Primo Vision

Digital in vitro embryo development monitoring and archiving system

Use and Maintenance Manual

Primo Vision

Contents

1. General introduction

1.1. General description

Primo Vision is a time-lapse based, in vitro embryo monitoring and archiving system that can be used for high-accuracy, in-incubator monitoring of embryos.

The system's main parts are:

1. The inert, compact, sealed digital inverted Microscope Unit (Primo Vision Digital Microscope) is to be installed inside any regular incubator,
2. The Controlling Unit, controlling the Microscope Unit(s) and running the Primo Vision software, is used outside the incubator,
3. Microwell (WOW) embryo culture Petri dishes,
4. 3-D mouse (optional).

The main advantage of the system is the complete embryo safety. This is served by:

1) The Primo Vision Digital Microscope's exceptionally large field of view that images ALL the embryos of the patient on the same picture.
2) The finely tuned illumination and optical system.
3) The electric control that is solely dedicated to the Controlling Unit outside the incubator, so Microscopes inside the incubator are switched off completely in between the image acquisitions.
4) No movement of embryos, so they are kept in completely undisturbed environment for the whole culture period.

A custom-made Petri dish, with individually identified microwells for each embryo, is placed into the incubator to the dish holder of the Primo Vision Digital Microscope. The Digital Microscope takes images of the embryos during their in vitro development by user-defined frequency. All the embryos are seen on the image at the same time. Focusing and scanning is simply executed from outside the incubator, using the software running on the external Controlling Unit. The photos taken by the Microscopes, are presented on the screen connected to the Controlling Unit and are archived in a dedicated subfolder where they are used for:

1) Supporting decision on embryo transfer,
2) Creating different types of time-lapse movies of the developing embryos,
3) Specific and precise manual analysis of embryo development both morphometric and dynamic (detection of cleavage times, multi-nucleation, vacuolization, fragmentation, ploidy, PB extrusion, etc.).
4) Providing source for remote analysis.

By this system, embryos can stay in the incubator completely undisturbed, but still under the most

thorough control during the whole period of in vitro development, providing maximum amount of information, in order to achieve optimal embryo selection while accurately recording all moments of embryo development.

1.2. Warnings

- Do not install or operate the equipment unless you have first read this Manual!
- Handle with care – the equipment contains a sensitive optical system.
- Primo Vision Microscopes arrive in so-called „Parking Mode". If the Microscopes are transported later, their inner mechanics should be set into Parking Mode again, in order to avoid any possibility of damaging the optical system, caused by the transportation shocks. Transportation with the optics not set in „Parking Mode" may lead to loss of warranty. Parking Mode can be set when closing the Capture software. Primo Vision Microscope leaves Parking Mode automatically after being recognized by the Capture software.
- Do not open the equipment housing!
- **Always remove Primo Vision Microscopes from the incubator before running the incubator sterilization program: the Microscopes and their cables are NOT heat resistant!**
- Always sterilize the Primo Vision Microscope with 96% Ethanol or other approved disinfectant before use!
- Primo Vision Microscope is drip-proof but not waterproof – do not immerse in liquids!
- When cleaning the protective glass plate of the objective, always use a non-scratching soft cloth suitable for monitor cleaning.
- Connect the USB cables of the Primo Vision Microscopes ONLY to the Controlling Unit's Microscope USB sockets! Connecting it directly to a PC might ruin both the PC and the Primo Vision Microscope. (see in the „Controlling Unit" section)
- Before use, after connecting to the USB cable, place the Primo Vision Microscope in the incubator for at least 6 hours to let it warm up to operating temperature.
- When giving commands (Live mode; Start capture) for the Primo Vision Capture Software, be patient! It takes 15 - 20 seconds for these functions to be activated, as all electric currents are switched off in the Controlling Unit, and it needs time to activate!
- Please do NOT change the Power options of your Primo Vision Controlling Unit - the computer should never go to sleep or hibernated phase, never shut down the hard drive. When the computer goes to sleep or hibernated status it stops capturing images!

- Monitor with HDMI cable is not part of the Primo Vision system, but is necessary for system assembly. System was designed to work on 1920x1080 screen resolution. Below this resolution proper operation is not guaranteed.
- For assistance with technical issues, please contact the local distributor. The Manufacturer, the Supplier or the Distributor assumes no liability for any damage to the equipment or property caused by non-compliance with this User and Maintenance Manual.

1.3. Contents of the Primo Vision System package

1.3.1. In case of purchasing the first Primo Vision Microscope Unit(s), the system contains the following items:

No	Item	Quantity
1,	Primo Vision Microscope	1 - 6 (optional)
2,	External Controlling Unit with 6 USB slots, and with installed Capture and Analyze software	1
3,	Primo Vision Analyzer software install CD (for installing the software onto one independent computer)	1
4,	Keyboard and mouse	1
5,	3D mouse	1 (optional)
6,	WOW dish for embryo culture	20 / Microscope Unit
7,	Extension USB cable to connect Primo Vision Microscope to the Controlling Unit	1 - 6 (optional)
8,	Power cable for the Controlling Unit	1
9,	Use and Maintenance Manual	1

1.3.2. If purchasing additional Primo Vision Microscope Unit(s) for extending the existing system, the package contains:

No	Item	Quantity
1,	Primo Vision Microscope	1 - 5 (optional)
2,	Extension USB cable to connect Primo Vision Microscope to the external Controlling Unit	1 - 5 (optional)
3,	WOW dish for embryo culture	20 / Microscope Unit

2. Usage

2.1. Main parts of the Primo Vision system

The Primo Vision system consists of 1-6 Microscope Unit(s), an external Controlling Unit that runs the installed Capture and Analyze software, and micro-well culture dishes for in vitro culture of the observed embryos. USB and power cables are also included.

2.1.1. Primo Vision Microscope

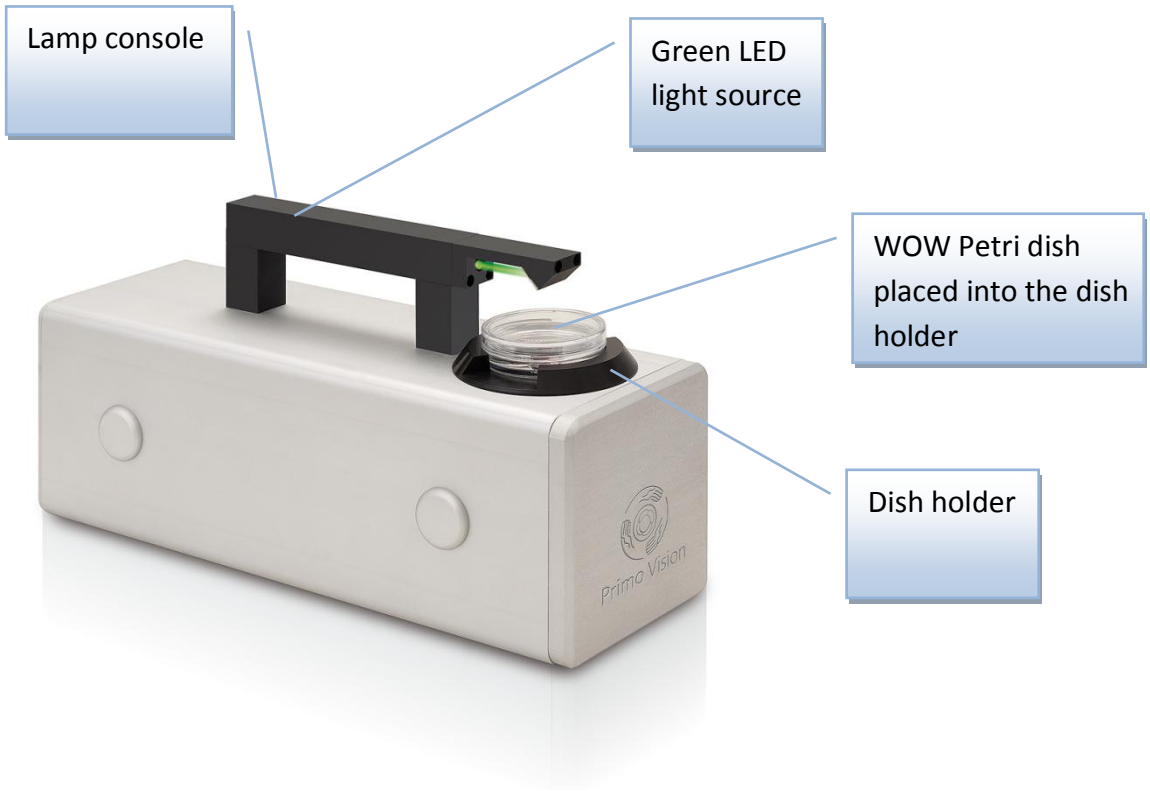
Primo Vision Microscope is a special, compact, airtight, digital inverted microscope, designed for safe and comfortable in-incubator use. The Microscope Unit's housing incorporates the custom-made, high - precision optical system, a camera and fine mechanics for focusing and scanning. On the top of the Microscope`s case the lamp console and the dish holder are located. On the back panel of the housing, a sealed mini-USB connector can be found.

Lamp of the Primo Vision Microscope is designed to minimize the light intensity, it illuminates only when „Live mode" is turned on in the software (when the WOW dish is placed in - light is necessary for positioning), and when the system acquires images. The Primo Vision Microscope uses a homogenous green LED light as light source.

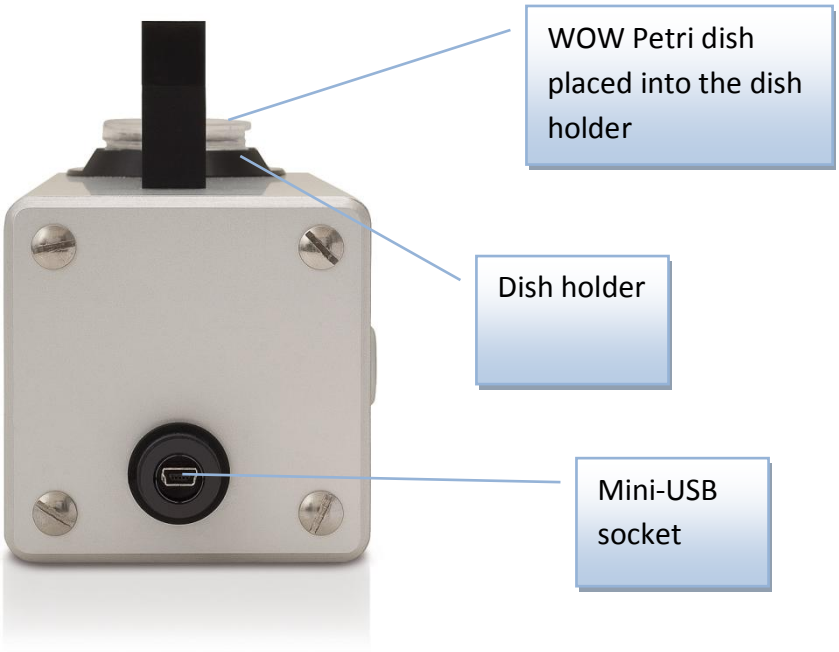
The optical system, the LED and the CCD are all designed and harmonized to provide exceptionally high resolution at a very large field of view while maintaining the highest embryo safety.

Electric current (maximum of 5 volts) is controlled by the external Controlling Unit, which lets electricity into the Microscope only for a few seconds to capture images, or for a maximum of 2 minutes when the Live mode is active. The Primo Vision Microscope is powered through the provided USB cable connected to the mini USB socket on the back panel of the Microscope.

Primo Vision Microscope Unit



Back panel of the Primo Vision Microscope



Before first use, disinfect the external surface and the USB cable with 96% ethanol. Following wiping, let the ethanol evaporate, or further wipe with distilled water inside a laminar hood before placing the unit(s) into the incubator. Disinfection is also possible with other approved solutions to be used inside the incubators, complying with current rules and regulations.

To connect the Microscope with the provided USB cable, unscrew and remove the protective cap covering the Microscope Unit's mini USB socket, remove its rubber insulation ring as well, connect the USB cable to the same socket, and screw on tightly the USB cable's protective cap. Then place the unit into the incubator, lead the USB cable through the factory made access port on the side/back of the incubator, and let it warm up and equilibrate for at least 6 hours.

The factory made access port on the side or back of the incubator, where the USB cables of the Microscopes are led through, is to be plugged back by a clean paper towel or sterile tissue. As there is a minimal positive pressure inside the incubators, there will be a slight airflow out from the port, so it should not be blocked (this, independently from the use of any device inside the incubator, is a general rule that applies to all incubators).

Markups on the Microscopes

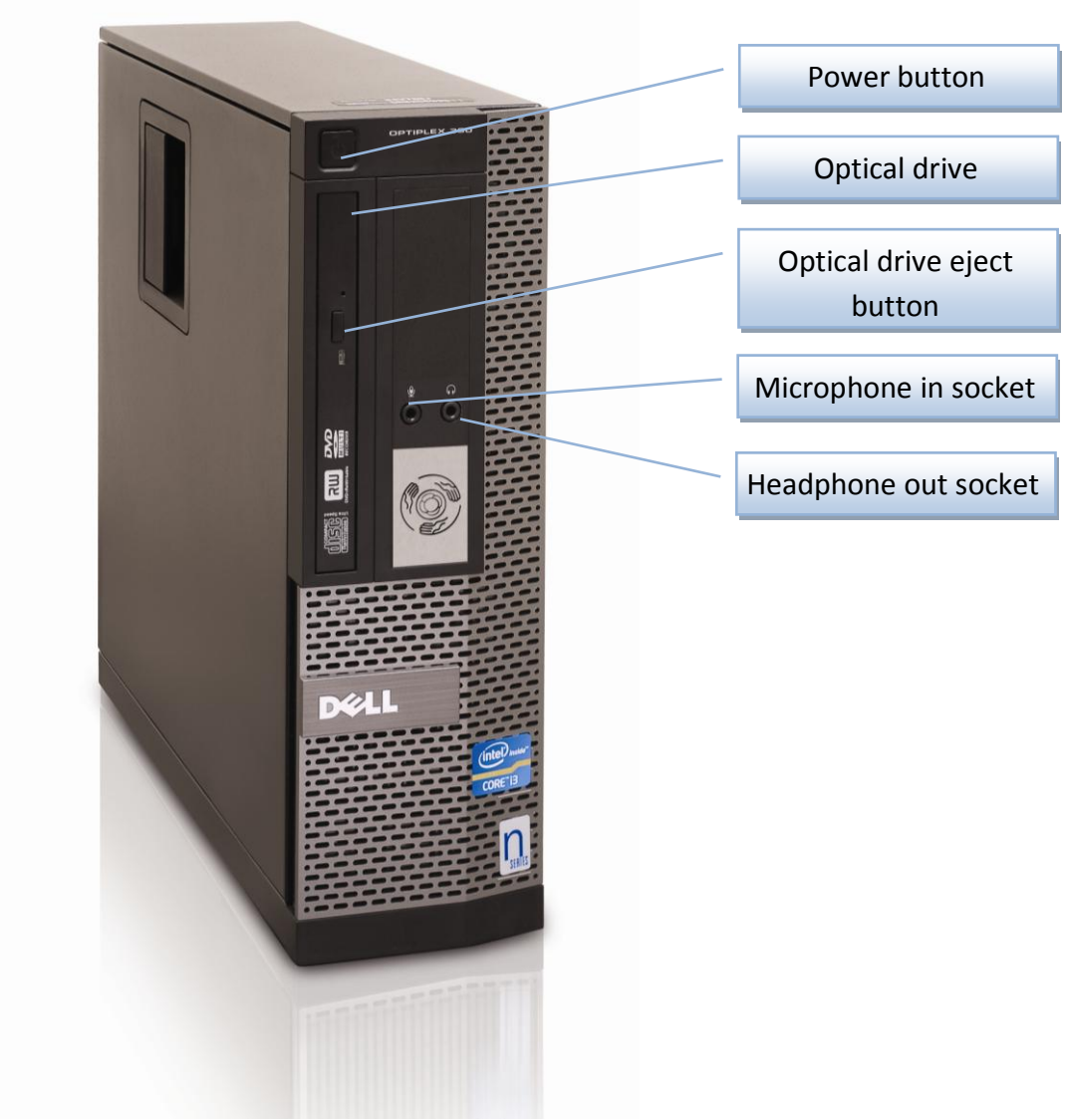
The back panel shows serial number and other relevant information, while the front panel shows a 4-digit number. That is the burnt-in identifier of the Microscope, which is automatically recognized and presented by the software. If a time-lapse project is running on a given Microscope, this identifier is shown in the project data on the HOME screen, as well as on the screen of the given Microscope.

2.1.2. Controlling Unit

Primo Vision Controlling Unit functions as the central controller of the Primo Vision system: controls the connected Microscope Units, stores the images taken by the Microscopes, and runs the provided software. A proper monitor (min. 1920x1080 (HD) resolution), is needed for the operation.

The power button is located on the top of the front panel of the Controlling Unit. The optical drive is found below this button. Next to the optical drive, the microphone-in and the headphone-out slots are located.

