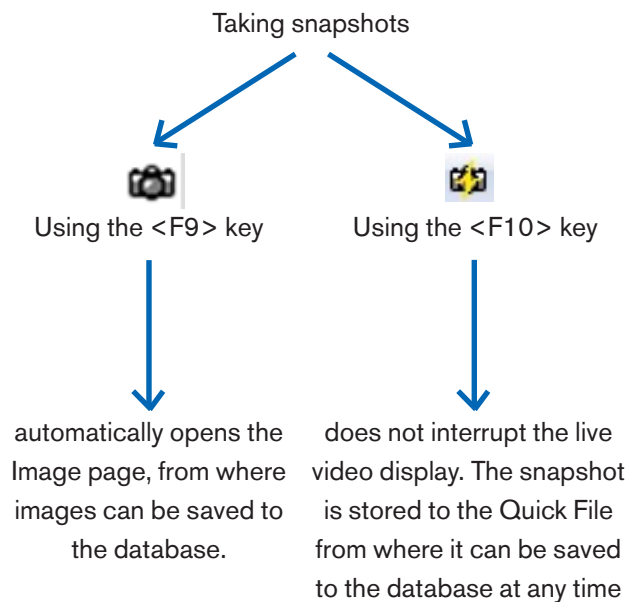


After switching the laser into active mode and selecting the irradiation time, LaserShot M / NaviLase can be triggered by clicking , by pressing the laser release button of the computer mouse or optionally with an external foot switch.

Taking Snapshots

A snapshot of the live video image can be taken by clicking the camera button at the main toolbar, in the Video menu or by pressing the <F9> key. This will automatically open the Image page. Now some Measurements can be added. For saving the image select the button or return to the Video page. When changing page, the Storage Wizard prompts to save image data to the database.

Taking a snapshot of the live video image without interrupting the video display is accomplished by pressing the <F10> key. The snapshot image will be stored to the Quick File from where it can be saved to the database by clicking the batch transfer button .



Calibration side bar

For quick recalling a pre-defined calibration set due to changing the objective or intermediate magnification, select the corresponding button within the left sidebar of the EyeWare window (Fig. 17).

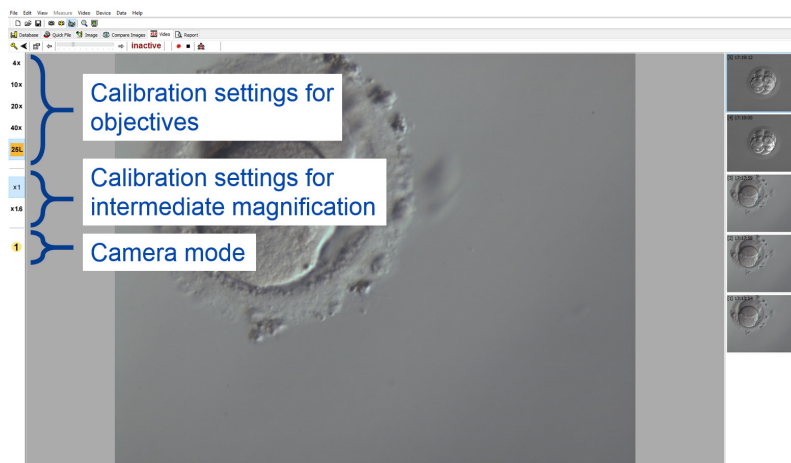


Fig. 17: Display of calibration settings for objectives, for the intermediate magnification, and the camera mode within the left sidebar

Active calibration settings are displayed by a colored button corresponding with the color code of the lens. Likewise, the camera settings in use are indicated by an active button within the left sidebar and displayed at the status bar.

Note: The calibration values are saved with each image, both in the Database and in the Quick File. Each time an image is recalled from either location, there will be a notification to restore the calibration setting that is compatible with the recalled image (Fig.18).

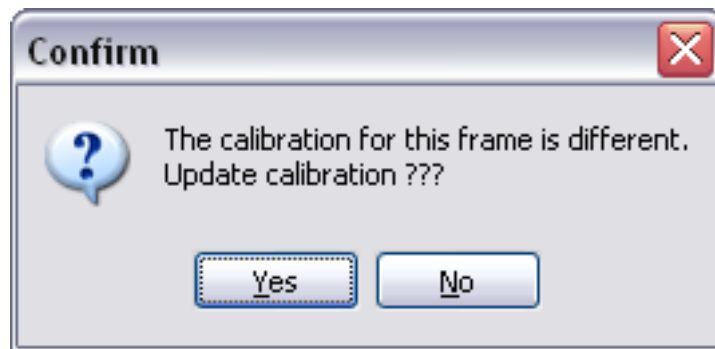


Fig. 18: Confirm dialog box

When switching back to the live video stream, the calibration settings which are indicated on the left sidebar become active again.



WARNING

The recipient end user of the LaserShot M /NaviLase systems should not unpack and install it upon receipt. Unpacking, installation, setup and end-user training of LaserShot M systems must be carried out by appropriately qualified technical staff authorised by Vitrolife GmbH.

Image gallery sidebar

Any image being taken by using the quick snapshot function by pressing the <F10> key will be displayed in the right sidebar on the Video image page (Fig. 19). The image gallery sidebar provides instant display and easy navigation between acquired images which have been stored to Quick File.

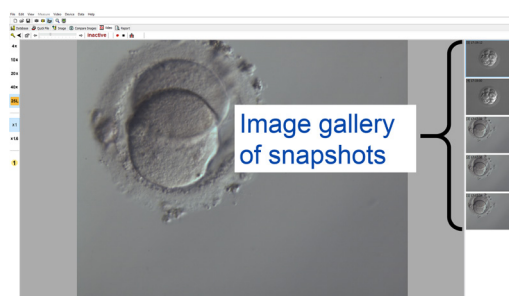


Fig. 19: Video page with image gallery sidebar

The Full Screen Mode: LaserShot M and dynamic operation of NaviLase

The dynamic features of NaviLase are only available in the full screen mode of the EyeWare (Fig. 20). Also LaserShot M can be controlled here. The full screen mode can be accessed by pressing the <F11> key. Pressing the <F11> key again allows returning to the classical view for the operation of NaviLase in the static mode.

The left sidebar of the full screen view contains control elements for taking snapshots and capturing videos. By activating the button "Arm laser" all relevant elements for the control of the LaserShot M or dynamic NaviLase laser function can be accessed ,i.e. adjusting the pulse time, selecting the working mode of the laser (NaviLase only), and triggering the (first) laser pulse(s).

All control elements for the camera mode and the appropriate calibration settings for the objective and intermediate calibration in use are located within the left sidebar.

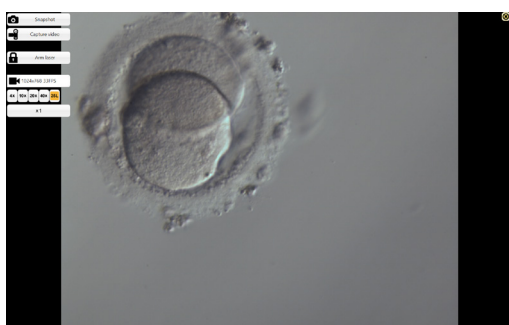


Fig. 20: Full screen mode for NaviLase dynamic operation

Taking Snapshots

Snapshot images acquired by either clicking the "Snapshot" button or by pressing the <F10> key are displayed within the image gallery. Individual Snapshot images within the gallery can be enlarged by moving the mouse cursor across the image of interest (Fig. 21).

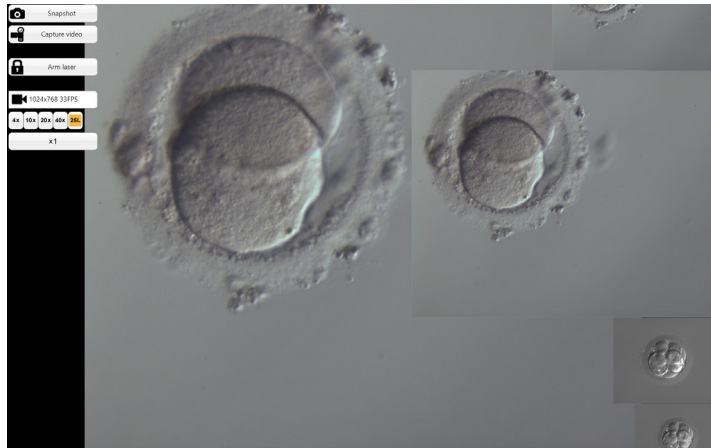



Fig. 21: Full screen mode with image gallery sidebar zoom function

Arm Laser

Clicking to  activates the LaserShot M or NaviLase laser and provides access to the respective control elements.

Clicking "Start"  initiates the laser action

NaviLase control elements for dynamic operation (not available for LaserShot M)

All control elements required for the dynamic operation of NaviLase (Fig. 22) can be accessed after activating the "Arm laser" field.

As a first step, the laser working mode has to be selected. Moving the mouse cursor across the field 'Hole' activates a drop down menu. Available working modes are the positioning of a hole, of a straight line or of a curved line to anywhere within the working area.

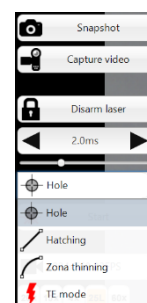


Fig. 22: Selection of laser dynamic working modes

Hole (NaviLase dynamic operation)

After selecting "Hole" as the designated operation mode, the electronic laser target gets activated. It indicates the current position of the laser impact and the expected hole size, depending of the selected pulse time. By using the drag & drop function of the mouse the electronic target can be moved to the intended position within the working field (Fig. 23). Adjustment of the expected hole size can be done by modifying the "Pulse time" either by moving the slider, clicking to the arrows or turning the mouse wheel.

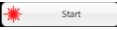

Clicking "Start"  initiates the laser action and converts this field into an emergency stop  button which may be used stall the laser action at any time.

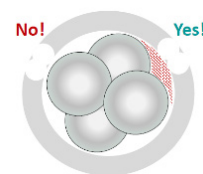


Fig. 23: NaviLase operational mode for drilling single holes at a position of choice



PVS (PERIVITELLINE SPACE) MAX. RULE



For maximum safety and minimal invasive reasons, always select the spot to perform the laser drilling where the distance between cell membrane and zona pellucida is the largest



Hatching (NaviLase dynamic operation)

The "Hatching" mode offers the possibility to apply a defined number of laser pulses along a straight line. It is ideal for drilling small openings, either for assisted hatching or for biopsies

After selecting "Hatching" as the designated operation mode, the electronic laser target gets activated. It shows the current position of the laser impact (indicated by a red dot in the center) and the expected hole size including a short line. By dragging & dropping the red starting spot of the line the electronic target line can be moved to the intended position within the working field (Fig. 24). The length of the line is defined by the distance between the blue starting and the yellow stopping spot. The distance can be adjusted by dragging each of the spots to their confining positions. Adjustment of the expected hole size can be done by modifying the "Pulse time" either by moving the respective slider, clicking to the arrows or turning the mouse wheel.

Clicking "Start"  initiates the laser action and converts this field into an emergency stop button  which may be used stall the laser action at any time.

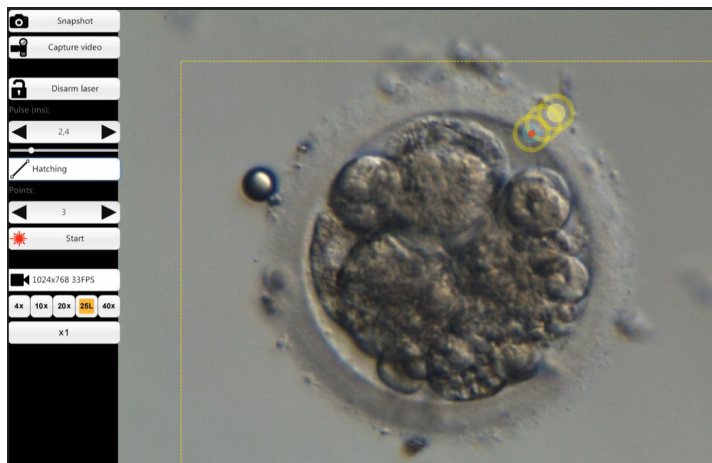


Fig. 24: NaviLase operational mode for drilling holes along a predefined straight line

Zona thinning (NaviLase dynamic operation)

The "Zona thinning" mode offers the possibility to apply a defined number of laser pulses along a curved line.