Front view of the Controlling Unit

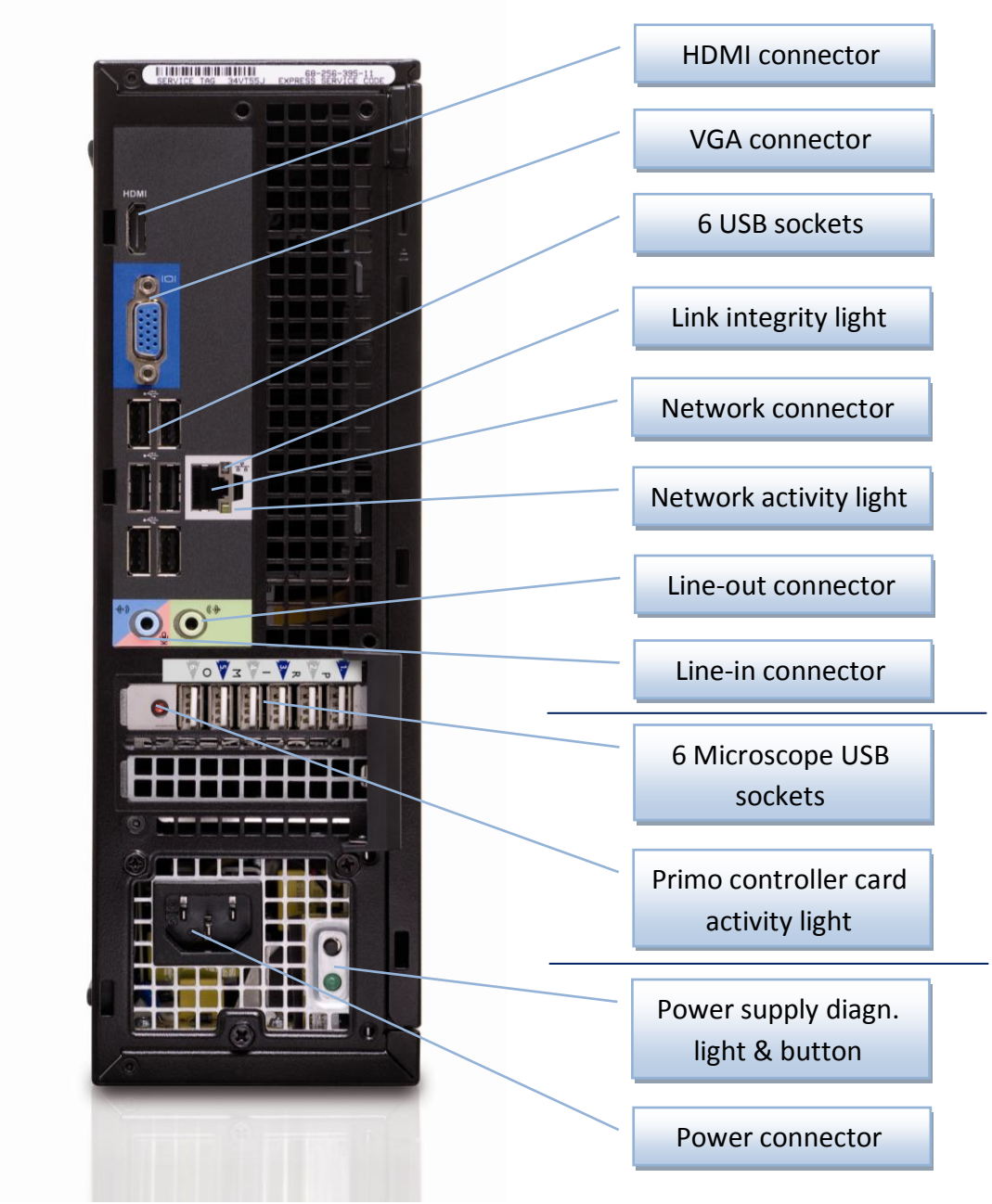


On the back panel of the Controlling Unit, three groups of sockets are seen. In the first group, the HDMI connector and the VGA connector can be found on the top, which can be used for connecting the monitor. Below these six USB sockets for system accessories, and the network connector is located. Finally, the line-out and the line-in connectors can be found under the USB sockets.

In the second group, six Primo Vision Microscope USB sockets are located, each for one Miscroscope unit, as one Controlling Unit can handle up to six Microscopes. On the left side of the Microscope USB sockets, the Primo Controller Card activity light is seen: it illuminates when the card is ready to use, after turning on the Controlling Unit; flashes in every 2 sec when the card is in use; and flashes in every 0.5 sec when any of the USB interfaces is malfunctioning.

In the third group, the power connector and the power supply diagnostic button and light can be found.

Back view of the Controlling Unit



The Controller Unit is equipped with Windows XP Embedded POS-Ready operating system, which runs the Capture and Analyzer software, and has 500 GB storage capacity.

2.1.3. Short description of the installed Capture and Analyzer software:

Primo Vision Capture Software: The Capture software allows control of each Primo Vision Microscope individually, by setting light intensity, defining image capture frequency, and archiving

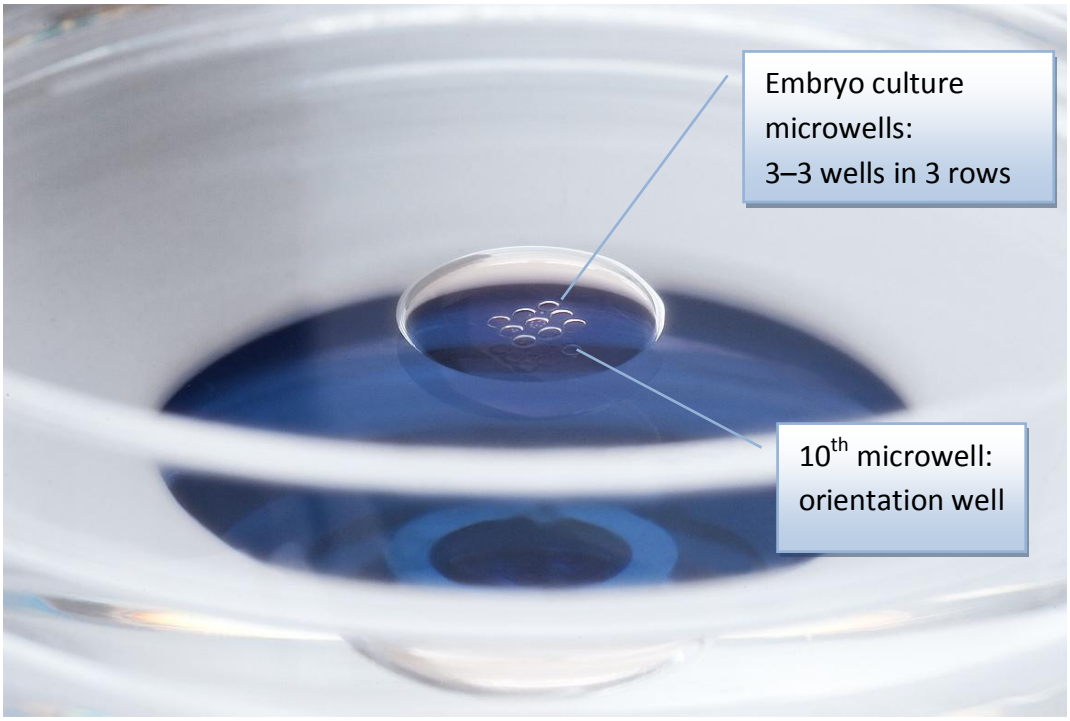
all the incoming photos into the dedicated subfolder which is created automatically at initiation of any time-lapse project. The software displays the image of the embryos, located in the WOW Petri dish on the microscopic stage of the actual Primo Vision Microscope. The software can manage up to six Primo Vision Microscopes individually. This software provides the opportunity of remote access, setting focus, imaging in multiple focal planes, and zooming into individual embryos.

Primo Vision Analyzer Software: This second software provides extra features for the manual analysis of embryo development. The images taken by the Primo Vision Capture Software are automatically stored and used for compiling a video suitable for processing. By the use of the program-generated time-lapse movie of the embryo development, user can define cleavage times and the occurrence of special/suspicious/important events (uneven cleavage, fragmentation, re-absorption of cellular fragments, multi-nucleation, vacuolization, etc.). The software is capable of generating graphs, showing embryo developmental dynamics and also data tables with the cleavage/event data. All these files are saved into the project`s (patient's) folder, together with an automatically generated report file. The information provided by the help of this software supports decision-making on embryo competence, and sets fundaments for the selection of the right embryo for transfer.

2.1.4. The microwell culture dish (WOW dish)

Well-of-the-well (WOW) Petri dishes are custom-made sterile, CE marked and officially 1cell mouse embryo tested, disposable, specially adjusted micro-well culture dishes for in vitro embryo culture in Primo Vision System.

When operating the Primo Vision System, it is crucial to identify each embryo individually, and to keep each embryo in the field of view of the Primo Vision Microscope. For this reason, it is recommended to use a special Petri dish which contains „wells" for proper placement of the embryos. The arrangement of these microwells allows easy adjusting, tracking and identifying the embryos and also confers improved culture conditions for the time of the culture period as it was published in many scientific papers (originally described by Vajta, in 1998).



The microwells form a matrix of 3 rows with 3 wells each (or 4x4 in other dish types), plus 1 extra (orientation) well. This extra well serves to identify the position of the dish. Use this orientation well to orient the dish by positioning it under the lamp console with this well pointing to the opposite side, away from the lamp console of the Primo Vision Microscope. In this case, the top row on the computer screen will correspond to the 1st row of the 3x3 matrix. Always use the dish in this orientation to ensure the reliable identification of the embryos.

2.1.5. Extension USB cables

Extension USB cables are 3 m long and are made to connect the in-incubator used Primo Vision Microscopes and the external Controlling Unit. The cable ends up in a standard USB plug (to be connected to the Controlling Unit's Microscope USB socket), and a mini USB plug (to be connected to the Microscope), the latter covered with a screw-on cap. The cap is for protection against incubator's humidity; the protector cap of the USB plug is to be screwed on tightly after inserting the mini USB plug into the socket of the Microscope.

Due to the small size of the USB plugs, the cable can be easily led through the factory-made access port on the side or back of the incubator. Once the cable is led through the port, the opening should be plugged by a tissue or paper towel. As there is a minimal positive pressure inside the incubators, there is a slight airflow out from the port, so it should not be blocked completely. This is a general characteristic of all incubators.

Some incubators do not have a factory made opening on the side/back. In this case, the USB cables may be led through the side of the incubator door.

2.1.6. Power cable

The provided power cable functions as power source for the Controlling Unit.

2.1.7. Other requirements for system assemblage

1) 24h power supply,
2) Monitor (1920x1080 or higher resolution).

These items are not included.

2.2. System installation

Primo Vision System is ready for operation out of the box, after connecting the Microscope(s) and the accessories to the external Controlling Unit and starting the software.

1. Before first use, disinfect the external surface of the Microscope and the cable by 96% ethanol. Following wiping let the ethanol evaporate, or further wipe with distilled water inside of a laminar hood before placing the unit(s) into the incubator. Disinfection is also possible with other approved solutions to be used inside the incubators, complying with the given lab's regulations.
2. Unscrew and remove the protective cap covering the Microscope Unit's mini USB socket. Also remove the cap`s rubber insulation ring as well as it might attach to the Microscope`s socket.

Insert the mini USB plug of the USB cable into the USB socket of the Microscope Unit, screw on the cable's protective cap, tighten it and then place the unit into the incubator, leading the USB cable through the factory made port on the side/back of the incubator. Connect the USB cable of the Primo Vision Microscope to the Microscope USB socket of the Controlling Unit outside the incubator. Repeat the preceding steps with each Microscope Unit.

Warning!

- The Microscopes should be placed into the incubator at least 6 hours prior to use, to let them warm up and equilibrate.
- The protective cap of the Microscope Unit's mini USB socket must be removed when using the Microscope inside the incubator; this cap is not for intra-incubatory use. It is for protection against dust and injury during transportation or storage. Make sure, that the rubber insulation ring belonging to the cap is also removed!
- The mini USB should be connected to the Microscope
- The cap of the USB cable should be fastened (in order to avoid vaporization of the socket) before placing the Microscope into the incubator!

3. Connect the monitor (with HDMI cable), the keyboard and the mouse (with their USB cables), and the 3D mouse (optional) to the sockets on the left side of the back panel of the Controlling Unit.

   Warning!

   - The monitor is not included in the system. Equipment of user's choice can be connected. System was designed to work with HD monitor, so at least a 1920x1080 screen resolution is required for the proper function.

4. Connect the power cable of the Controlling Unit, and then connect it to the electric circuit through a 24h power supply. Press the power button on the Controlling Unit front panel.
5. Launch the Primo Vision Capture Software. Open the Microscope port where you have connected a Microscope, by clicking on the „Microscope control" button. Click on the „Connect Microscope" button and wait for the device to be connected. Once the Microscope is connected, the positioning process will take place. Please wait for this process to be completed. Once the positioning is completed, Live mode is on and you can start fine-tuning your image.

2.3. System maintenance

2.3.1. Cleaning

Since the equipment is operated in a sterile/clean environment, disinfect the entire external surface and remove eventual impurities on a regular basis as it complies with your lab's protocol.

Warning!

- If your incubator uses an integrated sterilizing program, remove Primo Vision Microscopes before running it, and follow the previous steps for disinfecting the Primo Vision Microscopes.

When sterilizing the unit, proceed as follows:

- Exit the software, disconnect the Primo Vision Microscope from the Controlling Unit, and remove the unit from the incubator.
- When cleaning the Primo Vision Microscope, only use 96% Ethanol (or other disinfectant in compliance with the adequate laboratory rules and regulations) by either applying it with a cleaning cloth dampened with 96% Ethanol or by spraying 96% Ethanol onto the unit.

  Warning!

  - Though the equipment is drip-proof, it must never be immersed in any liquid.
  - While cleaning with 96% Ethanol, the use of open flame is prohibited!
- When sterilizing the equipment, carefully clean sides as well as the lamp console, and the cable.
- Following wiping let the ethanol evaporate, or further wipe with distilled water inside of a laminar hood before placing the unit(s) into the incubator
- Place the sterilized Primo Vision unit back into the incubator as soon as possible.
- The damp air in the incubator will cause some initial condensation on the Primo Vision Microscope, but once warmed up it will evaporate from the surface. In some cases, the evaporating precipitation might leave traces on the glass surface of the Microscope, which will be seen on the image at Live mode. This shall be wiped off by a non scratching tissue paper (eg. proper kimwipe). From then on, the operation of the unit can be regarded as normal.

2.3.2. Data storage and archiving

During operation, the equipment creates large image files, so make sure the computer has sufficient free storage capacity at all times. The weekly disk space requirement of ONE Microscope unit is maximum app. 13 GB. The application will warn you if there is not enough free disk space; in this case, back up your data to free up disk capacity. Stored data can be exported through the network, provided, that the system is connected and properly set up. For further instructions see Chapter 3.2.7. Capture / Settings / Remote access, and please ask your IT department.

Archiving is also possible with a regular USB drive, an external HDD or any other equipment that can be connected through the USB port, but in this case be sure that there is no time-lapse project running in the same time. The Microscopes can produce very dark or even black images when there is USB communication in the same time. This is not deterministic, depends on file size, computer load and Microscope capturing frequency but it is strongly suggested to use network connection for file transfer.

3. Software

The Primo Vision System comes with custom-made software, which contains the Capture software for photographing and subsequent archiving of the images of the embryos, and the Analyzer software for evaluation of the images taken. The software is pre-installed on the Controlling Unit. The current documentation contains description of the following software version: Primo Vision Embryo Analysis and Capturing System version 4.2.

3.1. File and folder structure

The software creates file and folder structure automatically.

All Project data are saved and stored in a separate folder. Folder name begins with the Project ID provided by the user at the start of a project, followed by the date of creation. Folder contains time-lapse images and the following subfolders:

.db_Patient name	–	database of the project
Scan	–	older of the scanned images
Videos	–	video files created by the software
Reports	–	report files created by the software

3.2. Capture

Primo Vision Capture software can manage up to six Primo Vision Microscopes individually. The software displays the image of the embryos placed in the WOW Petri dish onto the microscopic stage of the actual Primo Vision Microscope. To start the Primo Vision Capture and/or Analyzer software, start the Controlling Unit, and click on the Primo Vision Capture and/or Analyzer icon.

3.2.1. Home

Home screen gives a full view of your Primo Vision time-lapse embryo monitoring system. Status of all the 6 Microscopes can be seen here and Analysis of a previous project can be start from here as well.