After selecting "Zona thinning" as the designated operation mode, the electronic laser target gets activated. It shows the current position of the laser impact (indicated by a red dot in the center) and the expected hole size including a curved line. By dragging & dropping the blue starting spot of the line the electronic target line can be moved to the intended position within the working field (Fig. 25). The length of the line is defined by the distance between the starting and the yellow stopping spot. The distance can be adjusted by dragging each of the spots to their confining positions. The radius of the curved line can be adjusted to the perimeter of the zona pellucida by moving the pink spot to the appropriate position. Adjustment of the expected hole size can be done by modifying the "Pulse time" either by moving the respective slider or by clicking to the arrows.



Fig. 25: NaviLase operational mode for drilling holes along a predefined curved line

The degree of overlapping between adjacent circles and hence the shape of the line produced by the laser can be determined by adjusting the number of pulses whilst keeping the pulse time (the hole size) at a defined value (Fig. 26).

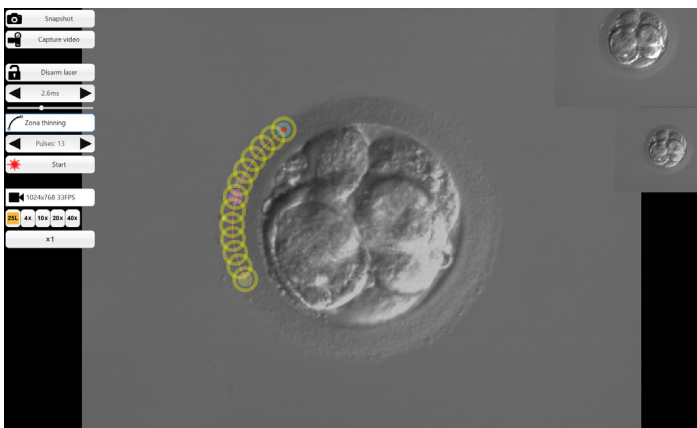




Fig. 26: NaviLase operational mode for drilling holes along a predefined curved line with high degree of overlapping of individual shots

Clicking "Start"  initiates the laser action and converts this field into an emergency stop button  which may be used stall the laser action at any time.

TE mode (NaviLase dynamic operation)

The "TE" (trophectoderm) mode offers the possibility to apply a preselected number of one to three laser pulses along a straight, vertical line. It is used to support a trained operator during trophectoderm biopsy for the step of releasing intracellular bonds between mechanically stretched trophectoderm cells. The TE mode compensates for higher absorption of laser energy given by the larger size of a blastocyst (compared to opening zona pellucida of a zygote).



TE MODE: RESTRICTIONS IN USE

The TE mode must only be used by experienced users trained in doing trophectoderm biopsies. The laser puls(es) can assist the release of mechanically stretched intracellular bonds between trophectoderm cells for biopsy. The TE mode must never be applied to the zona pellucida.

Consult the Indications for Use chapter for recommended procedures how to perform TE cell biopsy, including opening sizes and laser pulse length (pp 22).

After selecting "TE mode" as the designated operation mode, the electronic laser target gets activated. It shows the current position of the laser impact (indicated by a red dot in the center) and a line along which the preselected number of shots will be applied.

The number of shots applied in TE mode can be pre-selected in the classical view of EyeWare as shown in Fig. 27. From the menu select Device -> NaviLase -> TE mode to select 1, 2, or 3 shots.

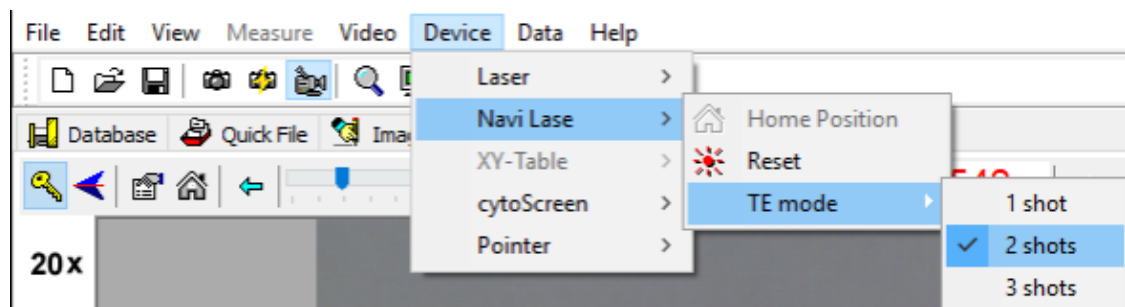


Fig. 27: NaviLase preselection of laser pulses in TE mode

For operation in full screen mode, the electronic target line can be moved to the intended position within the working field by dragging & dropping the red starting spot of the line (Fig. 28). Adjustment of the laser impact can be done by modifying the "Pulse time" either by moving the respective slider, clicking to the arrows or turning the mouse wheel.

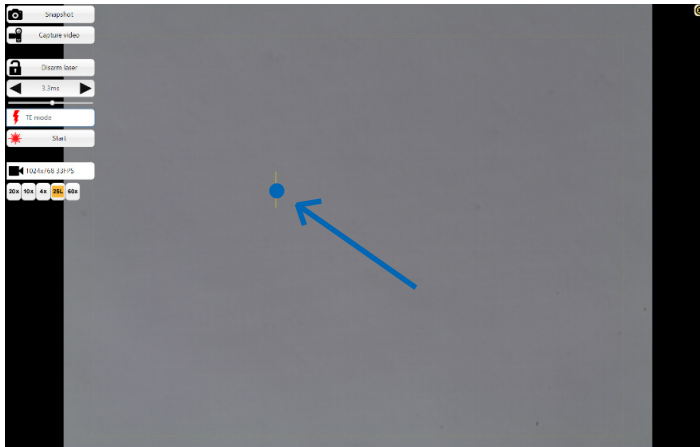





Fig. 28: NaviLase target line in TE mode (see arrow).

Clicking "Start"   initiates the laser action and converts this field into an emergency stop button  which may be used stall the laser action at any time.

TE mode using LaserShot M

The "TE" (trophectoderm) mode compensates for higher absorption of laser energy given by the larger size of a blastocyst (compared to opening zona pellucida of a zygote). It offers the possibility to apply a laser pulse to support a trained operator during trophectoderm biopsy for the step of releasing intracellular bonds between mechanically stretched trophectoderm cells.



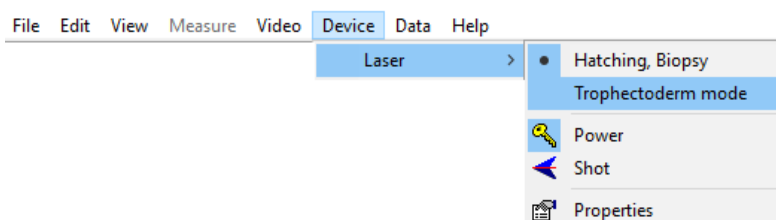
TE MODE: RESTRICTIONS IN USE

The TE mode must only be used by experienced users trained in doing trophectoderm biopsies. The laser pulse can assist the release of mechanically stretched intracellular bonds between trophectoderm cells for biopsy. The TE mode must never be applied to the zona pellucida.

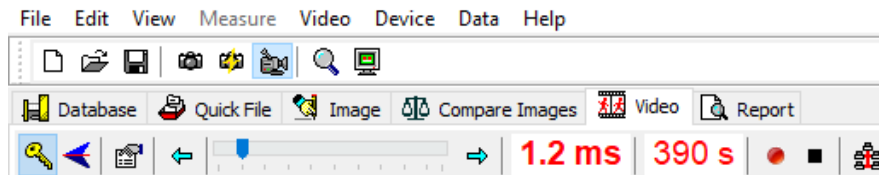
Consult the Indications for Use chapter for recommended procedures how to perform TE cell biopsy, including opening sizes and laser pulse length (pp 22).

To select the TE mode in the classical view of EyeWare:

Select Device -> Laser -> Trophectoderm mode from the menu



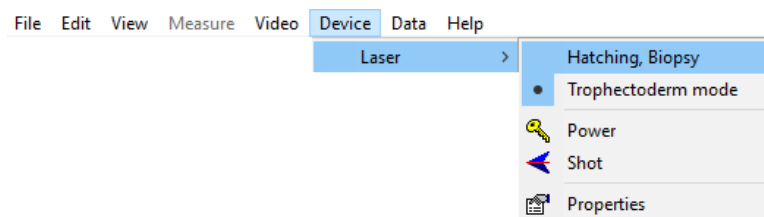
After selecting "Trophectoderm mode" as the designated operation mode, the display of laser pulse length changes from green to red color. This is to indicate that TE mode is active and the last pulse length used in TE mode has been saved and is displayed. The Hole Size Predictor (circle) is inactivated automatically. The laser target is indicated by a crosshair.



Use the laser to support in TE biopsy as recommended in Indications for Use (pp. 19)

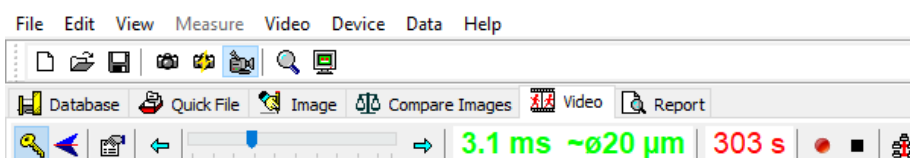
After finishing TE cell treatment, switch back to hatching/blastomere biopsy mode:

Select Device -> Laser -> Hatching, Biopsy from the menu.



Alternatively, the laser is reset to hatching mode automatically after switching the laser off and on again using the key symbol from the laser tool bar.

Check that the display of laser pulse length is displayed in green color to indicate hatching mode. The last pulse length used in hatching mode has been saved is displayed. The Hole Size Predictor is reactivated automatically.



REPEATED LASER SHOTS

Repeated laser shots to the same position of the embryo could result in an increased risk of embryo damage. In case of risk of applying repeated laser shots to the same position of the embryo the laser action can be immediately stalled by pressing the emergency stop button.

Quick File Page for Rapid and Temporary Storage of Images

The Quick File provides a clipboard-like storage of images without associated measurements. It can be useful for quickly saving an image without following the complete procedure of the Storage Wizard. For recalling an image from Quick File and displaying it at the Image page, e.g. for reviewing measurements or saving to a file or to the database, highlight the desired image and double-click it.

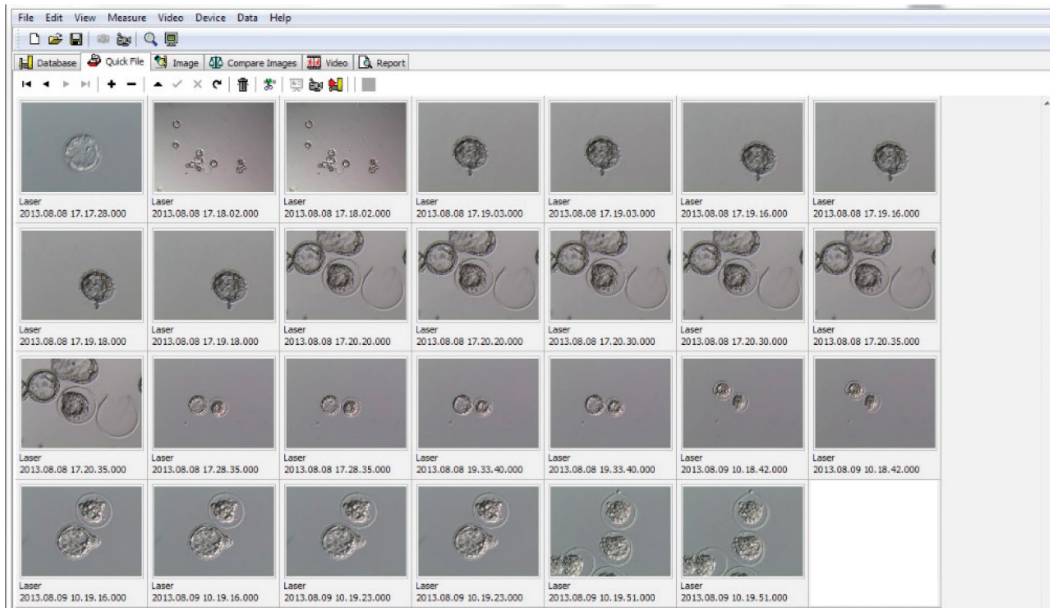


Fig. 29: Quick File page

There is no limitation in number of images saved to Quick File. However, a large number of images makes it more difficult to identify a specific one. When using the Quick File, it is recommended to empty the Quick File gallery after completing the storage of all images which are intended to be saved (Fig. 29) at the end of the day.

The Quick File Toolbar

The Quick File toolbar mainly includes the functions similar to the functions of the Database toolbar. For information regarding a specific button move the mouse cursor over any button to get a short description of its function or consult the chapter Database Page. Additional functions are:

Delete Function



For deleting selected items of the Quick File click on the trash can icon. Keep the [shift] and/or [CTRL] key pressed and select all desired images.

Batch Transfer to the Database



Using the database batch transfer button, more than one image can be transferred to the database at the same time with the Storage Wizard. More than one image can be selected by keeping the [shift] or [Ctrl] key pressed and highlight all desired images. After the transfer of all selected images to the database has been completed, a dialog box prompts to select whether or not stored images should be deleted from Quick File.

Compare Images Page

The Compare Images page provides the possibility to display and compare up to four selected images. In order to transfer images to the Compare Images page, select images from Quick File by right-clicking and assign the corresponding position this particular image is thought to have on the Compare images page (Positions A,B, C, or D) (Fig. 30). EyeWare does not evaluate the images selected for comparison.

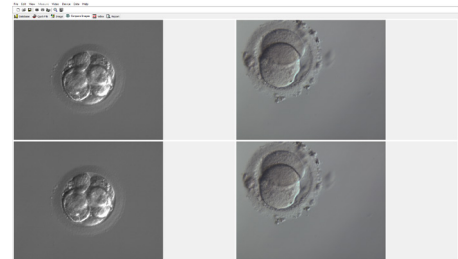



Fig. 30: Compare Images page

Image Page with Measurement Feature

The Image page is mainly used to assign measurements to newly acquired snapshots

(Fig.31). Additionally, existing images can be loaded from either the database (double click to the respective image at the Database page) or from files (select File > Open Image) and displayed. Changes can be saved either to the database by the use of the Storage Wizard (select  or change page) or to file (selecting File > Save Image). The measurement function allows the determination of the cell diameter, the hole diameter and the thickness of the zona pellucida.

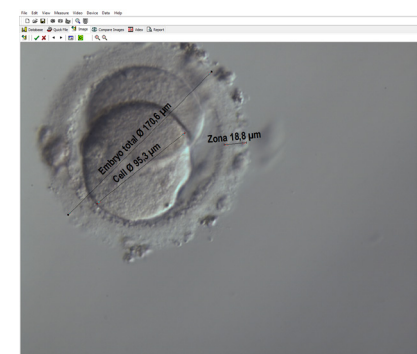


Fig. 31: Image page with measurements

Measurement Toolbar

For information regarding a specific button move the mouse cursor over any button to get a short description of its function.


Measurement Function

By using the measurement function new measurements can be applied. In order to determine the size of an object, click to the position where the line should start and hold the mouse button down until the end point is reached. The length of the drawn line is displayed in μm . The measurement function can either be activated by clicking the measurement button or by pressing the <Ins> key.

After selecting the drawn measurement line by clicking, it can be edited with different operations, like cut / copy / paste / delete, resizing, moving etc. The type of measurement can be modified from the Measure menu.

Calibration of EyeWare is required for carrying out measurements. Calibration depends on the microscope set-up and is set at "calibration setup...". Please make sure to select the proper calibration settings before applying any measurements.

Apply changes to the database

Clicking  saves any modifications to the database, which then are displayed at the measurement category of the database page.

Clicking  cancels changes and reverts the image to the last version stored.

Navigation

Using the arrows allows scrolling through the gallery of images of a patient in a selected examination or through the gallery of images at the Quick File page, depending on the location from which the image has been accessed.

Type of Measure Line

The type of measure line can be modified in the *Measure* menu. Either a predefined label (cell diameter, cell total diameter, hole diameter, zona pellucida thickness) can be selected (Fig. 32) or a new type of measure can be created using the measure customization dialog.

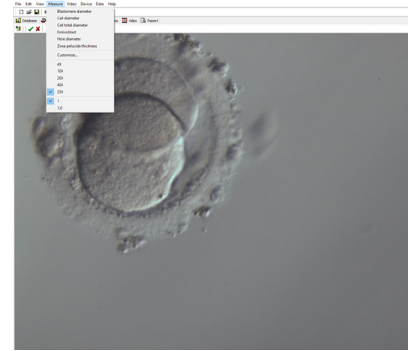


Fig. 32: Defining the type of measure in the Measure Menu

Measure Customisation Dialog

A new Measurement application can be created and a list of defined measurements can be set up (Fig. 33).

A new Measurement application is created by clicking New App, then the measurements are defined as follows:

- its name - for the printed report
- its short label - for the measure lines displayed on the image
- its long label - for the display in the menu

If a measurement is identified with diameter "TRUE" the program will calculate area and volume, assuming the object is spherical. Adding the "&"-character to the long label allows creating keyboard shortcuts, by defining the character following the "&" as the shortcut key. For example: &Cell Diameter will define "C" as shortcut in the measure menu (Cell Diameter).

The copy (Ctrl C) and paste (Ctrl V) short-cuts can be used to copy the Ø - symbol.

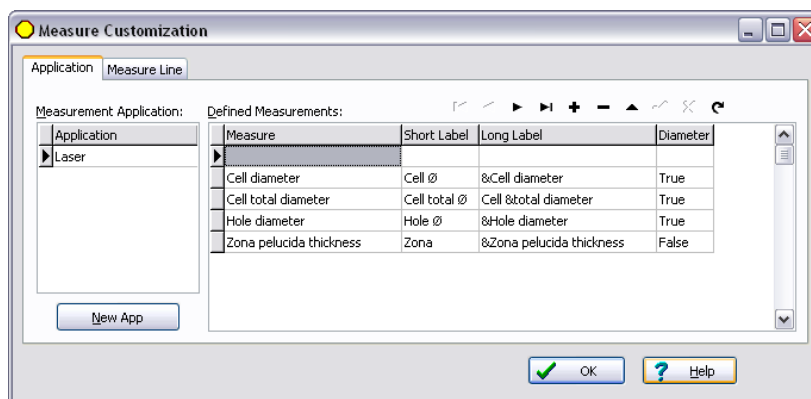


Fig. 33: Measure customisation dialog, measure application setup

Measure Line

For the individual appearance of the measure lines their color, arrow color, arrow fill color, shadows and text font can be configured (Fig. 34).

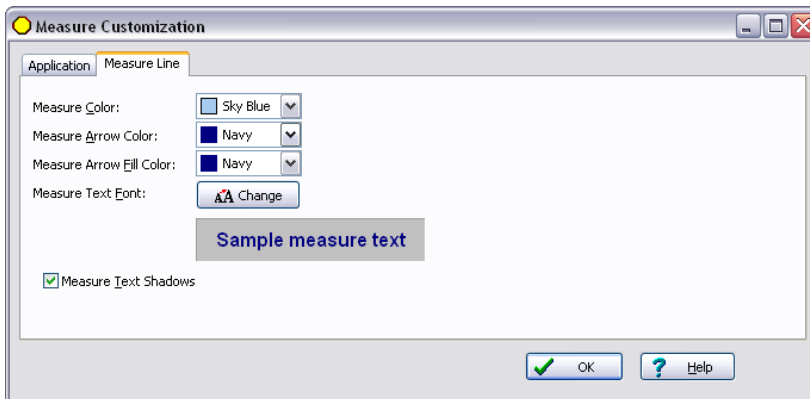


Fig. 34: Measure customisation dialog, measure line properties

Storage Wizard for Associating Images with Patients

For simplifying the process of storing a new image to the database of EyeWare, the Storage Wizard guides through the steps necessary to save an image. Please note every image intended to be kept has to be saved to the database.

1. Deciding where to save the image

It can be chosen between storing the image temporarily to Quick File or associating the image with a patient, respectively.

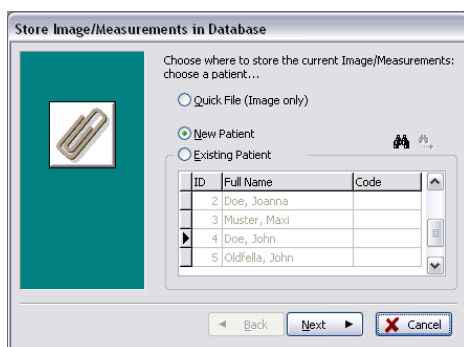



Fig. 35: dialog saving images

In case an image is supposed to be associated with a patient data set either choose New patient to generate a new patient data set (please proceed to step 2), or select an Existing patient from the list. In case of a large patient population, use the search function, by clicking . Now search for ID, Last Name, First Name, Birthday or Code (proceed to step 3).

2. Adding a new patient

When adding a new patient file, the database allows entering a limited amount of information about the patient.

- Last Name
- First Name
- Birthday
- Code

For the field 'Code' either the ID number of the main patient tracking database or any other unique identifier can be chosen.

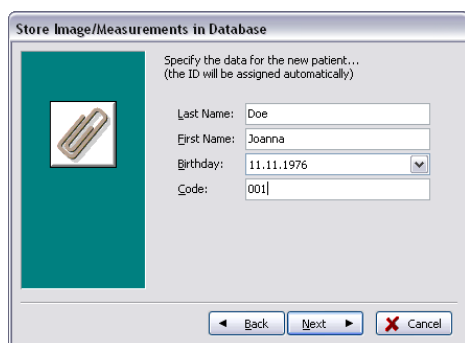


Fig. 36: dialog saving images

3. Selecting the examination

With each image of a patient, an examination is associated. The examinations are used to organize the images of the respective patient. One patient can have different examinations and one examination can contain different images.

For a new examination, choose New Examination. Otherwise, select an existing examination and directly proceed to step 5. Whenever saving an image to a new patient, no examinations will initially exist. Therefore, a new examination needs to be added according to step 4.

4. Adding a new examination

An examination is mainly determined by the date at which it is carried out. In addition, the name of the examiner and the examining lab can be added in order to keep track of all relevant examination data.

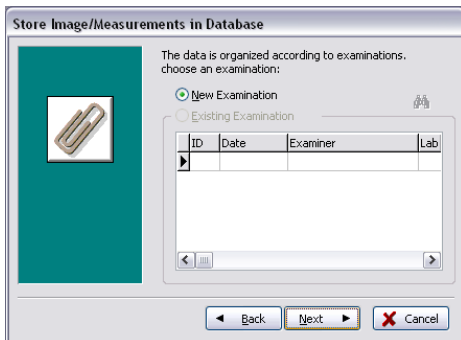


Fig. 37: dialog saving images

5. Finish

An examination is mainly determined by the date at which it is carried out. In addition, the name of the examiner and the examining lab can be added in order to keep track of all relevant examination data.

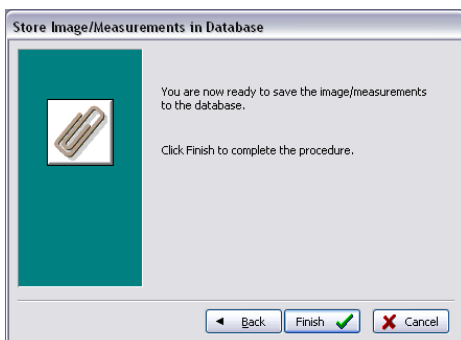


Fig. 38: dialog saving images

The Database Page for Managing Data Sets

The Database page allows recalling and managing data sets, based on patient data and examination records. Navigation through patient data sets and examination data sets can be accomplished by using the respective navigation bar for each of the sections. (Fig. 39).