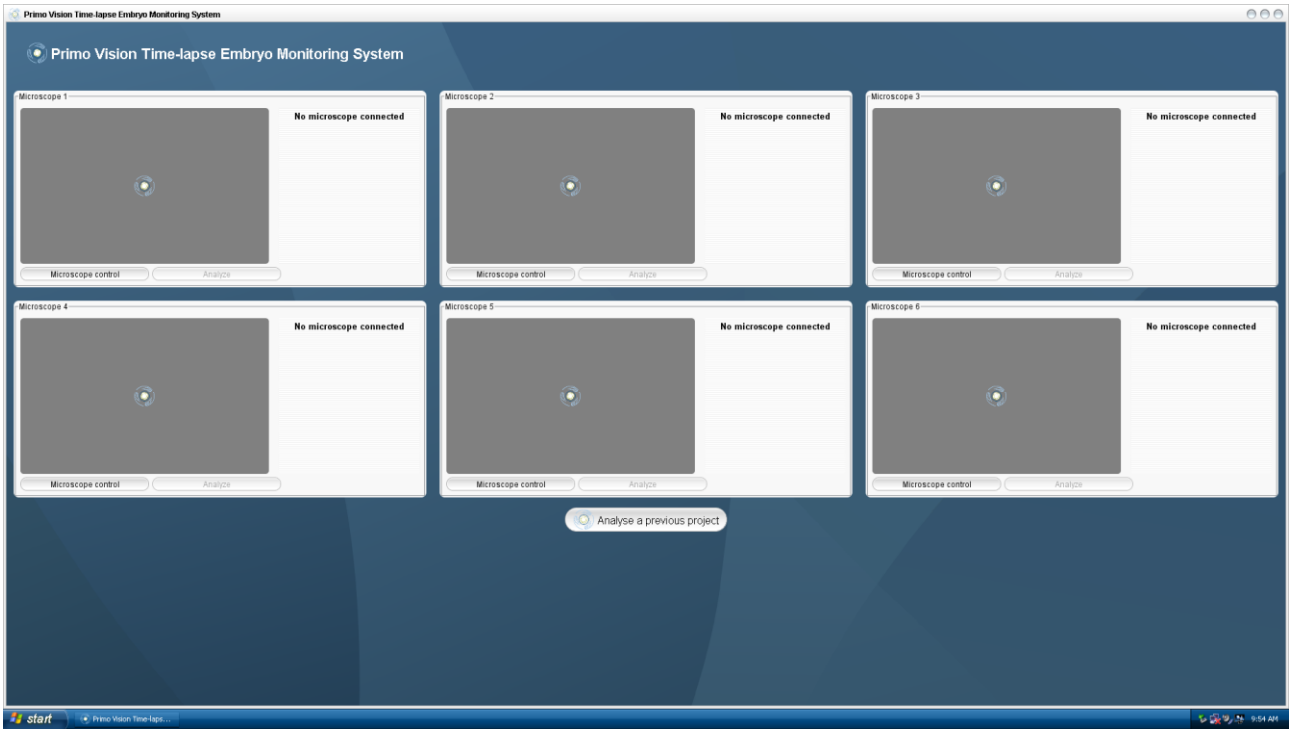
Each Microscope panel consists of the following elements:

- Status of the Microscope

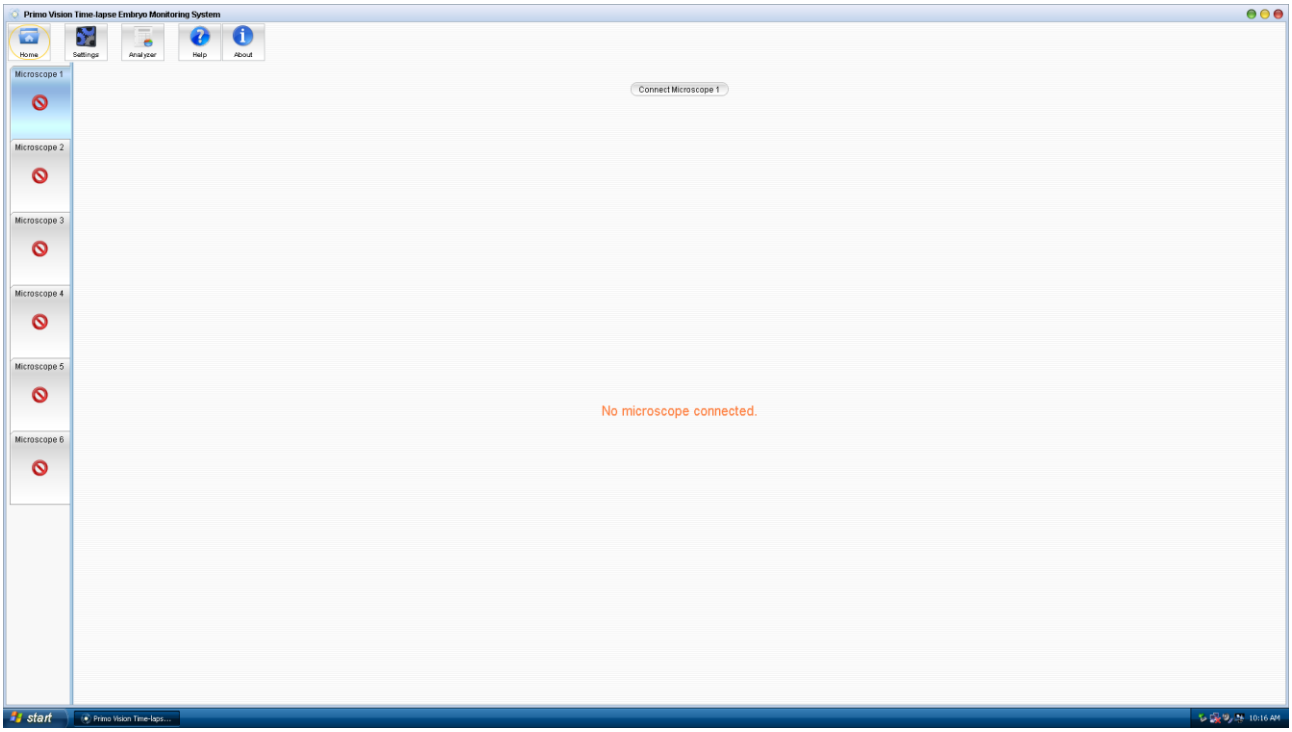
  - no Microscope connected
  - Microscope available, or
  - the data of a currently running project
- Image field – shows the last image of a currently running project
- Microscope control – instant access to the Microscope controls
- Analyze – opens the currently running project for analysis

3.2.2. Starting the Microscope Units

Upon starting-up, the image of the six Microscopes will appear.



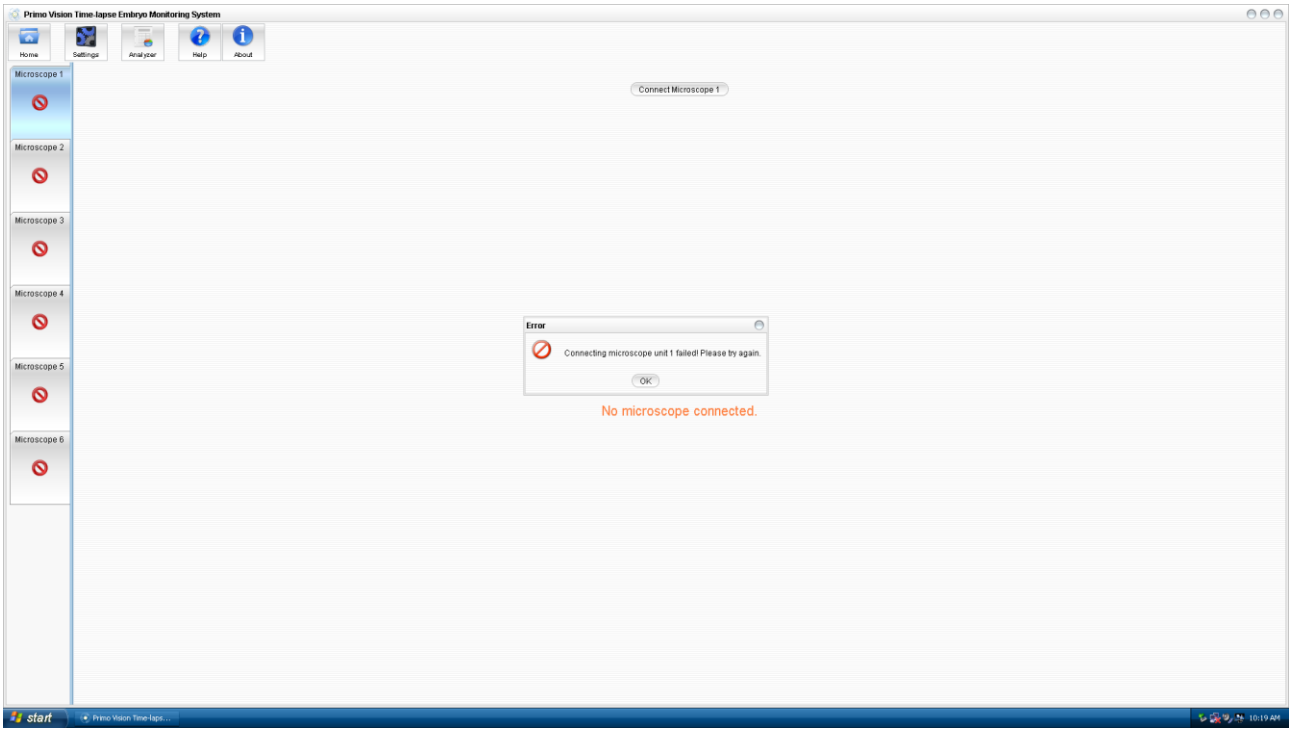
To start a project with the first Microscope, click on the „Microscope control” button bellow the image of the given Microscope. On the next screen click on „Connect Microscope 1” button.



It takes 15 - 20 seconds for the Microscope to be activated as all electric currents are switched off in the Controlling Unit. At first use after software startup, the activated Microscope performs a positioning action. When these processes are finished, the Microscope is in Live mode, where the zooming and focusing can be done, and light intensity and scan parameters can be set (see details in Chapter 3.2.3. „Live mode – focus and zoom settings, light intensity, scan test"). After setting these parameters, click on „Start Project" button to start the time-lapse sequence (see details in Chapter 3.2.4. „Starting a time-lapse project").

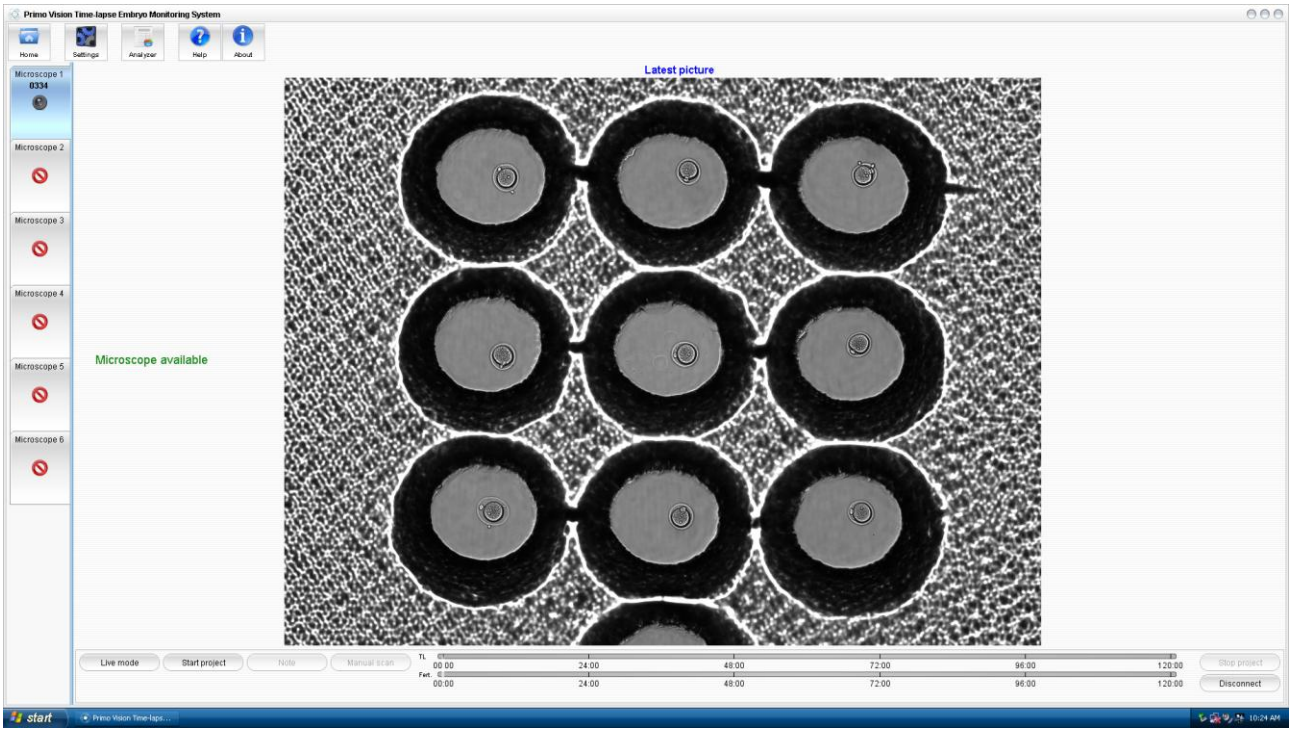
In order to start time-lapse sequences with the other Microscopes as well, follow the previous steps with each Microscope.

If the Microscope is not connected to the Controlling Unit, a pop-up window will appear with the text „Start Microscope 1 failed! Please try again.”



In this case check the USB plug and the number of the Microscope, close the pop-up window and click on „Connect Microscope 1” button again.

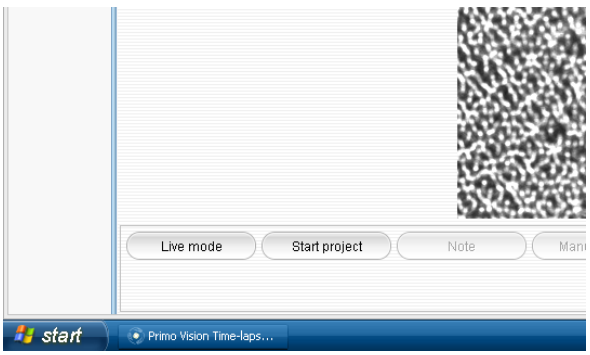
Microscopes will be shown in tabs on the left side of the screen.



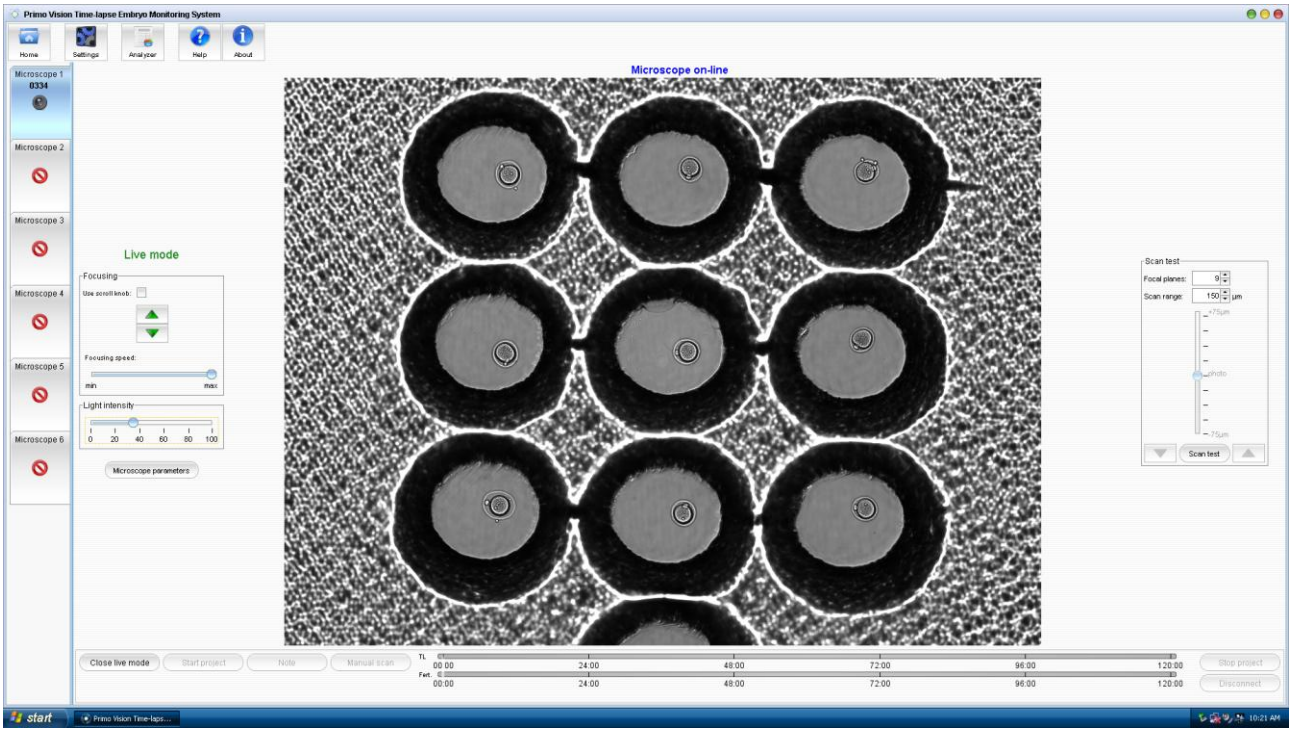
Click these tabs to select among the images of the Microscopes connected to the Controlling Unit. The software will only recognize lately connected Microscopes after activating and starting them.

3.2.3. Live mode – focus and zoom settings, light intensity, scan test

The Microscope Units are in an inactive status after connection and starting the software. Prior to starting the time-lapse photo sequence, positioning the WOW dish, setting the focus and the light intensity, zooming, and testing the scan settings is possible after activating the Microscope. This activation (Live mode) can be achieved by clicking on „Microscope control" button on the Home screen, or by clicking on „Live mode" button of the individual Microscope screens.

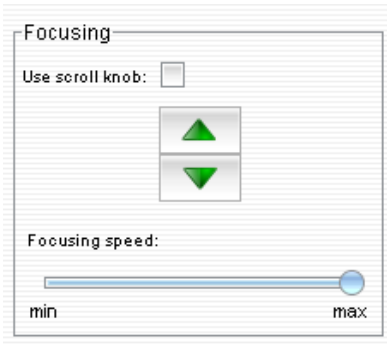


When the Microscope is in Live mode, and the WOW dish with the embryos is placed onto the dish holder of the Microscope, focus setting and zooming can be performed, completed with light adjustment.



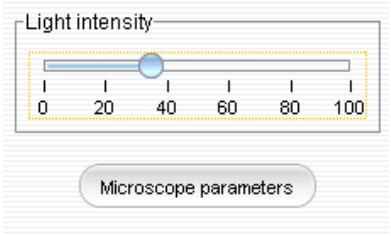
Controls of these functions are located on the left and right side of the screen (only in Live mode):

- Focus setting: Focus setting can be achieved by clicking (and / or pushing) on the arrows, which moves the optics up and down until finding the best focal plane. Moving the „Focusing speed" slider can set the speed of focusing.