The database of EyeWare is structured for routine medical use. For this intent a patient list is at the root of the database. For each patient the respective examinations are assigned. Each examination may contain one or more images or videos associated with one or more measurements (Fig. 40).

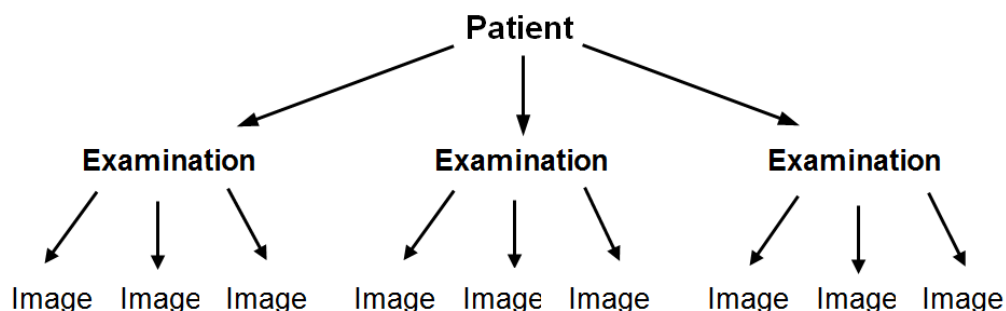



Fig. 39: Structure of the database

When a snapshot is saved to the database by use of the Storage Wizard, it is associated with both patient and examination data. The wizard also allows the entry of new patient/examination data.

In some circumstances, as in a non-patient related operation, it may be desirable to shortcut the Storage Wizard and to quickly save the image to Quick File. Please note that when using Quick File, no measurements are saved.

For recalling an image from the Database page and loading it to the Image page (e.g. for reviewing measures or saving to a file), double-click any image of the active examination.

With every image stored a comment may be associated. Click  to open the multiline editor. The software automatically timestamps every image at the time point of saving it to the database.

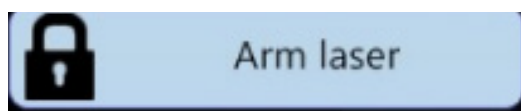
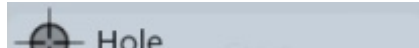


Fig. 40: Database page

The Database Toolbar



For information regarding a specific button move the mouse cursor over any button to get a short description of its function:

Data Navigation



Clicking the arrows allows scrolling through the highlighted category or directly getting to the first or last object.

Insert / Delete Function



The active object may be inserted or deleted.




Data Revision (edit, post, cancel, refresh)




After selection of a category a specific dataset can be edited. Subsequently, modifications can either be accepted or deleted. Clicking the refresh button will update the dataset.

Search Function



The search function offers the selection of different search criteria depending on the active category denoted by a bold title (Patient , Examinations , Images or Measurements ).

First the search category has to be selected by clicking to the respective icon. Then the search term to be looked for has to be entered. Clicking  will start the search (Fig. 41).

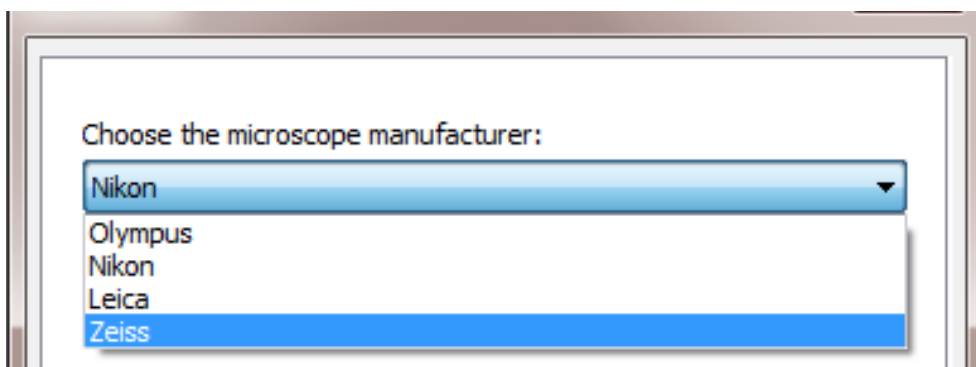
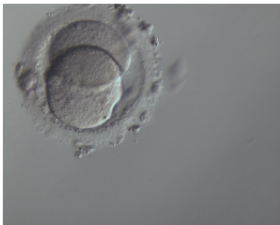


Fig. 41: Database search function

The icon for the search function is also shown in the Storage Wizard at step 1 and 3. Search function at step 1 corresponds with "Find in [patient]", step 3 with "Find in [examination]".

Report Page for Printing Examination Results

EyeWare allows to quickly printing out any set of examination results. The available report layouts are predefined. On the Report page, a preview of the examination report selected in the Database page is displayed below. This report will be printed as shown in the preview when the print command is issued after confirming the Print Dialog.

Examination Report			
Name:	Lastname, Firstname		IVF Lab
ID:	39		Street
Date of Birth:	01.03.1983		Town
Code:	ABCD1234		Telephone
			eMail
			contact
Examination ID	Examiner	Lab	Date of Examination
72	Laser	IVF Lab	28.01.2018
		Frame: 1	
		Timestamp: 28.01.2019 17:50:25	
		Comment: comment 1	

Available Report Layouts

Four different pre-designed reports are available. For changing the type of report layout you may select the desired page design at *File - Report - Template* from the menu.

Export of reports

The report may be exported as PDF, RTF, DOCX or HTML file. The address shown in the upper right of the report can be customized through *File > Settings > User Data*.

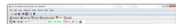
The Report Page Toolbar

For information regarding a specific button move the mouse cursor over any button to get a short description of its function.



Navigation Function

In case of a multipage report, navigation is possible by use of the arrow buttons.



Zoom Function

Zooms the preview to display the whole page, to 100% or to maximal width respectively.



Full Screen

Displays the report at full screen mode. At the upper most position of the screen, the report toolbar is shown upon moving the mouse to this area, allowing navigating between pages. To switch to normal view, press the <ESC> key or click on the door symbol in the toolbar.



TECHNICAL SUPPORT

If you need assistance at any time, please contact Technical Service. For contact details please refer to the section Customer Service.

Getting Started

Prior to the first use of the LaserShot M or NaviLase system or



- each time the whole microscope setup has been moved
- if the LaserShot M / NaviLase system was not used for a period of time
- after microscope maintenance
- after other major events, which could have induced movements and strong vibrations to the whole microscope and laser system

The position of the laser impact should be confirmed and the laser irradiation time should be determined to ensure safe treatment of embryos.

Starting EyeWare Software

Start the EyeWare software by double clicking the icon item on the computer desktop. Confirm the message and in order to initialize the live video display, select Video page.

Laser Aiming Verification Procedure

Take a cell which will not be used for any further treatment, e.g. an oocyte which failed to fertilize. The cell doesn't need to be intact, except for its zona pellucida. Starting the EyeWare software and selecting the Video page initiates the live video screen. Activate the laser by clicking . Select a cell and switch to the dedicated laser optics. The laser impact area is located in the center of the field of view and is indicated by a crosshair. (In case the crosshair modus is inactive or in order to change the design of the electronic target, select Video – Overlay – Crosshair and the preferred design in the menu.) Move the zona pellucida of the spare cell into the crosshair area and trigger a single laser pulse by clicking , pressing the laser release button of the computer mouse, or optionally by using an external foot trigger.

The Crosshair on the screen should be located straight at the center of the opening to be created by the laser (Fig. 42). If this is not the case, the position of the crosshair has to be moved to the exact position within the laser drilled opening.

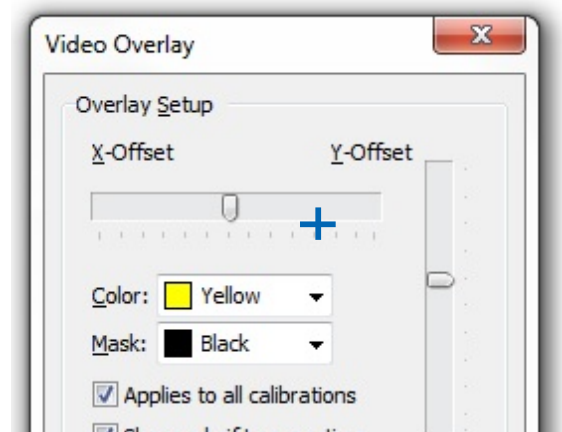


Fig. 42: Properly aligned electronic laser target

In order to match the position of the crosshair with the center of drilled hole, select *Video - Overlay – Customize* from the menu and correct the position of the crosshair in XY-orientation by scrolling the sliders.

After checking / aligning the crosshair in NaviLase home position, verify target position also in NaviLase dynamic mode as follows: position the spare cell in a corner of the NaviLase working area, move the laser target to the zona and trigger a single laser pulse. Verify position of the target indicator. It should be at the center of the drilled opening. If this was not the case, document deviation by taking a snapshot. Repeat above steps after moving the spare cell to the remaining three corners one after the other.



Caution: In case that the target indicator did not match with the center of the drilled opening in one or more cases, stop using NaviLase in dynamic mode. Contact customer service and provide the snapshots of the deviation(s). Only use NaviLase in verified home position until the error will be fixed.



INTEGRITY OF THE ELECTRO-OPTICAL SYSTEM

Wrong handling of the microscope or strong displacement of the electro-optical system, e. g. by mechanical shock can result in an incorrect position of the beam splitter system, magnifier lens position, camera, and the turret position, respectively. As a result of any of the above, aiming of the laser may no longer correspond to the crosshair position displayed at the video image and damage to embryos may occur if the laser is used in this condition.



INCORRECT LASER AIMING

Failure to follow the laser aiming verification procedure could result in wrongly located openings and therefore may damage the treated embryo.



REPEATED LASER SHOTS

Repeated laser shots to the same position of the embryo could result in an increased risk of embryo damage. In case of risk of applying repeated laser shots to the same position of the embryo the laser action can be immediately stalled by pressing the emergency stop button.

Relationship between irradiation time and opening size

According to the drilling strategy, a single laser pulse must generate a hole of appropriate size. The relationship between laser irradiation time and opening size is influenced by various factors such as the type of microscope, the kind of heated microscope stage (glass or metal), the type of heating system, the temperature, the culture dish, the culture medium, and finally the thickness, type and hardness of the zona pellucida.

The sole controllable parameter determining the opening size is the laser irradiation time. The laser energy is a function of the laser power and the irradiation time. Since the laser power is fixed, variation of the laser irradiation time will adjust the total laser energy, by which a drilled hole is introduced into the zona pellucida,

Due to the variability between laser irradiation time and the obtained drill hole size, the appropriate irradiation time for creating a hole of defined size cannot be precisely predicted or transferred from one laboratory to another. Instead, it has to be determined by a brief experiment on a spare cell for each individual microscope setup. This procedure finally results in a reliable electronic hole size indication displayed by the LaserShot M / NaviLase Hole Size Predictor (see chapter Calibration of the Hole Size Predictor for detailed information).

How to determine the “default pulse length setting” and verify the calibration of the Hole Size Predictor

The electronic overlay (green circle) of the Hole Size Predictor interactively indicates the expected opening diameter when selecting or changing a laser pulse length. Before starting to use the laser, verify the calibration of the Hole Size Predictor by following the steps below. Doing so, you can make yourself familiar with the “default pulse length setting” on your microscope. That is the pulse length which will generate an opening diameter of zona pellucida thickness (e.g. as will be used for assisted hatching or zona thinning):

1. Select the live video page, full-screen mode (F11), in the EyeWare software
2. Place a warmed dish (37°C / 98,6°F) containing a cell which will not be used for any further treatment on the heated microscope stage, e.g. an oocyte which failed to fertilize. The cell should be located at the bottom of the dish.
3. Locate the cell through the laser lens and focus on the zona pellucida.
4. Unlock the laser, using the corresponding software button.
5. Select “Hole” as working mode from the menu if using NaviLase.
6. Using the computer mouse and “drag & drop”, direct the circle indicating the laser target to an area on the zona pellucida (NaviLase).

When using LaserShot M, align the zona pellucida with the laser target circle using the cross moving table of the microscope.

7. Using the ms scale, adjust the laser pulse length in a way that the diameter of the green circle indicating the position and diameter of the laser holes corresponds to the zona pellucida thickness.

8. Release the laser by clicking the button or by pressing the footswitch while avoiding any vibrations of the microscope.

9. Verify that the diameter of the newly created hole is identical with the diameter of the circle indicating the position and diameter of the laser holes.

In case you observe a difference of the two diameters, re-check if all the following settings applied: temperature of the dish and microscope stage was 37°C / 98.6° F, the laser lens was used, the zona was located at the bottom of the dish.

Also read the following chapters about the influence of temperature and Z-positioning of the zona pellucida on the size of the drilled hole.

If the above treatment conditions were correct, see chapter Calibration of the Hole Size Predictor and perform a recalibration.

Important Notes on Laser Irradiation Time

Temperature

Conforming to good laboratory practice, the microscope to which the LaserShot M / NaviLase system is installed should be equipped with a heating stage that ensures temperature maintenance of the cells and the culture medium to 37°C / 98.6°F during the entire procedure.

The relationship between irradiation time and opening size is temperature dependent. Choosing inappropriate temperature settings or forgetting to switch on the temperature controller will cause unexpected opening sizes.



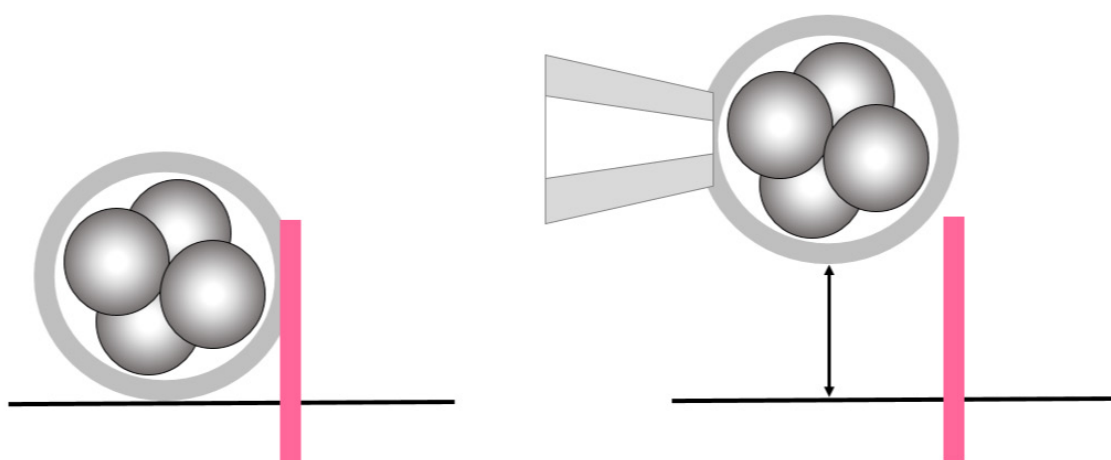
INFLUENCE OF TEMPERATURE ON DRILL OPENING SIZE

When using a heated stage during zona manipulation, make sure that it has been set to the correct temperature. Inappropriate temperature settings will lead to unexpected drill hole sizes. Lower temperatures result in smaller openings, while higher temperatures cause openings of excessive sizes that may lead to embryo damage.

Vertical (Z)-Positioning

Using a holding pipette to ensure the positioning of the embryo during laser treatment can be an advantage, but also introduces an additional parameter that needs the user's attention. For optimal results, the vertical (Z)-position of the holding pipette should always be as such, that the cell remains near the bottom of the culture dish during laser drilling.

The correlation between irradiation time and opening size is dependent on the vertical position (Z-direction) of the cell in relation to the bottom of the culture dish. Increasing the distance between the cell and the bottom of the culture dish will lead to increased absorption of the laser energy by the culture medium. Hence, wrong Z-positioning will result in unexpected opening sizes.



INFLUENCE OF Z-POSITION OF THE EMBRYO ON DRILL OPENING SIZE

Inappropriate Z-positioning will result in smaller drill holes and in reduced laser beam quality. It is recommended to keep the cell near the bottom of the culture dish during laser treatment.

Variation of the Laser Drilling Position and Strength

After strong displacement of the whole electro-optical system, e.g. a mechanical shock, the quality of the drilled hole has to be checked when using the parameters which had been adjusted previously (irradiation time and aiming of the laser). The laser drilled holes and openings will be of good quality if the laser impact is located near the center of the live video image on the monitor. This position may deviate slightly from the center of the monitor screen depending on the monitor used and combination of lenses.

Closing Eyeware Software

Close the EyeWare software by selecting File > Exit from the main menu or by clicking



INTEGRITY OF THE ELECTRO-OPTICAL SYSTEM

Wrong handling of the microscope or strong displacement of the electro-optical system, e. g. by mechanical shock can result in an incorrect position of the beam splitter system, magnifier lens position, camera, and the turret position, respectively. As a result of any of the above, aiming of the laser may no longer correspond to the crosshair position displayed at the video image and damage to embryos may occur if the laser is used in this condition.



DOUBTS ABOUT THE SUITABILITY OF THE LASER

In case of doubt about the suitability of the laser to create good quality openings do not continue to use the laser as there may be an increased risk of embryo damage. Please have your system checked by authorized support personnel or contact Technical Service. For contact details please refer to the section *Customer Service*.

PART III: ADDITIONAL INFORMATION

Advanced Functions of EyeWare, Advanced Database Functions, Program Settings and Camera Settings are listed in this chapter. Furthermore information about Maintenance, Troubleshooting and Customer Service is provided.

Advanced Image Handling Functions

Open Image and Save Image Dialog

EyeWare allows import of images and saving images to file. Supported file formats are Windows Bitmap files (*.bmp) and JPEG (*.jpg or *.jpeg). The Open Image and Save Image dialogs (Fig. 43, 44) are standard Windows functions.

Open Image

A preview of the selected image is displayed in the Open Image dialog. A zoomed preview can be displayed by clicking the magnifier icon.



Fig. 43: dialog opening images

Save Image

Click and highlight an image at the **Database** or **Quick File** page and select Save Image.

Save with Measurements

Images to which measurements have been added can be stored together with the measure lines from the **Image** page.



Fig. 44: Open Image and Save Image Dialog

Print

For printing a report from EyeWare, the printer must be chosen first. Be sure you have a printer installed and configured for this feature to function properly. Contact your system administrator if necessary.

The Print Dialog (Fig. 45) is a standard Windows dialog. For more information, refer to the Windows Help feature.

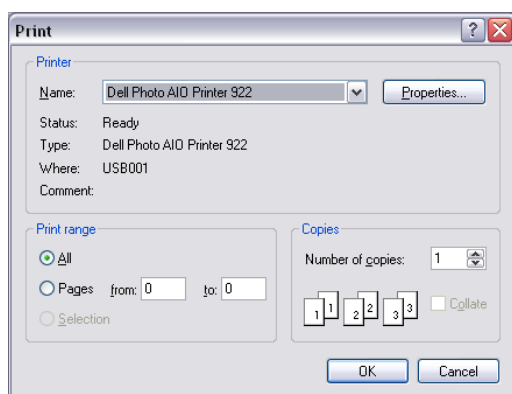


Fig. 45: Print Dialog

Photo Print

For printing a snapshot from the image page select Photo printing. For this function it is recommended to use a photo printer.

Send Image as e-mail Attachment

EyeWare automatically launches the e-mail program, composes a new mail and attaches the selected image file (the computer running EyeWare needs to support Microsoft Outlook®).

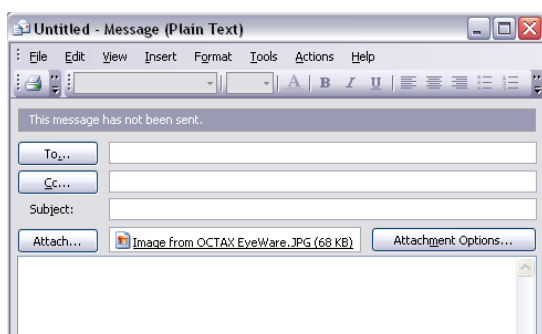


Fig. 46: Sending an image as an e-mail attachment



PLEASE NOTE

Your email software has to be properly configured to send and receive e-mails on this computer.

Program Settings

Several aspects of EyeWare are customizable. This allows the user to adapt some functions of the software according to specific needs. The Program Settings dialog allows modifying the following functions:

User Data

In this area, the end user contact information can be entered. There are up to 6 lines to be filled in with information such as name and address, telephone numbers, e-mail address etc. (Fig. 47).

Several language options are available.

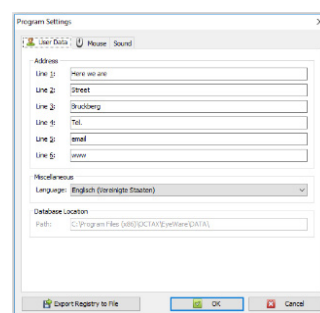


Fig. 47: Program Settings Dialog for User data entry

Mouse

This dialog allows configuring the properties of the computer mouse. While mouse pointer is located at the Test Area any click of one of the mouse buttons will reveal which mouse button corresponds to which button number. Tick the checkbox to select the mouse button that triggers the laser pulse. Additionally, a mouse button for taking a quick snapshot can be selected (Fig. 48).

Furthermore, a key from the keyboard can be selected to trigger the laser.

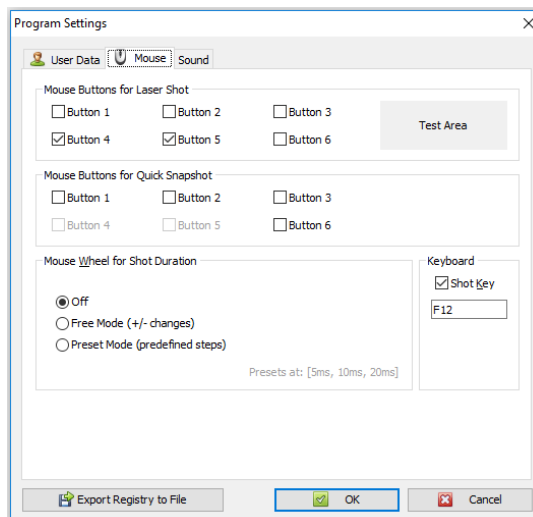


Fig. 48: Program Settings Dialog for mouse properties

Backup all Settings to REG-file

All settings of EyeWare can be saved to a backup file in the EyeWare system folder. Automatically, a file name is generated containing the computer name, date and time of storage (Fig. 49).

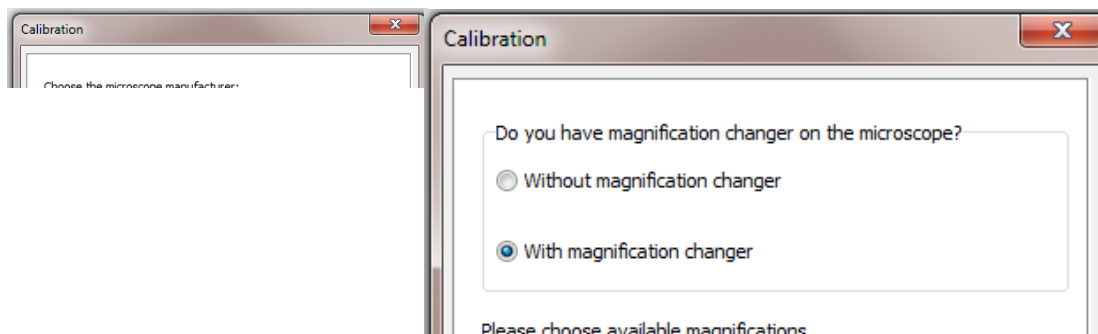


Fig. 49: Program settings are stored to the EyeWare system folder

In order to restore previous settings, select the appropriate file from Windows Explorer, double click and confirm the entry of the stored information into the registry. Now your previous settings are restored, including the settings of this dialog, and can be checked in the Program Settings dialog.