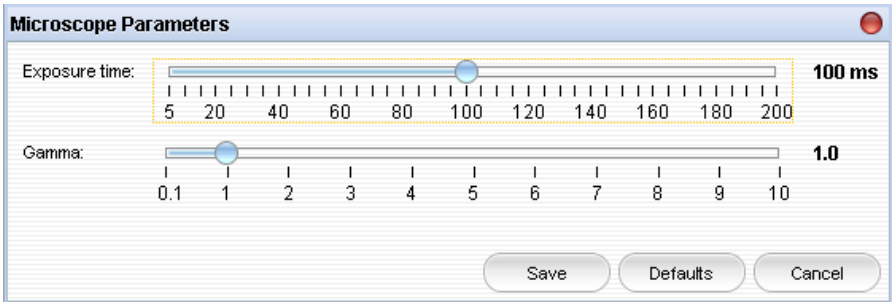


  Alternatively 3D mouse can be used for focus settings. After clicking on „Use scroll knob", twist the 3D mouse gently to find the best focal plane.

- Zooming: Move the mouse cursor to the image. By scrolling the mouse wheel zooming can be performed. If clicking on the enlarged image and moving the mouse (grabbing the image), the image can be moved in order to see each microwell. Using this enlarged image, fine focus adjusting can be achieved.
- Moving the „Light intensity" slider can set light intensity.



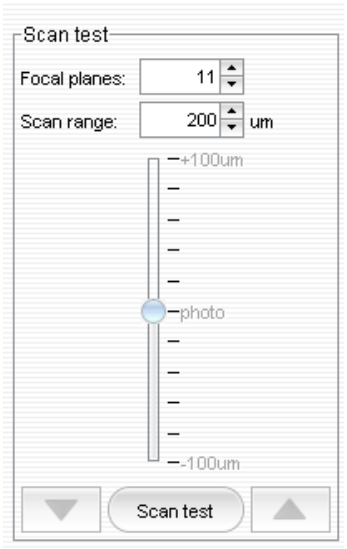
- For adjusting microscope parameters, click on „Microscope parameters" button, where exposure time and gamma can be set. The result of the changes executed will be seen on the screen in Live mode.



  - Exposure time (in milliseconds) can be set from 5 to 200 ms. Adjust this option to achieve proper brightness of exposed images.
  - Gamma can be set from 0.1 to 10 and enables digital gamma correction, which applies a gamma characteristic to the image.

  The changes made in microscope parameters settings can be saved by clicking on „Save" button, or can be cancelled; in this case the previous settings will be used. Defaults settings can be restored as well.

- Scan test: 3-11 different focal planes can be checked in Live mode by the help of the Scan test function. After setting the number of focal planes and the scanned range, click on „Scan test" button and by the help of the two arrows it is possible to see the images of the set focal planes.



This function can help in finding the best scan settings for the Settings menu / Time-lapse defaults (see details in Chapter 3.2.6. „Scanning" and 3.2.7. „Settings / Time-lapse defaults").

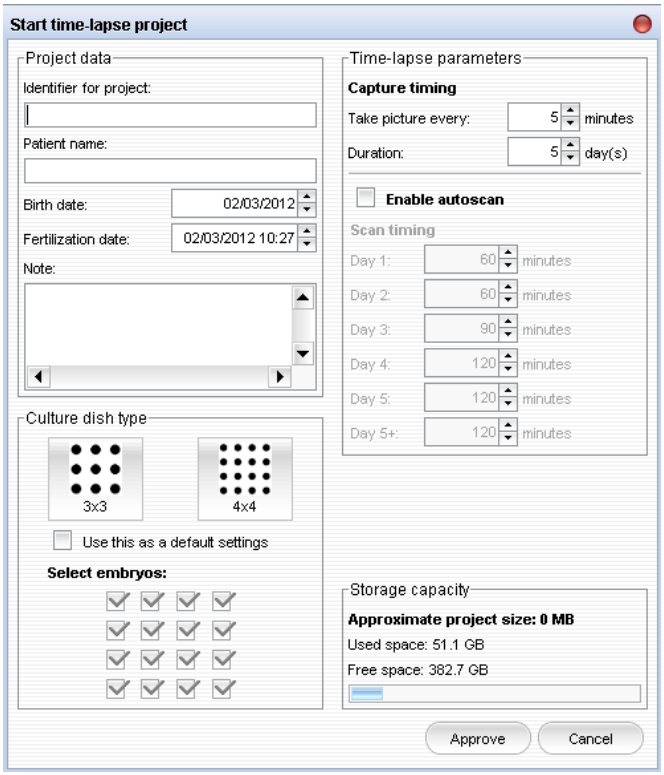
The number of focal planes is set to 7 by default and can be set between 3 and 11. The adjusted number of focal planes is imaged in the adjusted range. E.g. if 200 microns is set in 7 focal planes, then the software will scan the range +/- 100 microns from the adjusted focal plane and will make 7 images. The Microscope will move back to the preset focal plane after the scanning process.

When the best focal plane is found and microscope settings are completed, click on „Close live mode" button for inactivating the Microscope Units. When closing, the Microscope takes a photo of the embryos, and this photo will be shown on the screen. Live mode is turned off automatically after 2 minutes by default in order to ensure the undisturbed environment for the embryos and to minimize their light load. If necessary for a new dish positioning or focus or light setting, Live mode can be used any time during the time-lapse sequence.

3.2.4. Starting a time-lapse sequence

Up to six Primo Vision Microscope Units can be connected simultaneously to the external Controlling Unit. The program manages each Microscope separately, so setting of the parameters and starting photo sequences is performed separately for each Microscope.

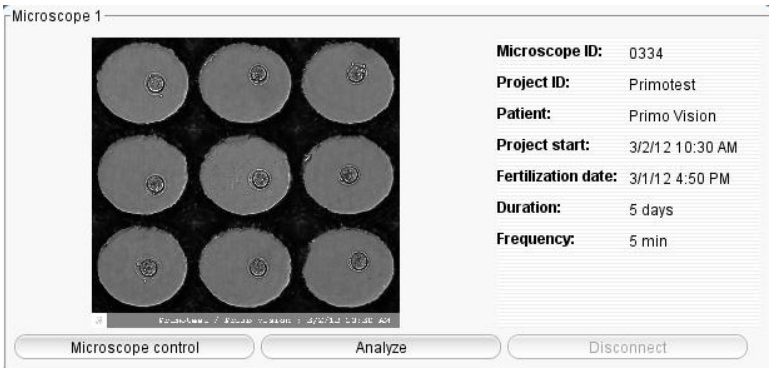
To start a photo sequence (a time-lapse project), first set the focus (and if necessary, light intensity, scan- and camera parameters) in Live mode, then, after closing Live mode, click on „Start project" button on the lower left part of the screen. A pop-up window will appear, where details of the project can be filled in, culture dish type and time-lapse parameters can be specified, and storage capacity can be checked prior to starting the time-lapse sequence.



- In the project data field, an identifier for the project, patient name and birth date, exact time of the fertilization shall be filled in. Notes can also be added in the „Notes" field. It is obligatory to specify the project identifier and patient name fields.

  The project identifier (supplemented with the date of starting the time-lapse sequence) will be the name of the subfolder where the images will be saved. (It is not necessary to write date in project identifier field, as the date will be saved in the folder name automatically.)

  If clicking on the „Home" button, the project identifier and patient name will be shown next to the picture of the given Microscope Unit, and also will be saved on the lower strip of each time-lapse image.

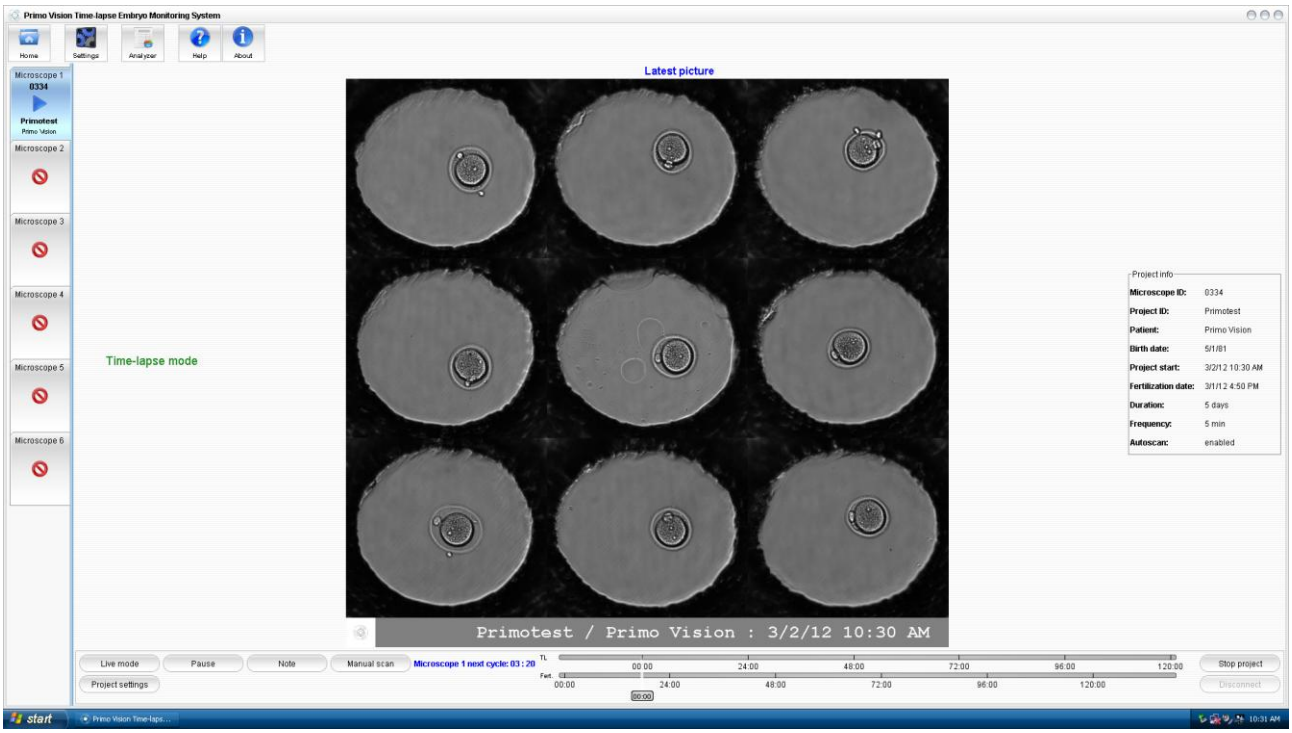


- By clicking on the adequate figure in the culture dish type field, the WOW dish type (9 or 16 microwells) used with the current Microscope can be selected. It is necessary to select the dish type to be used, as a montage of the microwells will be prepared by the software and only the image of the microwells will be saved. When the dish type is selected, the exact number and position of the embryos are to be selected by clicking on the checkboxes.
- In the time-lapse parameters field, the frequency and the duration of the capturing can be set, and by clicking on the checkbox of „Enable autoscan", the frequency of automatic three-dimensional scans can be set for each days of the time-lapse sequence. These images will be saved in the „Scan" subfolder of the given time-lapse project.
- In the storage capacity frame - based on the frequency and the duration of the capturing - the software estimates the size of the given project, whereby the available storage capacity can be checked.

When finished, click on „Approve" button; the time-lapse sequence is started now.

3.2.5. The running time-lapse sequence

The latest image of a given active Microscope is shown on the screen corresponding to the Microscope. Each image has a „stamp" on it stating the project identifier, patient name, and date and time of capturing. Zooming can be performed any time by moving the mouse cursor to the image and by scrolling the mouse wheel. If clicking on the enlarged image and moving the mouse (grabbing the image) the image can be moved in order to see each microwell.



Icons on the top of the window:

- Home: If clicking on „Home” button, the „Home” screen will appear, where the status of the Microscopes and the details of the running projects (Microscope and project identifier, patient name, date of starting the project and date of fertilization, and the duration and frequency of the time-lapse sequence) can be seen.
- Settings: Time-lapse defaults, remote access and project directory can be set here (See details in Chapter 3.2.7. „Settings”).
- Analyzer: The time-lapse images made by the Microscope of which picture active on the screen, can be analyzed by the Analyzer software by clicking on the „Analyzer” button. The same will happen if clicking on the „Analyze” button on the „Home” screen of the given Microscope.
- Help and About: The User Manual and the details of the producer can be reached if clicking on these buttons (see details in Chapter 3.2.9. „Help” and „About”).

The tabs of the connected Microscopes are shown on the left side of the screen. Click these tabs to switch among the images of the connected Microscopes. By default, the program always shows the image of Microscope 1. The tab referring to the Microscope of which image is seen on the screen appears as blue, while the other tabs are grey. Different icons indicate the status of the given Microscope:

- 

– No Microscope connected

  Only the number of the Microscope is seen.

- 

– Microscope available, no time-lapse project running

  The number and the burnt-in identifier of the Microscope is shown, which is automatically recognized and presented by the software.

- 

– Time-lapse project running

  The number and the burnt-in identifier of the Microscope, the project identifier and the patient name is shown.

- 

– An image is taken (animated icon)

  The number and the burnt-in identifier of the Microscope, the project identifier and the patient name is shown.

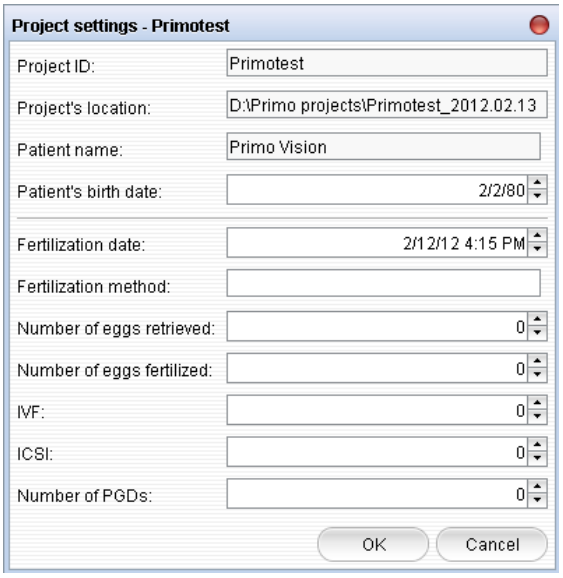
- 

– The time-lapse project is paused

  The number and the burnt-in identifier of the Microscope, the project identifier and the patient name is shown.

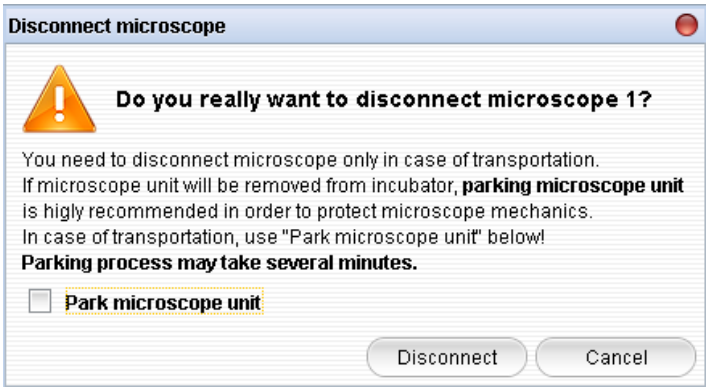
On the bottom of the screen:

- Live mode: Live mode can be reached any time during the time-lapse sequence by clicking on this button.
- Pause: By clicking on this button the capturing of the given Microscope can be paused. This function can be utilized when the WOW dish is to be removed from the Microscope Unit for any reason (changing of culture media, eliminating the non-developing embryos, etc.)
- Note: Notes can be added with the help of this button.
- Manual scan: If images of different focal planes are required at a given time, scanning can be performed instantly by clicking on this button (see details in Chapter 3.2.6. „Scanning"). Automated scanning can also be pre-programmed (see details in Chapter 3.2.4. „Starting a time-lapse sequence").
- Project settings: With the help of this button the following details of the project (except the first three lines) can be changed or specified.



Once the project is started, project ID, project's location and patient name cannot be changed.

- Next to the previous buttons, a timer shows the remaining time until the next capture below the image.
- On the right side of the timer two strips are shown; the upper strip shows the elapsed and remaining time from the beginning till the end of the time-lapse sequence, while the lower shows the elapsed and remaining time from the time of the fertilization (the time of the fertilization is to be set when starting the time-lapse project, or later on it can be specified by clicking on „Project settings" button).
- Stop project: To finish a time-lapse sequence, click on „Stop project" button, and then confirm your decision in the pop-up window. In case of exiting the capture software, the time-lapse project will be stopped as well.
- Disconnect: This function can be reached only in case if there is no running time-lapse sequence with the given Microscope. With the help of this button, an individual Microscope can be disconnected and parked if it shall be removed from the incubator. After clicking this button a window will appear, where parking of the Microscope can be set.



The same function can be reached from the „Home" screen as well, by clicking on the „Disconnect" button.

On the right side of the screen the details of the running project can be seen.

3.2.6. Scanning

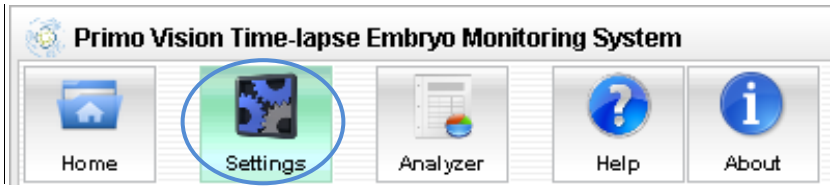
If images of different focal planes are required at a given time, scanning can be performed instantly by clicking on the „Manual scan" button on the lower part of the screen. Automated scanning can also be pre-programmed. The number of focal planes and the scanned range can be set in the Settings menu (see details in Chapter 3.2.4. „Starting a time-lapse project"). Images will be saved in the „Scan" subfolder of the given time-lapse project.