Camera Settings

The NaviLase system comes with an Eye USB2.0 camera. Exemplary, Fig. 50A, B show the camera properties of an Eye USB2.0 camera (1280x1024, 15 Hz). Usually, exposure, gamma and white balance should be set to automatic, whereas brightness and contrast can be adjusted manually.

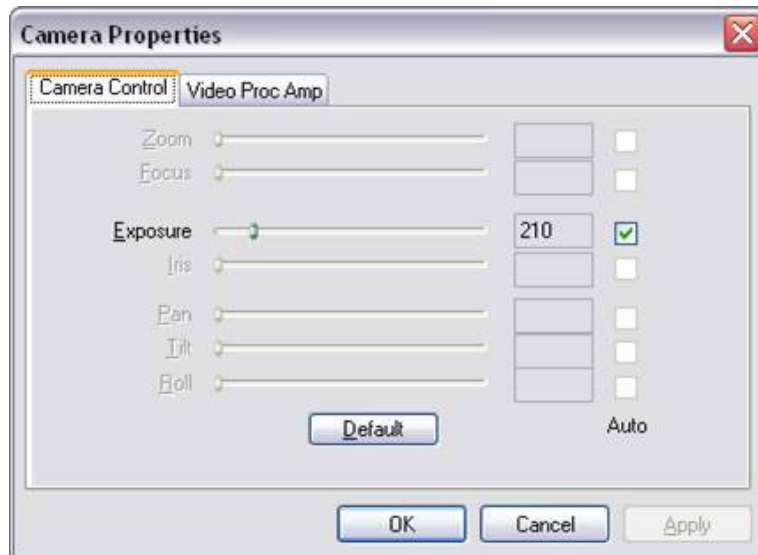


Fig. 50A: Camera Properties dialog of an Eye USB2.0 camera

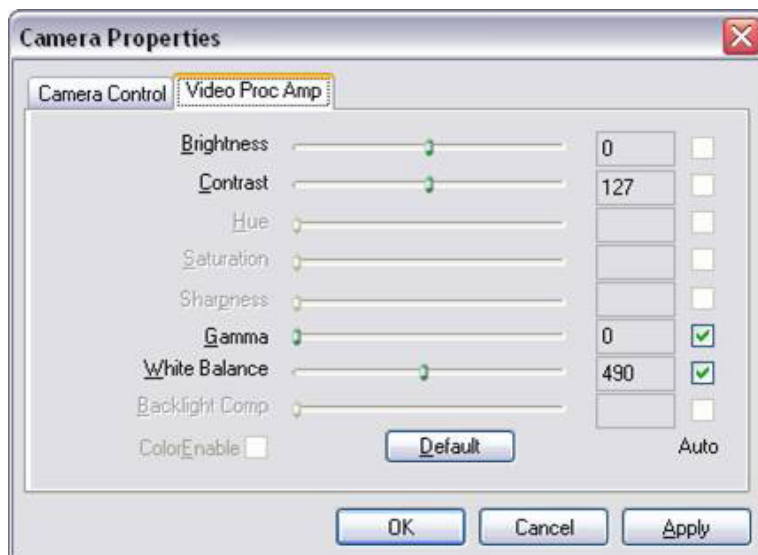


Fig. 50B: Camera Properties dialog of an Eye USB2.0 camera

Generating Support Request Data

EyeWare facilitates technical support. Open the menu Help and click Info... and then click Support Request (Fig. 51) and select the information that should be included to the support request. Alternatively use default settings. Always enter senders address details and a short description of the problem (Fig. 52). Then decide how to send the file.



Fig. 51: Support Request

Data for Support Request:	
EyeWare Data	
<input type="checkbox"/> Database	available
<input checked="" type="checkbox"/> Registry	selected
<input type="checkbox"/> Bug Reports	not available
<input checked="" type="checkbox"/> Dongle	selected
<input checked="" type="checkbox"/> Application File List	selected
<input type="checkbox"/> Setting Files (*.ini)	available
<input type="checkbox"/> Report Templates	available
<input checked="" type="checkbox"/> Log Files	selected
Hardware Information	
<input checked="" type="checkbox"/> Computer Overview	selected
<input checked="" type="checkbox"/> USB Device List	selected
<input type="checkbox"/> Complete Hardware Device List	available
Software Information	
<input checked="" type="checkbox"/> Windows Installation Overview	selected
<input type="checkbox"/> Windows Event Logs	available
<input type="checkbox"/> Active Directory Overview (upon Request by OCTAX)	not available
<input type="checkbox"/> Installed Software	available
<input checked="" type="checkbox"/> Running Processes	selected

Please, add a Short Description about your Problem:

Please enter comments here

Cancel Send as E-Mail HTTP Upload Save to File

Fig. 52: Support Request Options

- Send as e-mail (the computer running EyeWare needs to support Microsoft Outlook®): EyeWare automatically launches the e-mail program, composes a new mail and attaches the zipped support files.
- Save to file: Select where to save the System Data and wait until data are copied. Attach the file to an e-mail or send a disk with the System Data to Vitrolife GmbH. For contact details please refer to the technical service section.
- HTTP upload: The System Data are automatically uploaded to server via Internet connection. Some comments about the problem may be added before uploading the data. Please inform your local distributor or Vitrolife GmbH about further details.



PLEASE NOTE

Without any administrator rights some of the system data are not available for enclosure to the support request file. For including these data please contact your system administrator if applicable.

Maintenance

The LaserShot M system exhibits superior mechanical and optical precision, which makes realignment unnecessary during routine use.

LaserShot M and NaviLase are maintenance free systems. For the static LaserShot M there are no parts to be replaced in specific intervals or movable parts prone to disturbances. For the motion modules of NaviLase a preventive replacement after five years of use is mandatory.

A preventative maintenance of the laser is recommended every 12-18 months to ensure optimal performance of the laser.

System updates will be provided within short response times by technical staff of Vitrolife GmbH, Germany. For contact details please refer to Customer Service.

Make sure not to touch any optical components, i.e. the mirror block and the laser objective lens, respectively.

Reprocessing instructions Octax LaserShot M, Octax NaviLase

Reprocessing according to these instructions has been validated according to the FDA guidance document "Reprocessing Medical Devices in Health Care Settings" and shown to be effective.

Octax lasers and their components are not in contact with human embryos or adult patients when applied according to the indications for use. Despite Octax lasers are used in a clean IVF lab environment, contamination may occur from the following sources:

- dust accumulation through ambient air
- contamination of the laser lens or laser camera by contact with the skin (fingers)
- contamination with embryo culture media or oil for embryo culture by accidental spilling from a culture dish handled on the microscope stage (worst case scenario)

Sources of contamination as described above may include organic carbons, culture media components, oil, bacteria or fungi (skin contact). The contaminants may be solid (from skin contact), viscous (oil) or aqueous (culture media), or a biohazard (embryos).

WARNINGS

Warning: Never use abrasive disinfectants! Only use disinfectants based on quaternary ammonium compound tested for use in IVF laboratories for reprocessing.

Warning: Do not use any automated method for reprocessing.

CAUTIONS

Caution: Cover the laser module in the back of the microscope, the USB camera and cables, respectively, with surgical drape to protect from contamination.

Caution: Never remove any of the laser components from the microscope for reprocessing! Never change their position or orientation! Never apply pressure!

Caution: Never spray any of the laser components with disinfectant!

Caution: before starting with the reprocessing procedure, make sure all embryos are back to the incubators!

Caution: Do not remove or rinse disinfectant before drying.

LIMITATIONS OF REPROCESSING

If done according to the instructions, reprocessing will not impact the lifetime of the laser.

INSTRUCTIONS

Pre-treatment at the point of use before processing:

Accidental contamination with media or oil for embryo culture requires immediate removal of the liquids and cleaning with a lint-free cloth.

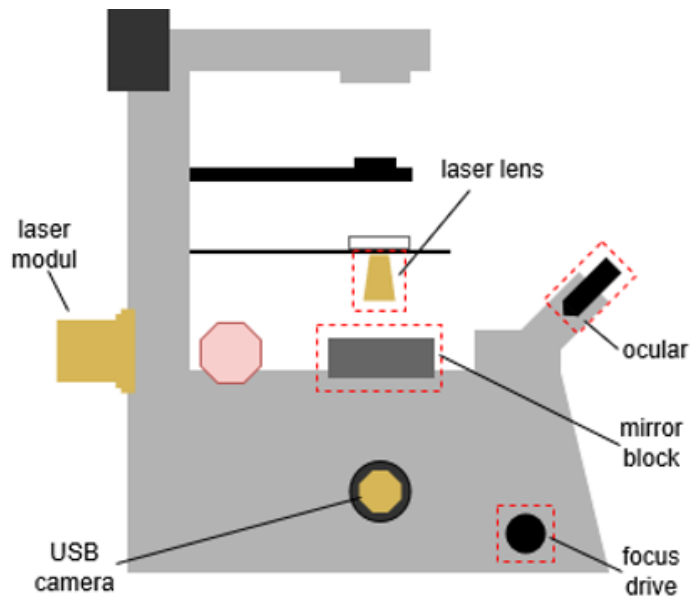
Preparation before reprocessing

See image below to identify areas to be cleaned according to your SOP:

Parts with regular user contact: marked with red square

Parts with limited user contact: USB camera and laser module at the back of the microscope.

Before starting to reprocess the laser components, remove and discard the surgical drape covering the USB camera, the laser module at the back of the microscope.



1. Accessories required for the reprocessing procedure: lint-free cleaning tissue, surgical drape

2. Disinfectant required for reprocessing procedure: agent tested for IVF laboratory use, based on quaternary ammonium compound (like Benzyl-alkyldimethyl chloride 2.6 mg/g)

Caution: before starting with the reprocessing procedure, make sure all embryos are back to the incubators!

Reprocessing: automated Not applicable

Warning: Do not use any automated method for cleaning.

Reprocessing: manual

1. Moisten a lint-free cleaning tissue with 10 ml of disinfectant.
2. The tissue should be dampened, not wet.
3. Wipe all surfaces of laser components four times up and down, carefully and slowly
4. Ensure that the full surfaces are completely moistened.
6. Let the disinfectant dry for a minimum of 15 minutes.
7. Following reprocessing of the laser module; USB camera, please recover with a new surgical drape.

Caution: Do not remove or rinse disinfectant before drying.

Drying: Let the disinfectant dry for a minimum of 15 min.

Inspection, maintenance and functionality testing:

Instruments have to be visibly clean after reprocessing.

Check that laser lens as well as microscope beam splitter are locked into position.

Perform laser aiming verification procedure on a spare cell after reprocessing (see Appendix).

In case the laser aiming verification procedure cannot be performed successfully or you have doubts about the functionality of the laser system, please contact technical service.

Packaging: Not applicable

Sterilization: Not applicable

Storage: Not applicable

Additional information: **Caution: Cover the laser module in the back of the microscope, the USB camera and cables, respectively, with surgical drape to protect from contamination.**

Contact to the manufacturer:

Vitrolife GmbH
Dr. Pauling Str. 9
84079 Bruckberg, Germany
support.de@vitrolife.com
Tel: +49 8765 939 900

Troubleshooting Guide

In case some technical problems occur during operation of LaserShot M/ NaviLase please check possible causes of defects according to the list below before contacting the technical support.