To find the best settings for scanning, use Scan test function: in Live mode 3-11 different focal planes can be viewed with the help of the Scan test function. After setting the number of focal planes and the scanned range, and clicking on „Scan test" button, with the help of the two arrows it is possible to see the images of the previous scan settings. The actual focal plane of which image is on the screen, is shown on the figure above the „Scan test" button.

The scans will be saved in a different subdirectory in the patient`s folder. All scanned images will have a **„**stamp**"** on them stating the scan parameters. These images can also be used for latter processing. They provide a possibility to locate important objects that are located outside of the pre-adjusted focal plane.

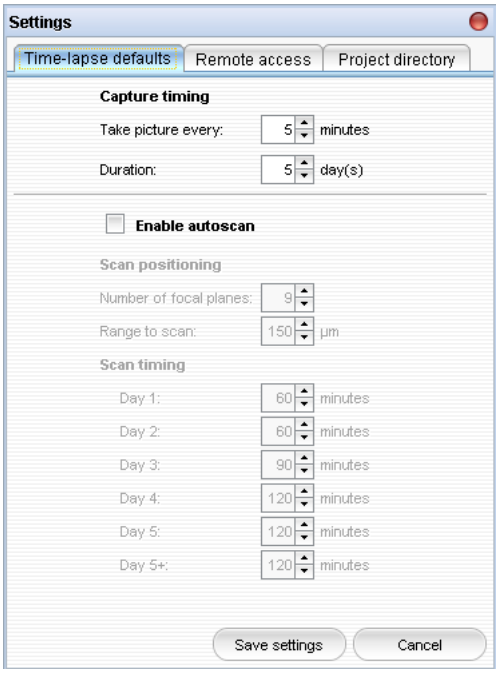
3.2.7. Settings

The following parameters can be set after clicking on the „Settings" button on the left upper part of the screen.



3.2.7.1. Time-lapse defaults

Duration and frequency of capturing, number of focal planes, scan range and scan frequency can be set in this menu point, which will be seen as default when starting the next time-lapse project. Alterations made in the default settings will not change the settings of running time-lapse projects; once the Microscope is in TL mode, settings can be changed only after stopping and starting the time-lapse sequence again.

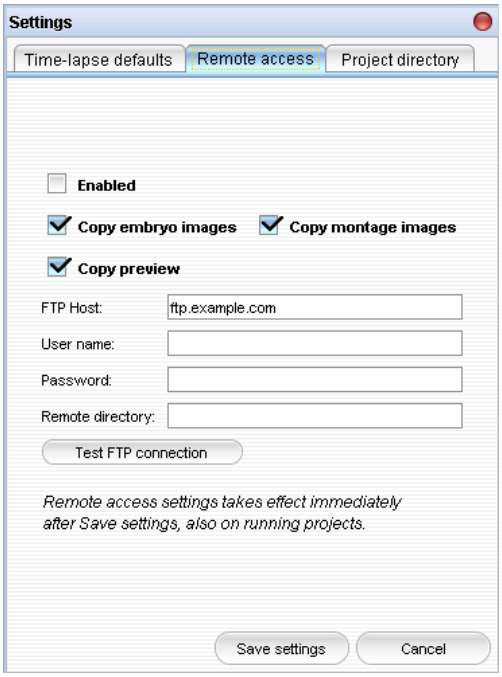


- Capture timing: frequency of the capturing can be set from 5 to 60 minutes. Duration of capturing is set to 5 to days by default and can be set between 1 and 30 days. Cycle time is 5 minutes by default and can be set between 5 and 60 minutes.
- Enable autoscan: a timing program for the scans can be initiated by selecting this function; in this case, scans will be done automatically at the pre-adjusted times.
- Scan positioning: number of focal planes is set to 7 by default and can be set between 3 and 11. The adjusted number of focal planes is imaged in the adjusted range. E.g. if 200 microns is set in 7 focal planes, then the software will scan the range +/- 100 microns from the adjusted focal plane and will make 7 images. The Microscope will move back to the preset focal plane after the scanning process. The optimal scan range and number of focal planes can be tested in Live mode, with the help of the Scan test function (see details in Chapter 3.2.3. „Live mode – focus and zoom settings, light intensity, scan test”.
- Scan timing: the minimal time that can be adjusted is 20 min. Increased scanning frequency can be recommended:
    1. in the first day of the cycle (scanning may be performed hourly) in order to properly spot pronuclei and multi-nucleation;
    2. on the 2nd and 3rd day of the cycle, scanning in every 2 hours may be sufficient to see multi-nucleation and to assess the cell number precisely, and to check the presence and degree of fragmentation.

When finished with time-lapse defaults setting, click on „Save Settings” button.

3.2.7.2. Remote access

By the use of this feature, images are transferred to the set remote location. Select „Enabled" if you want this function to be activated. It is important to harmonize this function with the remote location`s adjustments. For this, please contact your IT administrator.



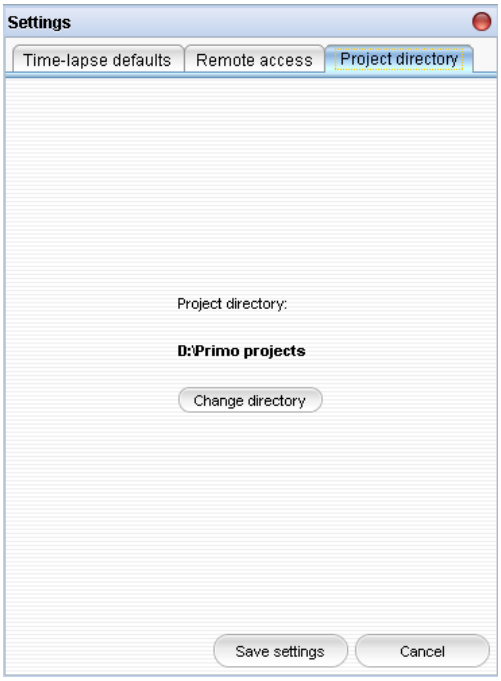
- By selecting the „Copy embryo images" and/or „Copy montage images" and/or „Copy preview" the transfer of the needed type of images can be selected. By selecting all the three image types, all the database created by the Capture software will be transferred to the FTP server. Check transfer capacity of your network connection before choosing its setting. For this, please contact your IT administrator.
- Parameters of the remote access can be set in the following fields:
    - FTP Host – IP address or the name of the FTP server
    - User name – the name of the user on the given server
    - Password – the set password on the given server
    - Remote directory – the directory of the FTP server where the user has the access
- With the help of the Test FTP connection the setup can be checked, and in case of inadequate settings the software warns the user to change them.

When finished with remote access setting, click on „Save Settings" button. It is strongly suggested to use network connection for file transfer since the Microscopes can produce very dark or even black images when there is USB communication in the same time. This is not deterministic, depends on file size, computer load and Microscope capturing frequency - for further instructions please ask your IT department.

Primo Vision Controllers are shipped with standard Windows setting regarding the network profile. It means that the computer is instructed to get IP address automatically from a DHCP server. When you attach the Controller to your local network and it doesn't see other computers it means that fix IP addresses are used or there are special restrictions in your IT policy - in this case please ask your IT department.

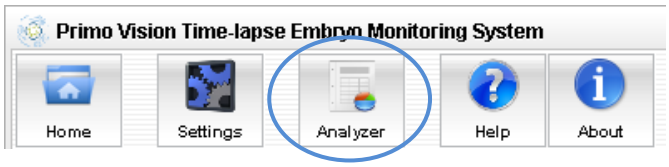
3.2.7.3. Project directory

The folder where each of the time-lapse projects' subfolder is saved can be set here.



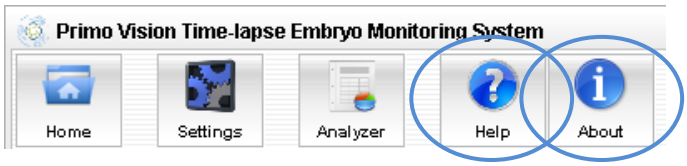
After clicking on „Change directory" button, a pop-up window will appear, where the new directory can be selected.

3.2.8. Analyzer



The Analyzer software (see details in Chapter 3.3. „Analyzer module") can be launched by clicking on the „Analyzer" icon.

3.2.9. Help and About



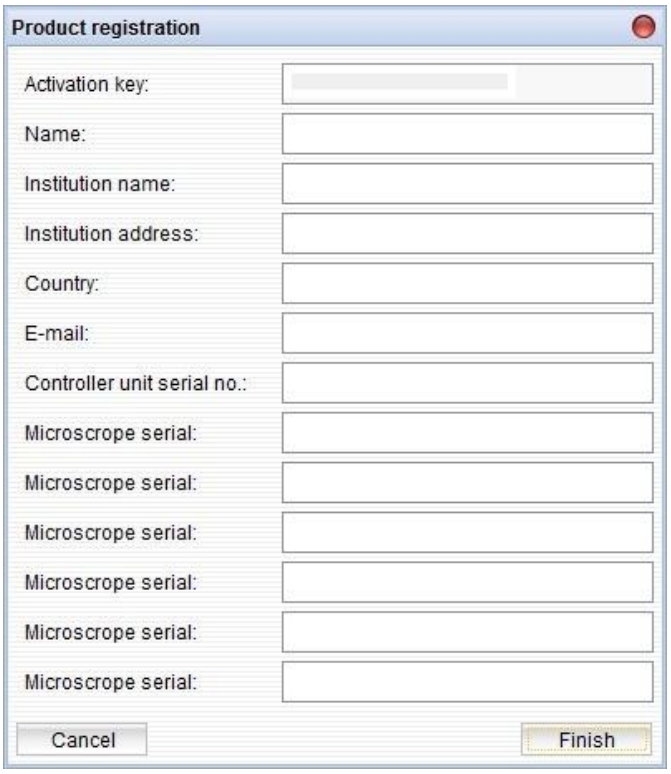
After clicking on the „Help” button, a pop-up window with the Use and Maintenance Manual of the Primo Vision system will appear.

If clicking on the „About” button, the details of the producer and the version number of the software can be checked.

3.3. Analyzer module

The Primo Vision Analyzer software creates time-lapse movies of the developing embryos and provides quick and comfortable possibility to manually analyze and compare the development of the different embryos, in order to support proper embryo selection. Inclusion- and exclusion-parameters can be set as well as any user-defined events of the embryo development with corresponding reference values. Size measurements are also possible.

The Analyzer part of the software can be used on other computers (not only the same where the time-lapse project is performed) as well. For this, please contact your distributor or our company. The standalone Analyzer software requires a registration that is available with the provided Primo Vision Activation Key only. Software runs for 14 days without a valid registration. The registration can be made online, by typing the license code into the activation key field. After clicking on „Next", the following pop-up window will appear:

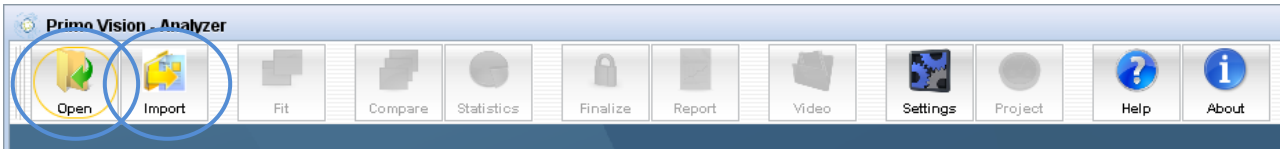


After filling in the fields here, click on „Finish" button; the registration is completed now.

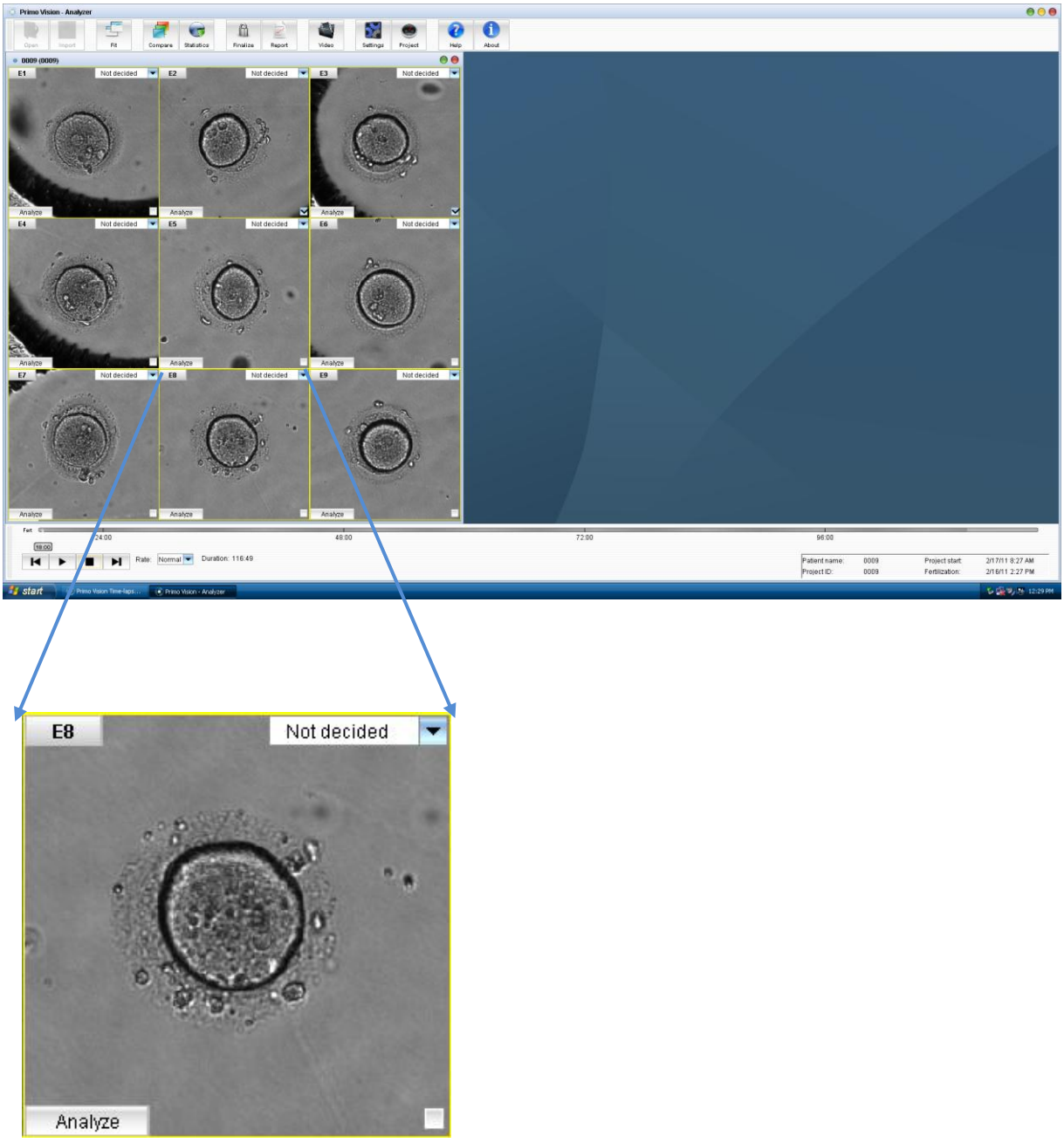
3.3.1. Loading of a photo sequence for analyzing

Running projects can be analyzed directly from the Home screen, or it also can be started from the capturing screen of the given active Microscope. Picture sequences can be loaded from the

analysis window as well, by clicking on the „Open” button. Previously made and partly or completely analyzed projects can also be opened, checked or re-analyzed.



After clicking on „Open”, select a folder from the existing projects, and load the selected one. The program will display the photo sequence, starting with the first image of the sequence. On this compiled embryo window each embryo has a parallel field, on which the needed buttons and checkboxes can be seen.



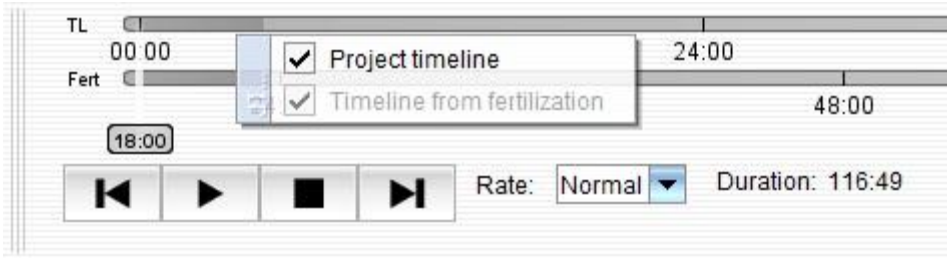
Description of the individual embryo image:

- Embryo number: The number in the left upper corner shows the number of the microwell in which the embryo developed. A color can be added if clicking on the box around this number, which color will belong to this embryo during analysis.
- Decision dropdown: In the right upper corner, the final decision about the embryo (transfer, cryopreservation, discard or not decided) can be selected.
- „Analyze" button: This button is shown in the left bottom corner, and it can be used to select the embryo to analyze.
- „Compare" checkbox: In the right bottom the „compare" checkbox can be found, which can be used for the purpose of comparison (see details in Chapter 3.3.3. „Compare") and for representing the embryos in the graphs of the „Statistics" menu (see details in Chapter 3.3.4. „Statistics").

The actual embryo images can be saved or the original image with all embryos of the same WOW dish can be seen by right-clicking on the image any time during analysis.

Description of the lower part of the screen:

- Timelines: On the lower part of the screen bellow the embryo images a timeline can be seen, which shows the time elapsed from the fertilization. If right-clicking on this timeline, another timeline can be selected as well, which shows the time elapsed from the beginning of the time-lapse sequence.



  If moving the mouse cursor above these timelines, the images can be played by scrolling the mouse wheel forward and backward.

- Video control buttons: The time-lapse sequence can be played with the „Play" sign bellow the timelines as well; when playing, this sign turns into a „Pause" sign. With the help of the „Stop" button the sequence can be started again from the onset. With the help of the two terminal control buttons the time-lapse sequence can be scrolled frame by frame.
- Rate: The speed of playing the sequence is set to normal by default, but can be changed between 16 times slower and 4 times faster with the help of the dropdown next to the control buttons.

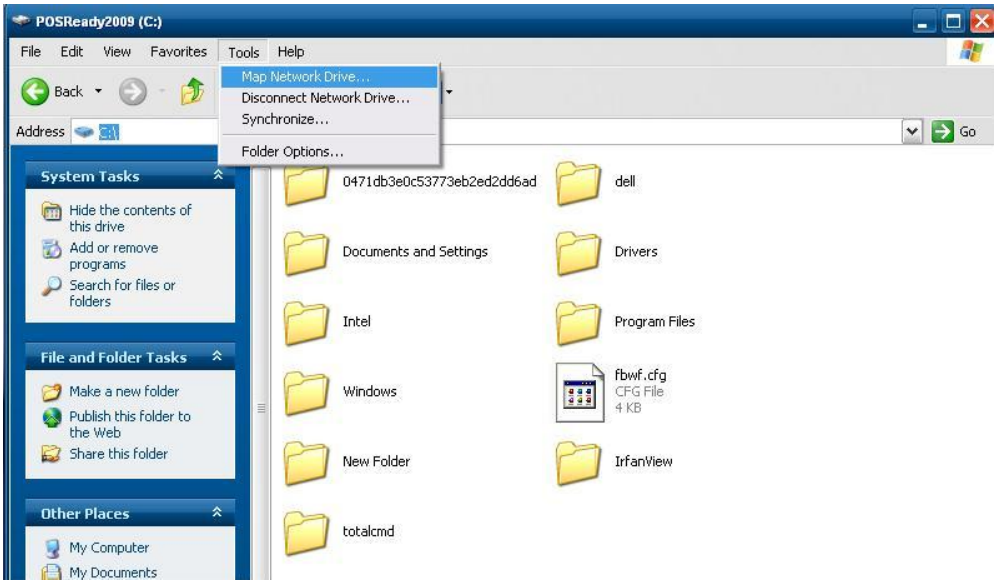
- Duration: On the right side of the dropdown the whole duration of capturing is displayed.
- Details of the project: On the right bottom part of the screen the patient name, project identifier, time of project start, and time of fertilization can be seen.

3.3.1.1. Opening time-lapse sequences captured with previous software versions

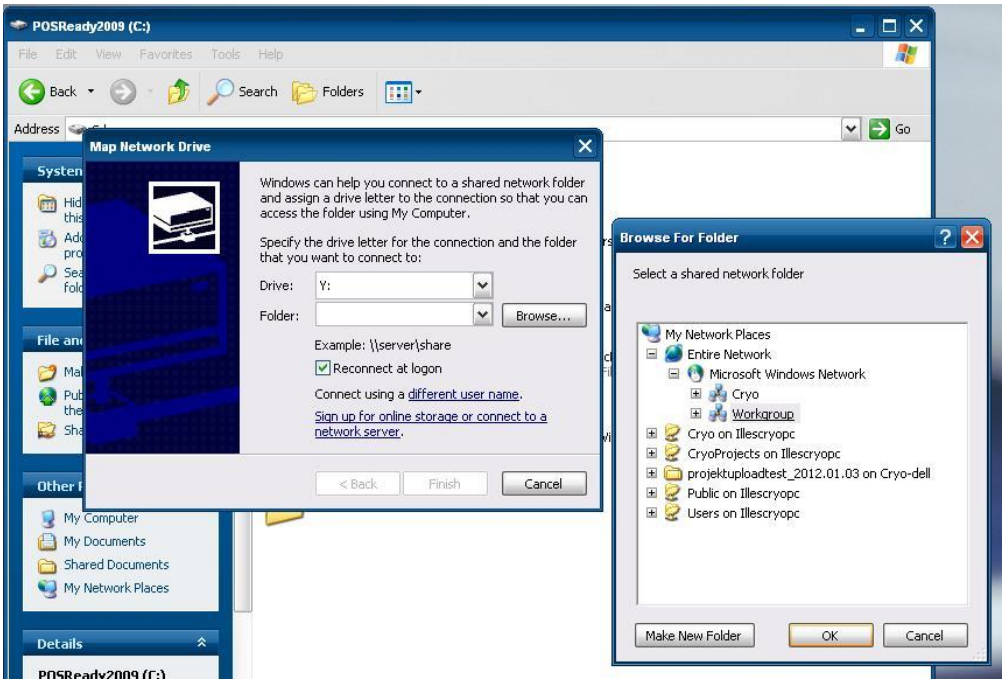
If the images are made with previous software versions, there is a possibility to import them by clicking on the „Import" button. Fill out the relevant information such as project ID, patient name and birth date, project start and fertilization date, and select the wow dish type and image file type.

3.3.1.2. Opening projects from the local network

Projects that have been transferred to the local network can be analyzed as well; for this purpose the project's folder is to be shared. In order to let the Analyzer software access the shared folder, open Windows Explorer on the PC that runs the Analyzer software and open the „Map network drive" in the „Tools" menu:



Assign a drive letter to the connection in the „Map network drive" settings, then click on the „Browse" button and select the previously shared folder.

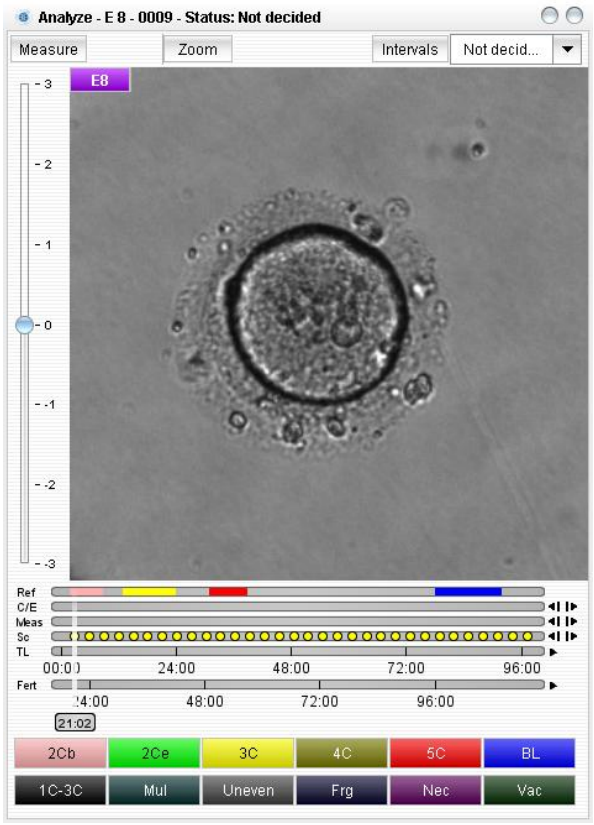


The Analyzer software now can be started and the project can be opened as described previously in this chapter. The shared folder will be available as a local drive (in accordance to the drive letter set in the „Map network drive” setup) after clicking on the „Open” button.

3.3.2. Selecting an embryo for analysis

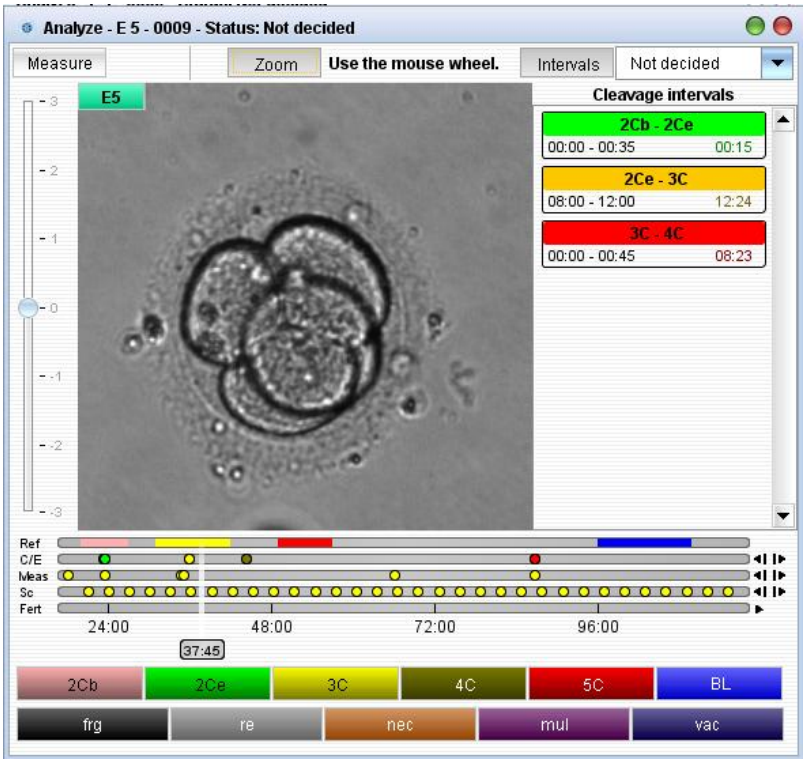
By clicking on the „Analyze” button in the lower-left corner of an embryo image, the enlarged image of the embryo will be seen.

3.3.2.1. Description of the „Embryo analysis” window



Buttons and functions on the top of the window:

- Measure button: To measure, click on „Measure” button on the left upper part of the enlarged embryo window; two cross-hairs will appear which can be moved by the mouse. The distance between the two crosses will be automatically measured and saved by the software. The diameter of the zona pellucida, the blastomeres, or any distances of the user’s choice can be measured.
- Zoom button: Click on „Zoom” button on the right upper part of the enlarged embryo window, move the mouse cursor to the image, and by moving the mouse wheel back and forward, zooming can be performed. If clicking on (grabbing) the enlarged image and moving the mouse, the image can be moved in order to see all parts of it.
- Intervals button: If clicking on this button, the intervals between the statuses, set previously in „Settings” can be viewed.



Regarding to the reference values and the tolerance rate, set in the „Settings", the legend of the given interval can be seen in the tones between green (the cleavage interval of the embryo is within the reference values), orange (out of the reference values, but within the tolerance rate), and red (out of the reference values and the tolerance rate).

- Decision dropdown: This function in the right upper corner indicates the final decision about the embryo (transfer, cryopreservation, discard or not decided). Based on this decision, the embryos will get a color-coded frame: green for transferred, blue for cryopreserved, and red for discarded embryos.

Strip on the left side of the window

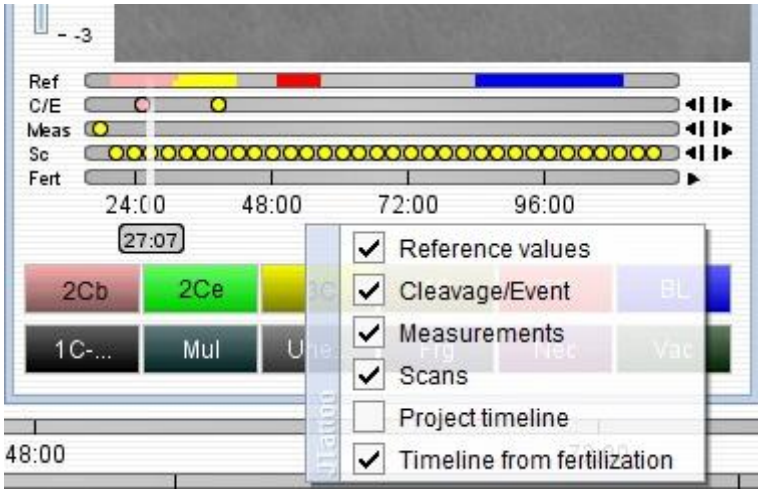
- Scan strip: In the time-points when three dimensional scan images were performed by the Capture software, the scan strip contains dots; each dot on the vertical strip refers to an image made in a distinct focal plane. Checking the images of different focal planes can be achieved either by clicking on the dots or by clicking on the endpoints of the strip. The time-points of scannings can be found easily with the help of the Scans (Sc) strip under the image of the embryo.

Strips under the image:

- Ref - Reference values strip: Reference values for the optimal time of cleavages can be set in the „Settings" menu; these values are shown on the Ref strip.

- C/E - Cleavage / Events strip: The developmental stages of the selected enlarged embryo can be identified and marked manually by the user; the times of status changes are shown on the C/E strip. See details of developmental status setting in chapter 3.3.2.2. „Marking the developmental status of the selected embryo”.
- Meas - Measurements strip: Times of the saved measurements are shown on the Measurement strip.
- Sc - Scans strip: During the time-lapse sequence, images of different focal planes can be taken by the Capture software. Times when these scanned images were taken are shown on the Scans strip.
- TL - Time from project start strip: The time elapsed from the beginning of the time-lapse sequence is shown here.
- Fert - Time from fertilization strip: Shows the relative time elapsed from the fertilization.

These bars can be customized by right clicking on them, and unselecting the not desired ones.



By clicking on the arrowheads on the right side of the strips jumping to the previous or next event can be achieved. If moving the mouse cursor above these bars, the images can be played by scrolling the mouse wheel forward and backward.

Status and event buttons at the bottom of the window:

- The current developmental state of the selected embryo can be marked by the user with the help of these status and event buttons (see details in Chapter 3.3.8. „Settings” and 3.3.2.2. „Marking the developmental status of the selected embryo”).

The actual embryo images can be saved by right-clicking on them any time during analysis.

3.3.2.2. Marking the developmental status of the selected embryo

The following steps should be followed for marking the developmental stages of the selected embryo:

1) Click on the „Analyze” button of the selected embryo. It will be displayed as enlarged. By pressing the „Play” button, the time-lapse movie will be played in the box. The video can be moved frame by frame by the appropriate buttons, or by using the mouse scroll wheel when the mouse points to the strips bellow the embryo image.
2) If the embryo reaches a stage of development that is previously set (see details in Chapter 3.3.8. „Settings”), pause the movie, and click on the selected „Status” button. The program will place a dot on the proliferation strip for the time of the event. If misplaced a mark, it can be deleted by clicking on the relevant Status button again.
3) Continue marking the next developmental stages until the end of the time-lapse sequence or until reaching the last status. In case of stopping an analysis before finishing it, and closing the project, the actual status will be saved, so by opening it again, it automatically loads previous analysis data.

Measurements can be made at any point of the sequence as well, and those time points will also be marked on the relevant proliferation strip.

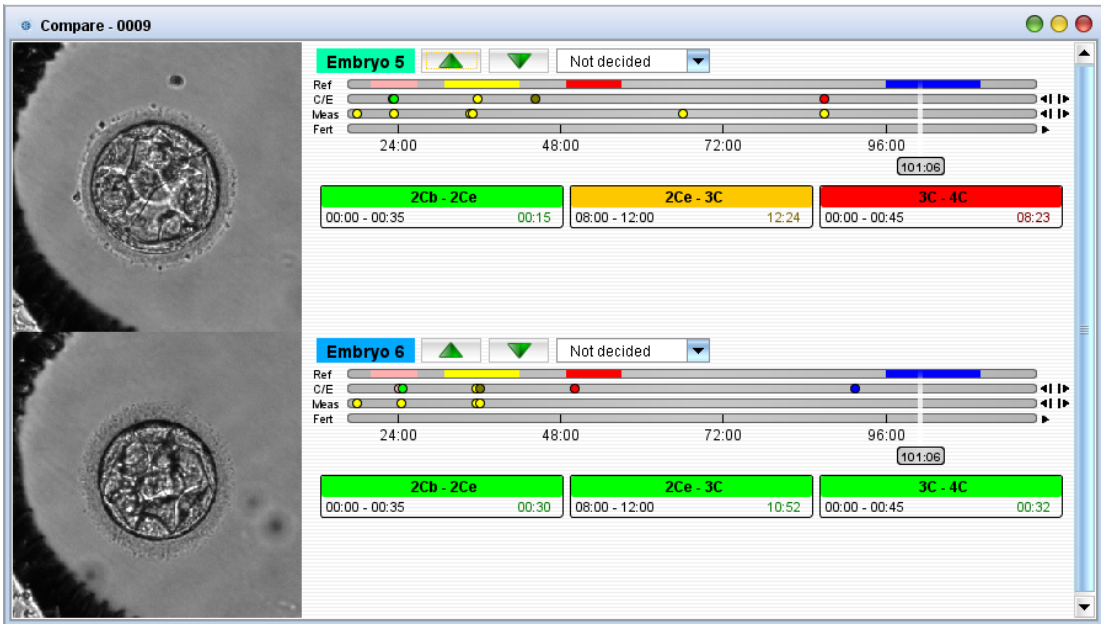
The images taken in different focal planes (the scanned images made by the Capturing software) can be seen in the time points when the scannings were performed. These time points can be found easily with the help of the „Sc” strip and the two arrowheads under the image. In these time points the scanned images can be viewed by clicking on the points on the vertical lane on the left side of the enlarged embryo image.

The developmental stages and the results of the measurements appear in a visible and comprehensive way in the „Statistics” menu in graph and table forms, too (see Chapter 3.3.4. „Statistics”). For presenting the given embryos on the graph, the „compare” box on the original window showing all embryos needs to be ticked.