Problem	Possible Cause	Recommendation
Misalignment Misalignment in "home" or "target pointer" position: crosshair on the screen is not located straight in the center of the opening you created	<ul style="list-style-type: none"> ▪ whole setup has been moved ▪ LaserShot M / NaviLase was not used for some time ▪ microscope maintenance ▪ major events which could have introduced movements and strong vibrations to the microscope and laser system 	1. Restart EyeWare, 2. Check the laser alignment in "home" or "target pointer" position 3. Correct overlay settings
	Golden laser objective is not in correct position	a. ensure objective is correct and right in place (clamped)
	1,5x/1,6x magnifier is not in correct position	b. Additional magnification is off (1x) and is clamped
	Light path selector lever is not in correct position	c. Beam splitter is in correct position and clamped
	Camera or c-mount is loose	d. Camera is tightly fixed (do not move it, only check)
		4. If the problem persists and misalignment is below 2 cm on the screen, move the overlay as described in the section Laser Aiming Verification Procedure
Misalignment in other locations crosshair on the screen is not located straight in the center of the opening you created		1. Restart EyeWare 2. Check the alignment in "home" or "target pointer" position 3. If the problem persists, contact Technical Support

unexpectedly small drilling sizes

drilled opening is suddenly smaller as intended

glass stage yes/no
culture media in use

laser irradiation time has to be adjusted as described in the section Laser irradiation time determination for appropriately sized openings

excessive z-position

for optimal results, the vertical (Z)-position of the holding pipette should always be such that the cell remains near the bottom of the culture dish during laser drilling.

temperature too low

increase temperature of culture cells and medium to 37°C

drilled openings are gradually getting smaller while irradiation time remains constant

optics of the laser are getting dusty

call the technical service

Crosshair not displayed or unexpected appearance of crosshair

- crosshair modus is inactive
- appearance of crosshair has been modified

select video – overlay – crosshair and the preferred design from the menu.

Decommissioning of LaserShot M / NaviLase

For decommissioning of the LaserShot M / NaviLase system please contact Technical Service. For contact details please refer to the section Customer Service.



In case the LaserShot M / NaviLase system is becoming obsolete, please assure appropriate waste disposal according to your local environmental regulations

Customer Service



TECHNICAL SUPPORT

If you need assistance at any time, please contact Vitrolife Technical Services.



Vitrolife GmbH

Roedersteinstr. 6

84034 Landshut / Germany

phone: +49 (0)8714306570

email: support.de@vitrolife.com

PART IV: QUICK GUIDE

The Quick Guide outlines in a tight and concise manner important and frequent operations.

Laser Aiming Verification Procedure

Start the EyeWare by double clicking icon on your desktop, read and confirm the message and select Video page.

- Activate the laser by clicking.
- Move the zona pellucida of the spare cell into the Crosshair area.
- Trigger the laser pulse.
- The Crosshair on the screen should be located straight in the center of the opening introduced into the zona pellucida (53A). If this is not the case (53B), the position of the Crosshair has to be moved to the exact position within the laser drilled opening.

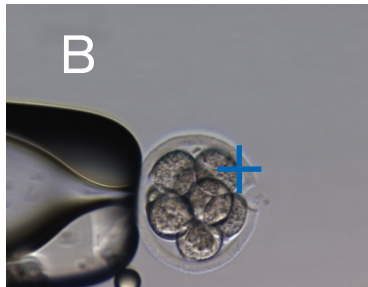
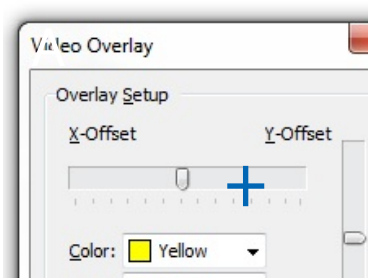


Fig. 53A / B: Cross Hair positioning

If required: correct the position of the crosshair in XY-orientation. Therefore select Video - Overlay – Customize from the menu (Fig. 54A) and scroll the sliders. Alternatively, click and highlight one slider and move the crosshair with the cursors of the keyboard into position. This allows more precise control (Fig. 54B)



Fig. 54: XY positioning of the crosshair. A: dropdown menu. B: Video Overlay window for moving the crosshair position.

Hole Size Predictor

The laser Hole Size Predictor is a convenient means of reliably indicating the expected hole size in correlation to the selected pulse time. Displayed as a green circle which is part of the electronic target overlay, it interactively changes its diameter depending on the pulse time selected. Vice versa the Hole Size Predictor allows adjusting the pulse time in accordance to the required hole size

In order to calibrate the diameter of the Hole Size Predictor based on the actual diameter of a drilled hole at a defined pulse time, select Video - Overlay – Calibrate the Hole Size Predictor from the menu and adjust the indicated hole size by clicking the arrows on the right side (Fig. 55). The diameter of the Hole Size Predictor can be calibrated in a range between -70% and +200% in steps of 5% relative to the current value.

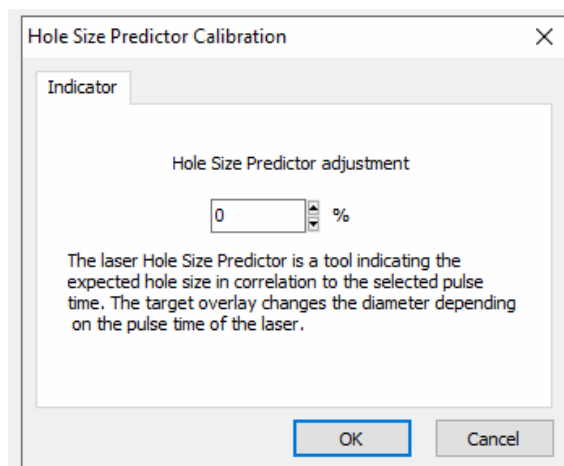


Fig. 55: Hole Size Predictor Adjustment

Working Principle LaserShot M - NaviLase

Insert the laser lense

LaserShot M / NaviLase Static mode

Open the Video page



Focus to zona pellucida at equator level and position embryo



Activate laser

2.8 ms

Adjust pulse time according to the indicated hole size



Trigger laser pulse

<F10> Take a Snapshot

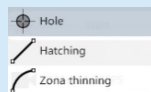
NaviLase Dynamic mode

<F11> Open full screen view

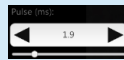
Focus to zona pellucida at equator level and position embryo



Activate laser



Select working mode



Adjust pulse time according to the indicated hole size



Trigger laser pulse



Take a Snapshot



Add some measurements



Store to database using the Storage Wizard



Database

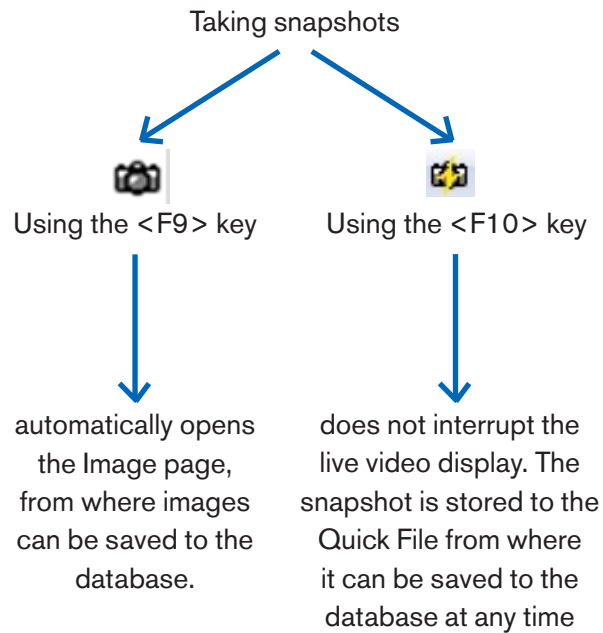
Select a patient and an examination in the Databasepage



Report

Switch to the Report page; preview and print the examination report

Taking snapshots



TECHNICAL SUPPORT

If you need assistance at any time, please contact Technical Service. For contact details please refer to the section Customer Service.

PART IV: APPENDIX

The Appendix contains further documentation

Target Pointer

The Target Pointer is an optional device for LaserShot M and NaviLase which can be used to localize the laser target by the position of a red LED dot when looking through the eyepieces of the inverted microscope. The Target Pointer also features an optical signal pattern of four red LED dots. This square pattern indicates the instant of releasing a laser pulse.

Key Features of the Target Pointer

The Target Pointer offers an optional indicator of the laser target in addition to the target overlay of the EyeWare software. Whereas the digital overlay can only be observed on the screen, the Target Pointer emits red LED light which is visible both on the screen as well as through the eye pieces of the inverted microscope.

A static LED module consisting of five red LEDs is built into the mirror block of the laser system. Four LED lights are arranged in a square pattern and one LED light is at the center of the square pattern. The Target Pointer LED module can be adjusted manually by two screws (see Fig. 56). Turning the screw(s) will shift the position of the Target Pointer LEDs on the screen in X and/or Y directions. This way, the position of the central red LED dot of the Target Pointer is superimposed with the digital laser target overlay on the screen to indicate the laser target of LaserShot M and of NaviLase in home position.

Working with the Target Pointer

Target Pointer function is activated or deactivated in the Device menu, section Pointer. To activate, check On and visible; uncheck box to deactivate Target Pointer function. When Target Pointer function is active, the red Target Pointer LED dot will be turned on automatically when LaserShot M / NaviLase function is activated using the Key symbol.



Caution:

Check that the central LED dot of the Target Pointer is superimposed with the digital laser target overlay on the screen and verify the selected hole size before using the Target Pointer for laser aiming.

Functional states of the Target Pointer LED lights when working with the laser (also see figures a-c below):

a. The central LED is turned on and indicates the laser target position when the laser is activated. The central LED stays on as long as the laser stays activated without being released.

b. When releasing a laser pulse, simultaneously the central LED is switched off and the square pattern of four LED dots is turned on for a 2 seconds interval. This optical pattern indicates the instant of laser impact and enables for unhindered view at the targeting area of the laser.

c. Following the 2 seconds interval in b., the central LED is turned on again (now visible in the center of the laser induced opening).

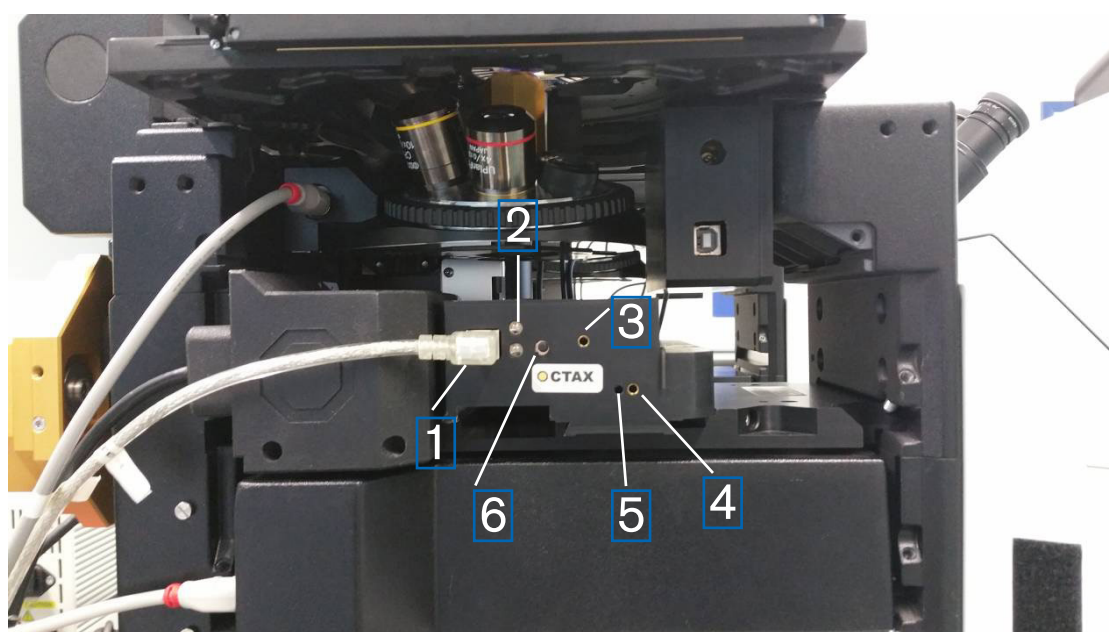


Fig. 56: Target Pointer

1. USB Port
2. Status LED
3. Y – adjustment (allen wrench key 1.5 mm required)
4. X – adjustment (allen wrench key 1.5 mm required)
5. Focusing of the red light dot (allen wrench key 1.5 mm required)
6. Switch

Adjustment procedure

First check if dioptré is set to ZERO for both of the eyepieces (Fig. 57). Indicators are seen on each eye piece lens.