3.3.3. Compare

With the help of the software, it is possible to compare the developmental dynamics of the embryos. The images with the measurement and proliferation strips and the cleavage intervals of the previously selected embryos (by ticking the „compare” checkbox) can be seen in the same

window, one beneath the other, enabling you to select the best quality embryo. For giving help in building the quality order of the embryos, it is possible to rearrange the order by clicking on the arrows next to the identification number of the embryo.



Regarding the reference values and the tolerance rate set in the „Settings", the legend of the cleavage intervals can be seen in the tones between green (the cleavage interval of the embryo is within the reference values), orange (out of the reference values, but within the tolerance rate), and red (out of the reference values and the tolerance rate).

3.3.4. Statistics

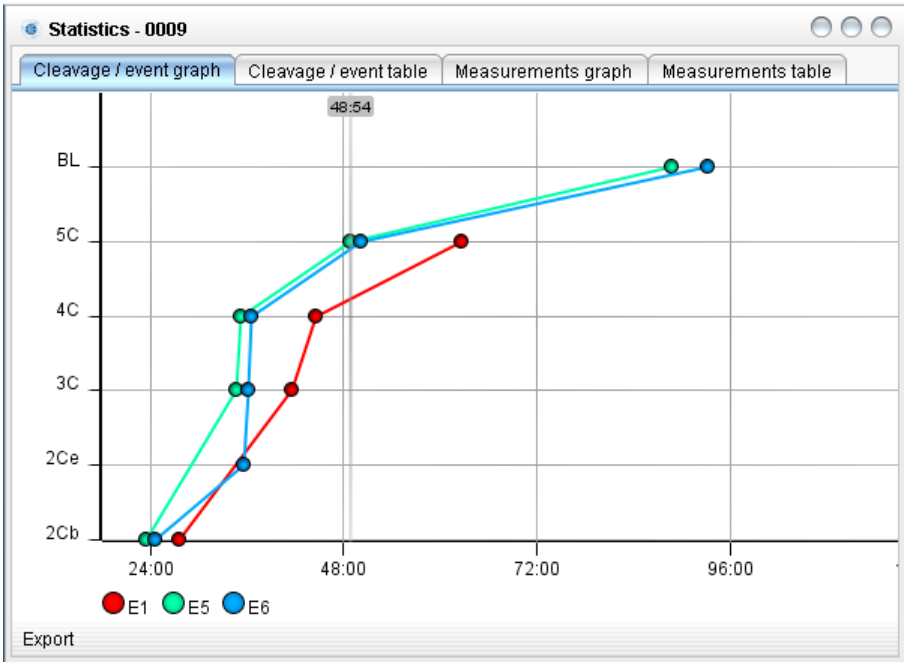
In this section, the program displays the development dynamics of the analyzed embryos, based on manual classification. With the help of the diagrams and data tables provided by the software, selection of the best quality embryo – based on development dynamics – can be obtained in a quick and comfortable way. In case of stopping an analysis before finishing it, and closing the project, the actual status will be saved, so by opening it again, it automatically loads previous analysis data.

3.3.4.1. Cleavage / Event graph

This menu item is to view embryo development in a graphical form. Only the previously selected embryos are represented on this graph (by ticking the „compare" checkbox). Embryo plots are color-coded; the color of the line is the same as the color of the identifier of the embryo during

the whole analysis. The graph indicates the developmental pace of the analyzed embryos; each horizontal line shows a developmental stage. The plots show the time (on the horizontal axis, elapsed from the start of the photo sequence) when the embryo reached the given stage.

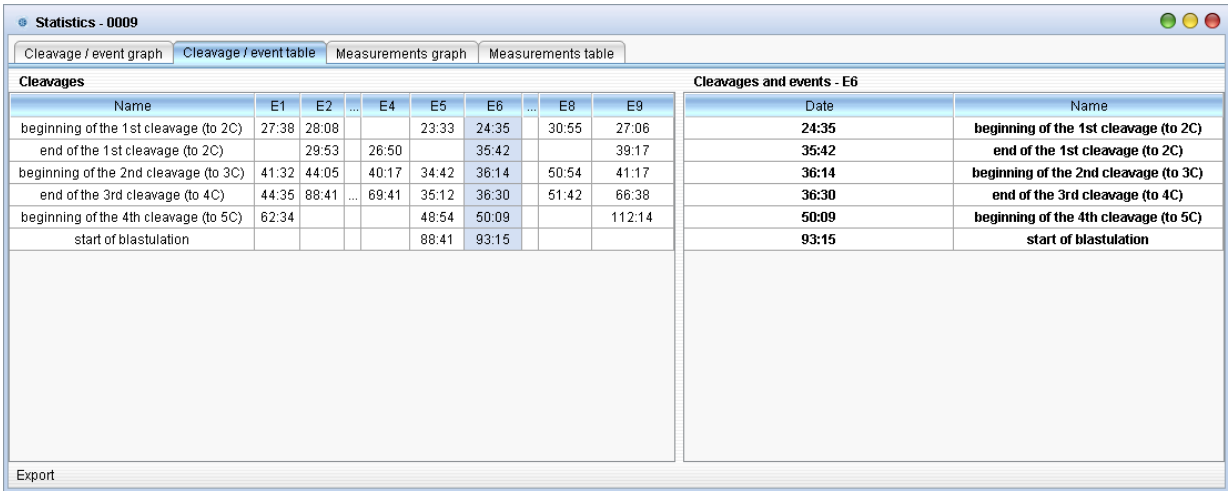
If moving the mouse cursor to an embryo identifier dot under the graph, the other lines referring to all other embryos will become grey, and only the selected embryo's line will be seen in color. If the mouse cursor is moved to a dot on the line referring to an embryo development, all the other lines referring to other embryos will become grey again, and if clicking on the dot, the exact time of reaching the developmental state belonging to the given dot will appear.



By clicking on „Export" button, the graph can be exported to the clipboard or to Microsoft Excel, and saved in the „Data" folder within the same folder where the captured images were saved (or to any other folder, defined by the user).

3.3.4.2. Cleavage / Event table

This menu item can be used to view the previously analyzed embryo development dynamics in tabular numeric form. The table shows the time elapsed until the embryo reaches the certain developmental stages. In the first table each column represents one embryo and each row represents a developmental stage; the elapsed time belonging to the given developmental stage can be seen in the cells. If marking a column by clicking on the first cell of it, the details of the marked embryo will be seen in an individual table on the right side of the window.

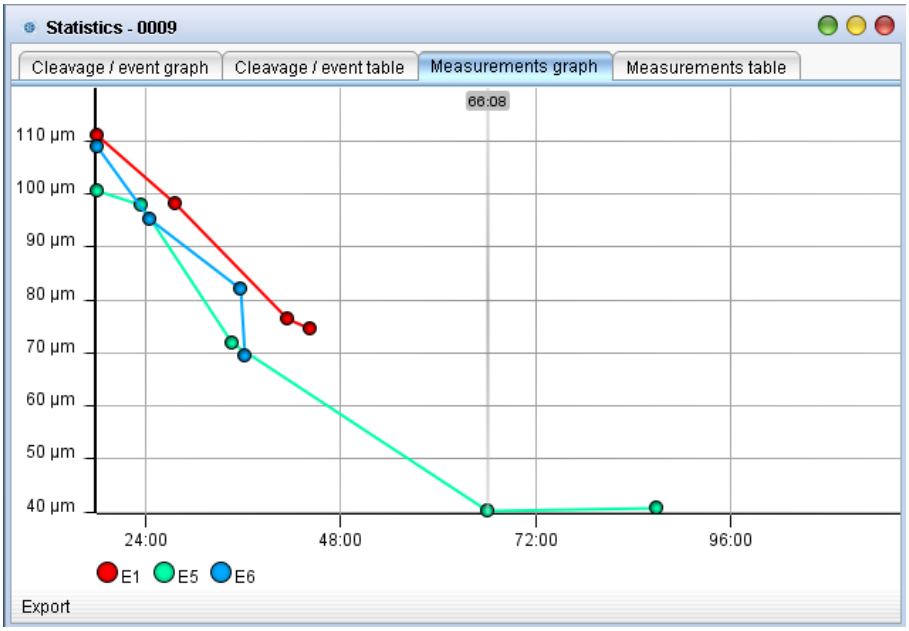


By clicking on „Export" button, the tables can be exported to Microsoft Excel, and saved in „Data" folder within the same folder where the captured images were saved (or to any other folder, defined by the user).

3.3.4.3. Measurements graph

This graph helps to compare the measured size of the embryos. The horizontal axis shows the time elapsed from the start of the analysis; the vertical axis shows the measured cell diameters (or other sizes of the user's choice). Each line represents an embryo; the color of the line is the same as the color of the identifier of the embryo during the whole analysis. Only those embryos are represented on this graph of which checkbox has previously been ticked in the compiled embryo window containing all the embryos.

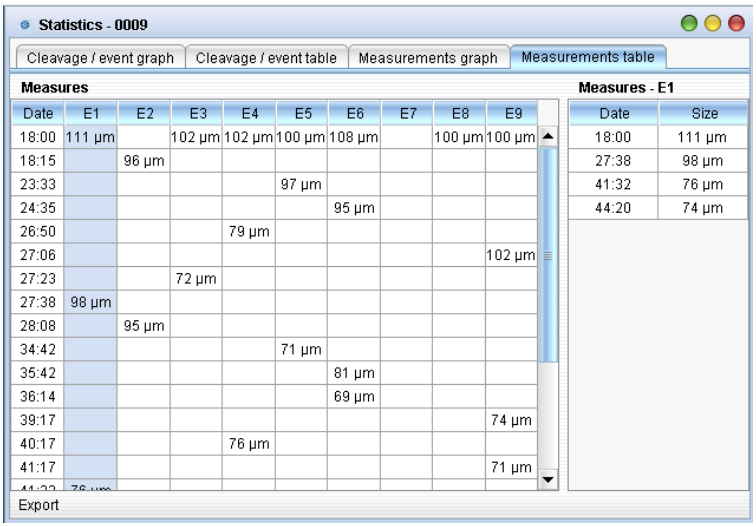
If moving the mouse cursor to an embryo identifier dot under the graph, the other lines referring to all other embryos will become grey, and only the selected embryo's line will be seen in color. If the mouse cursor is moved to a dot on the line referring to an embryo development, all the other lines referring to other embryos will become grey again, and if clicking on the dot, the result of the measurement belonging to the given dot will appear.



By clicking on „Export" button, the graph can be exported to the clipboard or to Microsoft Excel, and saved in „Data" folder within the the same folder where the captured images were saved (or to any other folder, defined by the user).

3.3.4.4. Measurements table

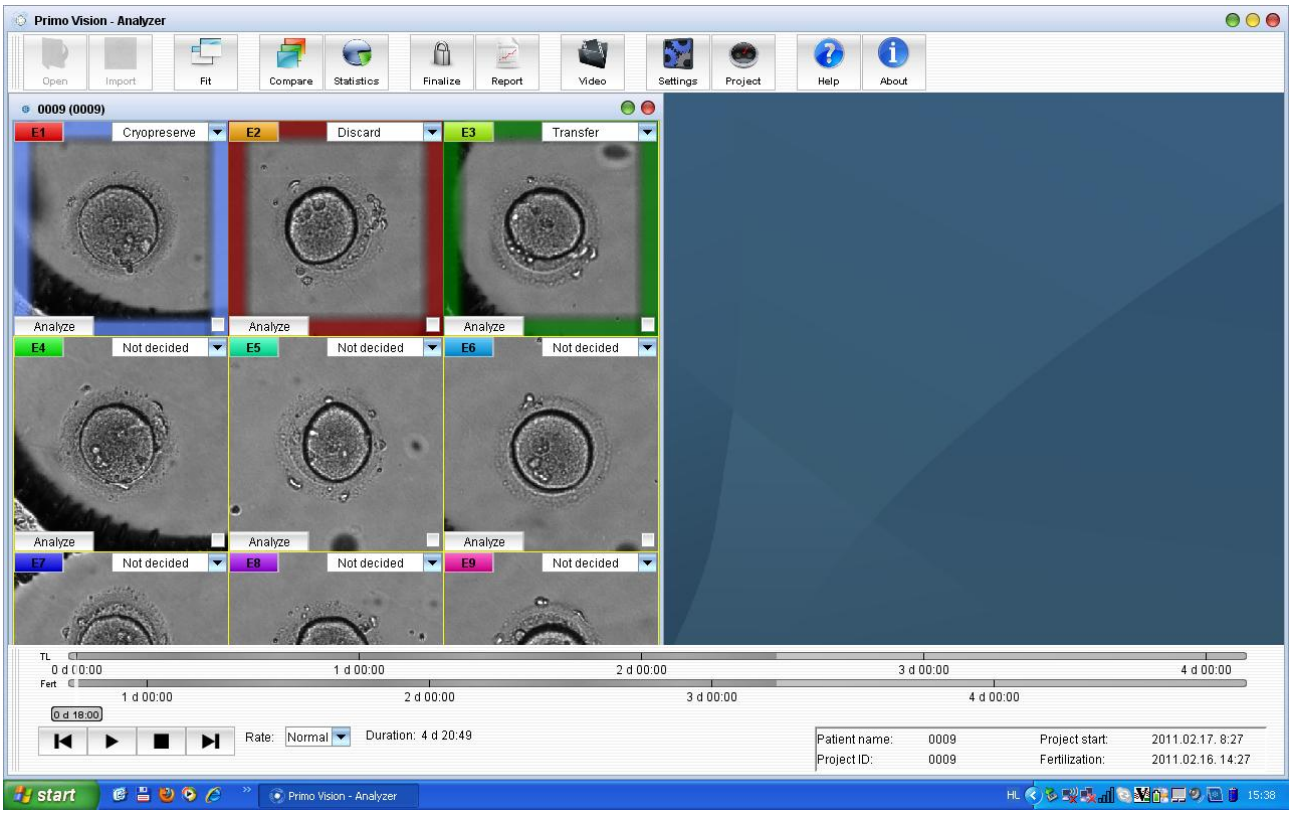
The table shows the time elapsed from the start of the photo sequence until the time of measurement, and also the results of the measurements executed. In the first table, each column represents one embryo and each row represents a measurement; the elapsed time belonging to the given measurement can be seen in the cells. If marking a column by clicking on the first cell of it, the details of the marked embryo will be seen in an individual table on the right side of the window.



By clicking on „Export” button, the tables can be exported to Microsoft Excel, and saved in the „Data” folder within the same folder where the captured images were saved (or to any other folder, defined by the user).

3.3.5. Decision making during analysis; finalization

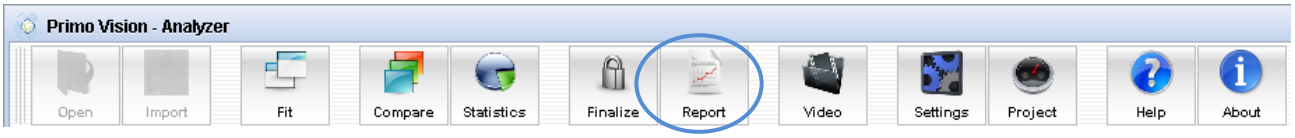
The decision about the embryo can be marked in the drop-down menu of the embryo image: „Transfer”, „Cryopreserve”, „Discard” or „Not decided”. These can be set and changed any time until the finalization. Based on this decision, the embryos will get a color-coded frame: green for transferred, blue for cryopreserved, and red for discarded embryos.



The „Finalize” menu point gives the possibility of deciding about the embryos following the time-lapse sequence and the analysis. The decision („Transfer”, „Cryopreserve”, „Discard” or „Not decided”) can be made of each embryo in the compiled embryo window or in the finalize window as well, and after clicking on „Finalize”, it will be saved in the patient’s folder.

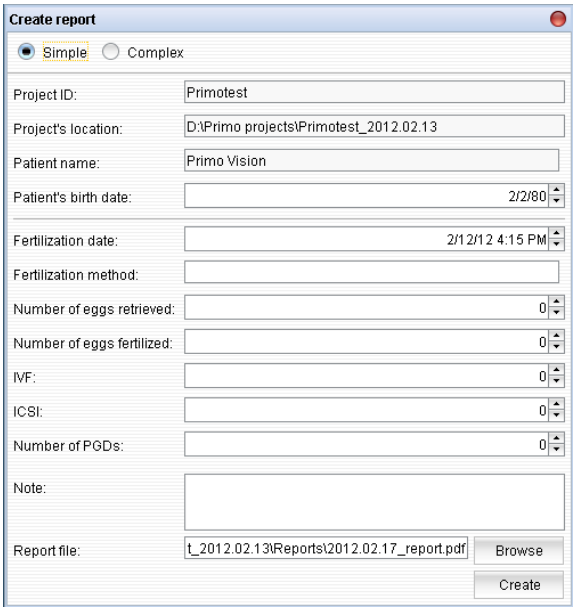
After finalization, the status of the transferred and discarded embryos cannot be modified.

3.3.6. Reporting



In this menu point a simple or a complex report with all the relevant data of the patient and the time-lapse cycle, together with the last image of the embryos can be created in PDF format. As a first step for creating a report, it is recommended to fill in the fields in the „Reporting" tab of the „Settings" menu point, in order to create an institution-specific report (see details in Chapter 3.3.8. „Settings").

After clicking on the „Report" button, a pop-up window will appear, where the following fields can be filled in, and notes can be added as well. Project ID, Project's location and Patient name fields cannot be modified.