Fig. 57: Diopter adjustment (example: Olympus inverted microscopes)

Then verify ase shot position matching with the cross hair on screen. If not, adjust laser cross hair position. See chapter "Alignment of the Crosshair". Activate laser and crosshair will appear. If there is mismatch between crosshair and red dot move the red dot to the crosshair position using the X and Y adjustment until red dot will match with the crosshair. Make use of the 1.5 mm wrench key. Therefore insert the tool into the small hole and turn the key clockwise or counter clockwise until the red dot moved to desired position. Start with correcting X position of the red dot (Fig. 58) and proceed with Y position (Fig. 59)

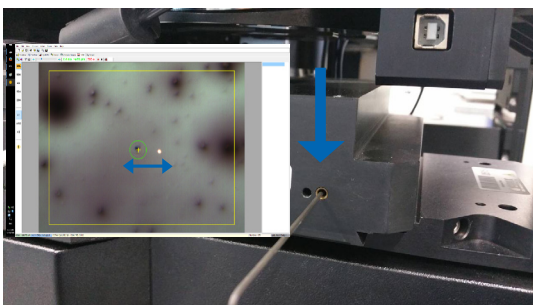


Fig. 58: X position adjustment

Correct Y position of the red dot

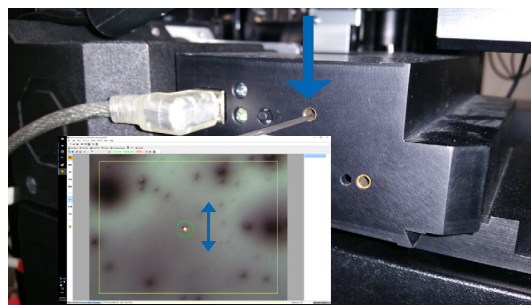


Fig. 59: Y position adjustment

If red light beam is seen very weak or widely spread (Fig. 60) correct the focus of the beam. Use 1.5 mm wrench key (Fig. 62) and turn clockwise and check how the spot changes its appearance. If spot is getting smaller continue turning the focus screw until red dot is set sharp pointed. If spot spreads wider turn into opposite direction until red dot is seen sharp (Fig. 61).

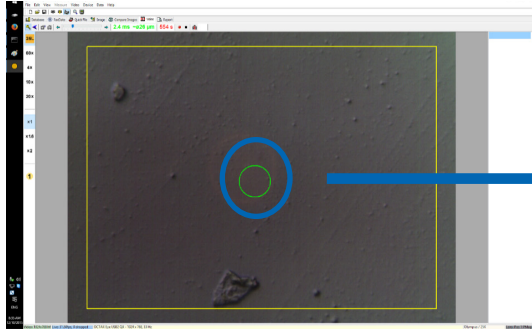


Fig. 60: widely spread red dot

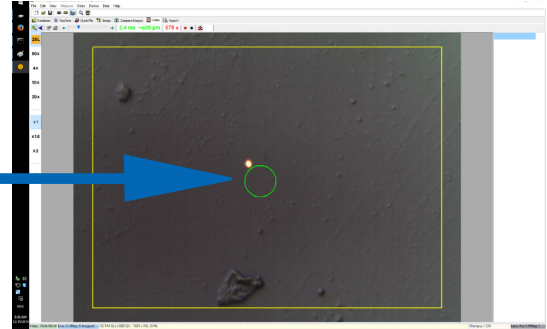


Fig. 61: red dot focused

If red LED light is not visible at all first turn off the light of the microscope or reduce to a minimum level of light in order to see the red light trough the eyepiece. If the red light is seen weak or widely spread focus the red light see chapter above.

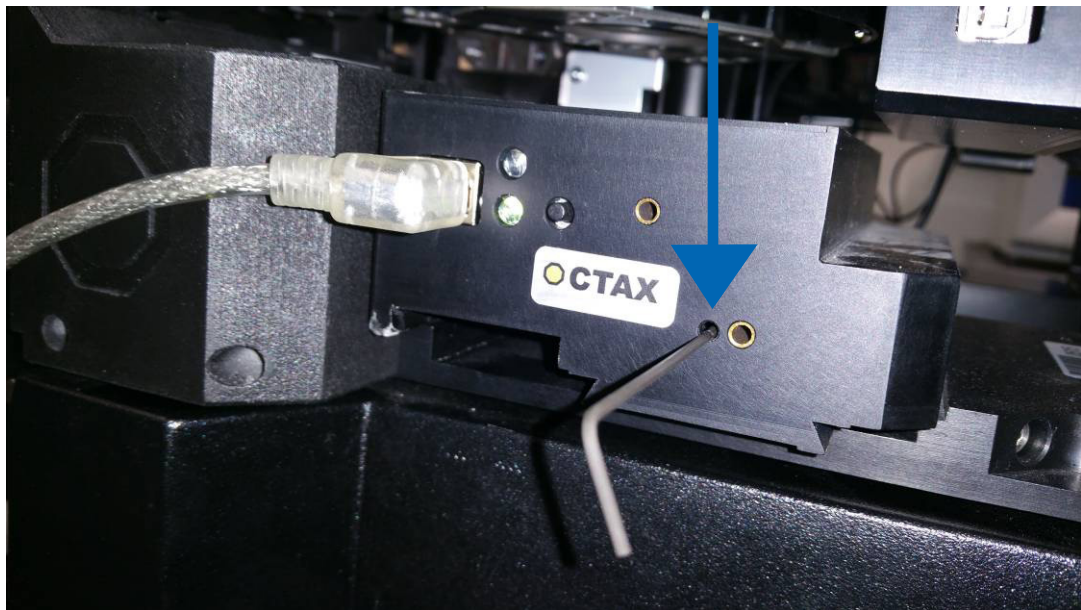


Fig. 62: Adjusting focus of red dot Target Pointer

If red dot is still not visible (Fig. 63) check the X and Y alignment. Focus at any specimen located at the bottom of the dish. Watch the spot trough eyepiece and turn the screws for LEFT/RIGHT and UP/DOWN slowly but steady several revolutions until red light gets back into the field of view.



Fig. 63: Target Pointer

Finally adjust red light dot to convenient brightness. Brightness is automatically corrected on screen but brightness of the beam seen through the eyepiece depends on user preferences. Therefore open the menu DEVICE / POINTER and click on CUSTOMISE. Move slider left for decreasing brightness and move to the right for increasing brightness (Fig. 64)..

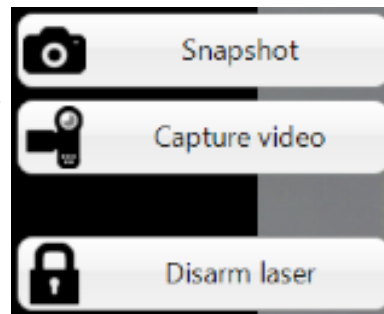


Fig. 64: Adjustment of Target Pointer brightness

After adjusting the Target Pointer position check that the central LED dot of the Target Pointer is in the center of a laser zona opening using a discarded cell (e.g. unfertilized oocyte):

- Focus on the zona pellucida and position the cell that the LED of the Target Pointer is located at half diameter of the zona thickness (Fig. 65a).
- Select a pulse length to create an opening of about the zona thickness (range: 0.4-4 ms)
- Trigger the laser (four square LED lights are visible shortly, Fig. 65b)
- Observe position of the central LED in the center of the created opening (Fig. 65c)

If the LED is not in the center of the new opening, check laser aiming verification procedure and repeat adjustment procedure of the Target Pointer afterwards.

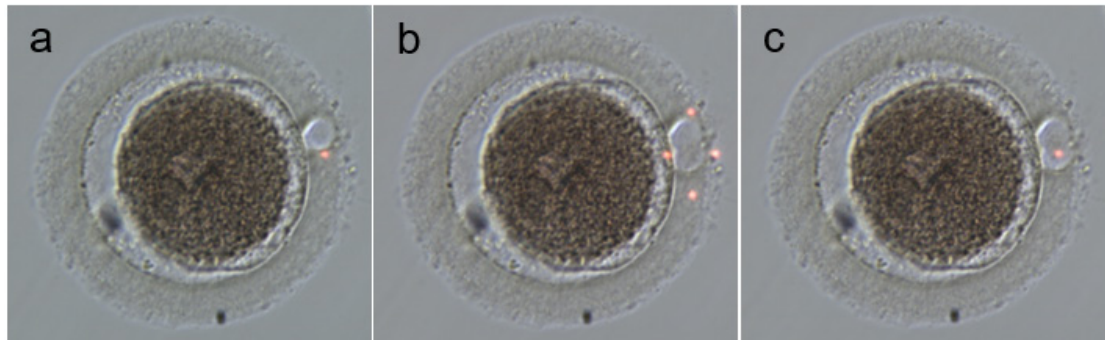


Fig. 65: Verifying laser target position as indicated by the Target Pointer: a, central LED dot indicating laser target position; b, square pattern of four LED dots, activated for a 2 seconds interval upon releasing a laser pulse; c, central LED in the center of the created opening.



Eye Safety of the Operator

Eye safety of the operator is guaranteed as the visible light is in the range of visible light.



Technical Support

If you need assistance at any time, please contact Technical Service. For contact details please refer to the section Customer Service.

Laser Module Specifications

Laser Radiation, do not view directly into light path using optical instruments

Laser:	1.48 μm Infrared Diode Laser, Class 1M. Definition of a class 1M laser according to DIN EN 60825-1 Ed 2.0, 2007-03: Class 1M lasers emit in the wavelength range from 302,5 nm to 4000 nm.
Power in focus:	100 - 250 mW (depending on microscope, optics, heated stage & operation mode)
Impulse time:	0.1 - 10.0 ms, in 0.1 ms steps (CPU Clock +/- 1,5 %)
Laser targeting:	live video display with crosshair and calibrated measurement grid overlay
Video display:	with EyeWare on computer monitor
Status indication:	LED on LaserShot M module, EyeWare user interface
Laser irradiation release:	via mouse or external foot trigger
Laser irradiation indication:	LED on laser module, EyeWare user interface
External connectors:	<ul style="list-style-type: none"> ▪ laser module is connected to computer via USB cable, the whole system (consisting of laser system, microscope, PC and monitor) must be connected to electric mains via the separator transformer and the multiplug installed together with the system. ▪ the multiplug must not be placed on the floor ▪ no other electronic components than the system components listed above, must be plugged into the multiplug. Plugging in additional components may lead to higher leakage current that specified by IEC60601-1 which can lead to insufficient performance of the system ▪ do not plug additional multiplugs or extension cords into the dedicated multiplug. ▪ the system components must not be plugged into any other plug than the dedicated multiplug. ▪ please be aware that the system component are not grounded due to the separator transformer used, do not plug any system component into wall mains power socket.
Power supply:	via USB, NaviLase option includes PS 5V/2A
Dimensions:	laser module: 11.5 x 11.5 x 9.8 cm (+/- 10%)
Operating conditions:	10° - +35 °C ambient temperature relative humidity 20%-80% (non-condensing), 735 - 1060 hPa
Transport conditions:	-20° - 70°C, relative humidity 20%-80% (non condensing), 735 - 1060 hPa
Storage conditions:	-20° - 70°C, relative humidity 20%-80% (non condensing), 735 - 1060 hPa
Objective:	25x ELWD biopsy objective for simultaneous micromanipulation and laser use, compatible with Hofmann modulation contrast
Compatibility:	compatible with major brands and models of inverted microscopes

Related Products



SALES

For sales information please contact your local Vitrolife distributor or



Vitrolife GmbH

Roedersteinstr. 6

84034 Landshut / Germany

phone: +49 (0)871 4306570

email: support.de@vitrolife.com

The following Accessories are mandatory parts of the laser system:

Order code	Product name
19310/9999	Separator transformer for Octax laser systems
19310/9998	Multi plug for Octax laser systems

The following Accessories are optional parts of the laser systems:

Order code	Product name
19310/4150	Target Pointer for laser systems
19310/0141	Breadboard for installing laser systems to Olympus IX53/73/83
19310/1148US	Foot switch for releasing the laser

Vitrolife Sweden AB
Box 9080
SE-400 92 Göteborg
Sweden
Tel: +46-31-721 80 00

Vitrolife GmbH
Roedersteinstr. 6
D-84034 Landshut
Germany
Tel: +49 871 4306570

E-mail

support.de@vitrolife.com

Internet

<http://www.vitrolife.com>