In case of selecting „Simple" report, a one-page PDF file with the institution name and logo on the top (if previously set in the „Reporting" tab of the „Settings" menu point) is created, where the date and method of fertilization, the start and the end of the time-lapse cycle, number of eggs retrieved and fertilized, number of PGDs, the decision (transferred, cryopreserved, discarded or not-decided) about the embryos and the added notes can be seen, together with the last time-lapse photo of the nine embryos.

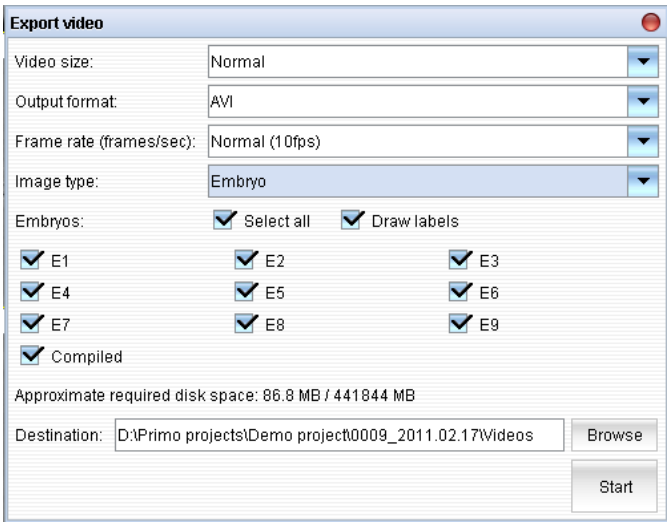
If selecting „Complex" report, the first page of the PDF file is identical with the simple report, but it contains additional pages with information about the individual embryos. Each page refers to one individual embryo. The last time-lapse image of the given embryo, and a summary datasheet can be seen with all the information (exact time of the cleavage and the first photo of the embryo following the cleavages, results of the size measurements) on the additional pages. The proliferation graph and the measurement graph is also attached to the individual embryos' page.

The reports are saved in the „Reports" subfolder of the project by default, but it can be modified by the user.

3.3.7. Creating and playing a video

By clicking on the „Video" button on the upper part of the screen, a video file can be created of the time-lapse photo series. If pressing this button, a pop-up window will appear, where the video size (normal or small), output format (AVI or MOV), frame rate (slow, normal or fast) and image type (well or embryo view) can be set. Videos of individual embryos can be created by clicking on the checkboxes of the embryos or by clicking on „Select all", or a video with all the embryos in the

field of view can be created as well by clicking on the „Compiled” button. If ticking „Draw labels” checkbox, the name of the patient and the embryo number will be „stamped” to each frame of the video file, and it will be seen during the whole video.



The estimated required disk space is indicated by the software, and the folder where the video will be saved can be set by the user. By default, the videos will be saved in the „Videos” folder within the patient folder (the folder of the given project).

If the criteria are specified, click on the „Start” button.

The names of the individual embryos’ video within the folder are „Date_E1”, „Date_E2”…, the name of the video with all the frames in the field of view will be defined only by the date of the video creation. The created video files can be played with most of the media players, e.g. VLC Media Player.

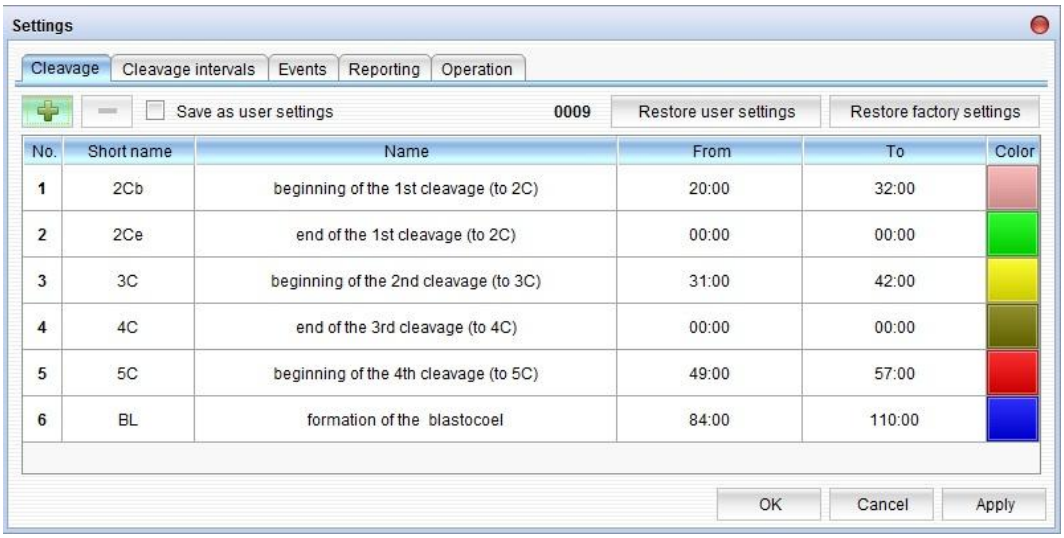
3.3.8. Settings

The following parameters can be set after clicking on the „Settings” button on the left upper part of the screen.

3.3.8.1. Cleavage

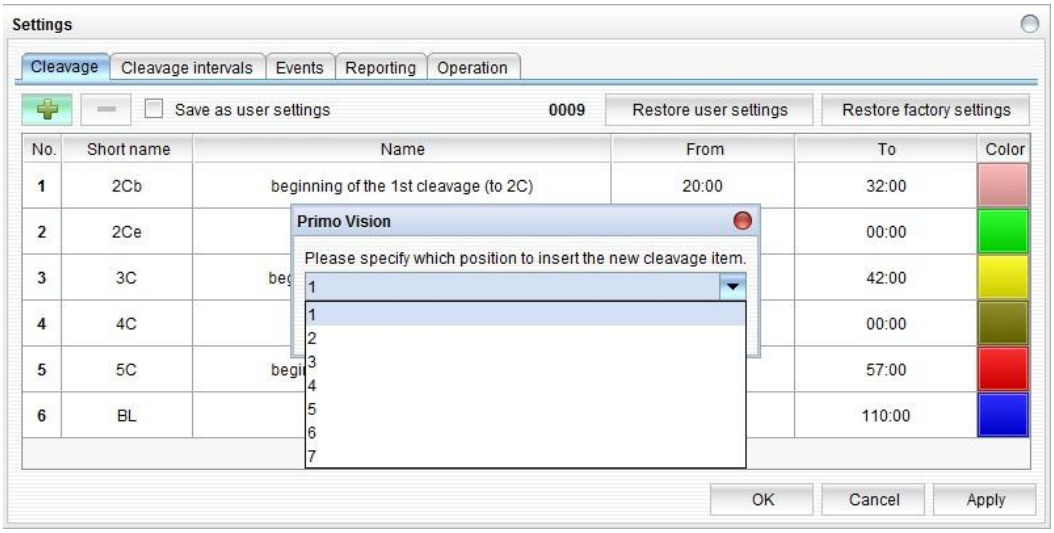
The sequential developmental stages can be defined under the „Cleavage” tab of the „Settings” menu. As these developmental changes normally happen only once in the time-lapse sequence,

and only in the given order (e.g. the sequence of the cleavages), these status buttons can be used only once during the analysis of the embryo, and the sequence of them is defined. The software comes with previously set default cleavage statuses and reference intervals, but it is possible to customize them as well. The developmental stages set in this window, will be displayed as buttons bearing the adequate abbreviations in the „Status" box, on the lower part of the enlarged image of the analyzed embryo.



In the top line of the window the following signs can be seen:

- „+" and „-": New cleavage statuses can be added or deleted by clicking on these buttons. When clicking on „+", the ordinal number of the new status can be given; the following statuses with superior ordinal numbers will be moved forward.



When selecting a cleavage status and clicking on „-", the selected status will be deleted.

- „Save as user settings" checkbox: If ticking this checkbox, the defined values will be saved as the default software settings, provided that the „Apply" button is used.

- „Restore user settings” button: if making changes in previous user settings, by clicking on this button, the software restores the user settings.
- „Restore factory settings” button: by clicking on this button, the software restores the original factory-set values.
- Each row of the table refers to one cleavage status, and each cell of the rows can be redefined by the user. The original default cleavage statuses are:

• 2Cb	beginning of the first cleavage (to two-cell stage)	20 h : 0 min	-	32 h : 0 min
• 2Ce	end of the first cleavage (to three-cell stage)	0 h : 0 min	-	0 h : 0 min
• 3C	beginning of the second cleavage (to three-cell stage)	31 h : 0 min	-	42 h : 0 min
• 4C	end of the third cleavage (to four-cell stage)	0 h : 0 min	-	0 h : 0 min
• 5C	beginning of the fourth cleavage (to five-cell stage)	49 h : 0 min	-	57 h : 0 min
• BL	start of blastulation	84 h : 0 min	-	110 h : 0 min

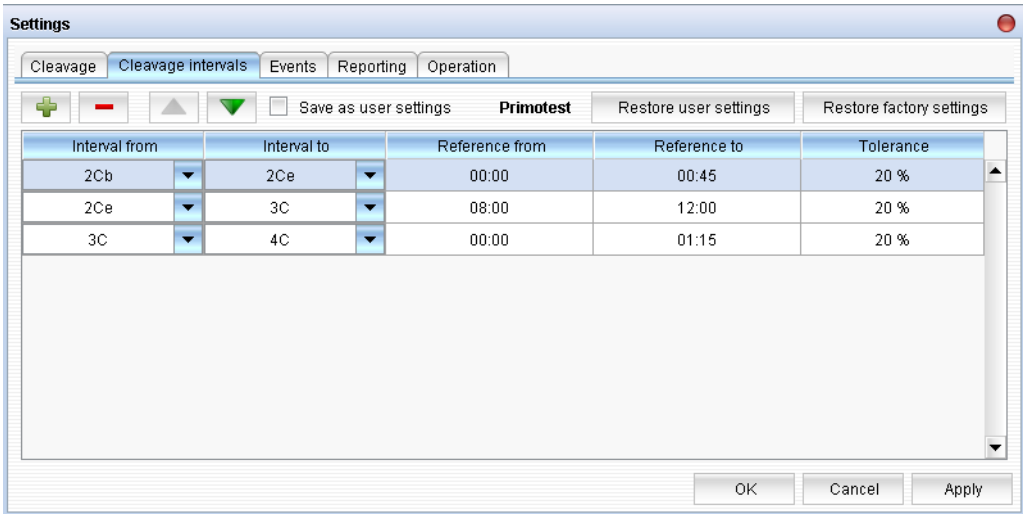
The columns of the table are the following:

- No.: an ordinal number regarding the given cleavage status is seen in this column.
- Short name: an abbreviation is defined for the cleavage status, which will be shown on the status buttons under the enlarged image of the analyzed embryo.
- Name: the name of the cleavage status is seen in this column.
- „From” and „To”: Reference values can be set for the cleavages by clicking on the „From” and „To” column of the table. The software comes with factory-set default reference values, based on published results in the field of time-lapse investigations, and based on the results of Primo Vision users as well. As optimal cleavage times are influenced by culture conditions, these values can be changed by the user.
- Color: A color can be attached to the given status by clicking on the last column of the table.

Click „Apply” to accept changes.

3.3.8.2. Cleavage intervals

This menu point can be used to measure the elapsed time between two cleavage statuses. The user has the chance to select the cleavage points / sequential events as starting and ending points of the interval, to set the references values for the interval and define the tolerance level. The cleavage intervals set in this window will be displayed on the right side of the analyzed embryo window (see details in Chapter 3.3.2. „Selecting an embryo for analysis"), and also in the „Compare" window (see details in Chapter 3.3.3. „Compare").



The structure of the upper row of the window is the same as described in the „Cleavage" tab of the „Settings" menu. Each row of the table represents one cleavage interval. The cells of the cleavage intervals table can be customized, but the default cleavage intervals are the following:

- 2Cb - 2Ce interval between the beginning and the end of the first cleavage (to two-cell stage) 0 h : 0 min - 0 h : 45 min 20%
- 2Ce - 3C interval between the end of the first cleavage and the second cleavage (to three-cell stage) 8 h : 0 min - 12 h : 0 min 20%
- 3C - 4C interval between the second and the third cleavage (to four-cell stage) 0 h : 0 min - 1 h : 15 min 20%

The columns of the table are the following:

- „Interval from" and „Interval to": In the dropdowns of these two columns the two cleavage statuses can be selected between which the elapsed time is wished to be measured.
- „Reference from" and „Reference to": Reference values can be set for the cleavage intervals by clicking on the cells of these columns of the table. The software comes with factory-set default reference values, based on published results in the field of time-lapse investigations,

and based on the results of Primo Vision users as well. As optimal cleavage times are influenced by culture conditions, these values can be changed by the user.

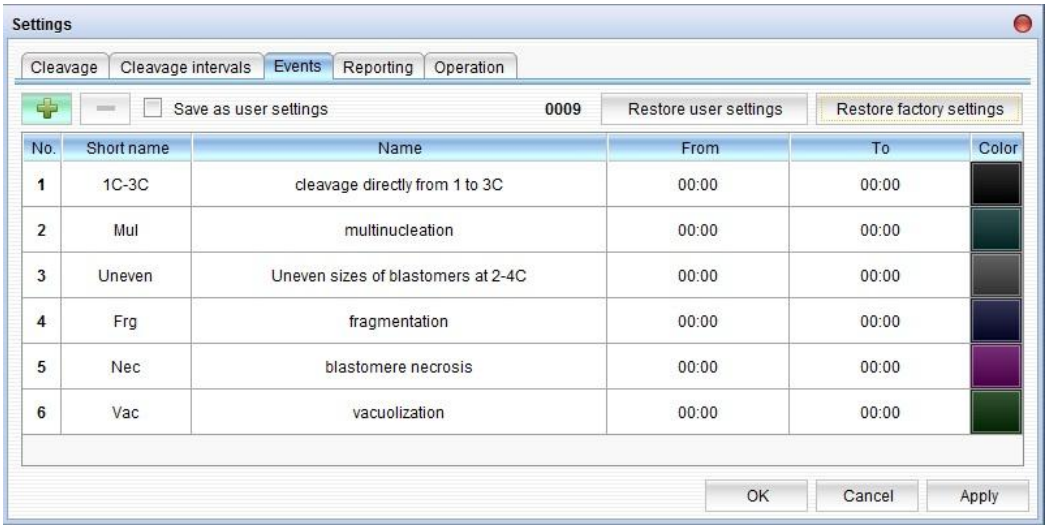
- Tolerance: A tolerance rate can be set here for the reference values of the cleavage intervals, in percentage.

When displayed on the right side of the analyzed embryo window (see details in Chapter 3.3.2. „Selecting an embryo for analysis"), and in the „Compare" window (see details in Chapter 3.3.3. „Compare"), regarding the reference values and the tolerance rate set in the „Settings", the legend of the given cleavage interval can be seen in the tones between green (the cleavage interval of the embryo is within the reference values), orange (out of the reference values, but within the tolerance rate), and red (out of the reference values and the tolerance rate).

3.3.8.3. Events

The non-sequential developmental stages can be defined in the „Events" tab of the „Settings" menu. These stages can be used to define those kinds of changes which are reversible and/or can happen more than once during the time-lapse sequence (e.g. fragmentations) and are independent from the cleavage sequence.

The events set in this window will be displayed as buttons bearing the adequate abbreviations in the „Status" box, on the lower part of the enlarged image of the analyzed embryo.



The structure of the window is the same as described in the „Cleavage" tab of the „Settings" menu. It is possible to customize the different events in this menu point as well. The original default events are the following:

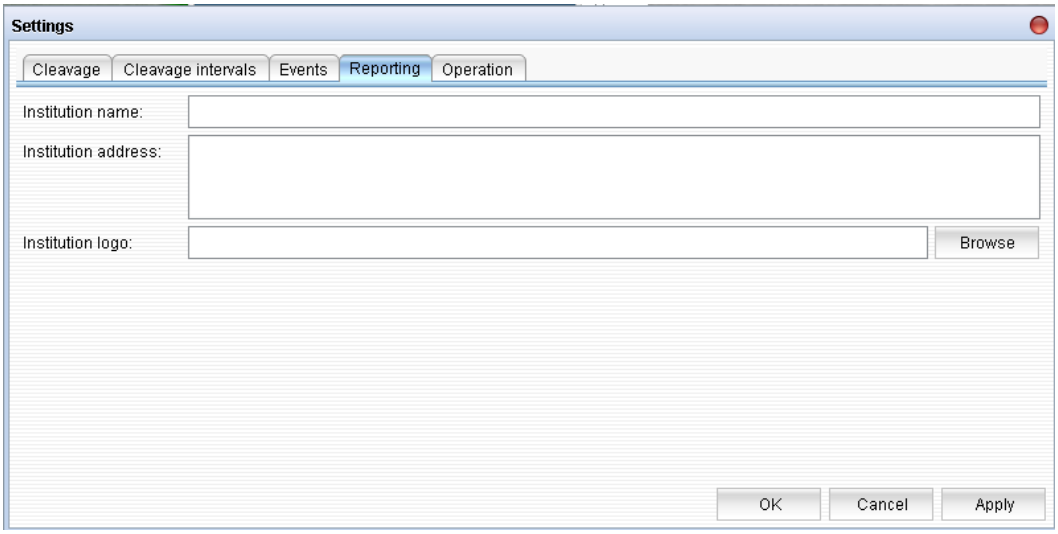
- 1C-3C: Cleavage directly from one to three cells
- Mul: Multinucleation

- Uneven: Uneven sizes of blastomeres at 2-4cell stage
- Frg: Fragmentation
- Nec: Blastomere necrosis
- Vac: Vacuolization

As these events are mostly unexpected events, reference values can be used only as research tools for user-defined special events in the „From" and „To" column of the table.

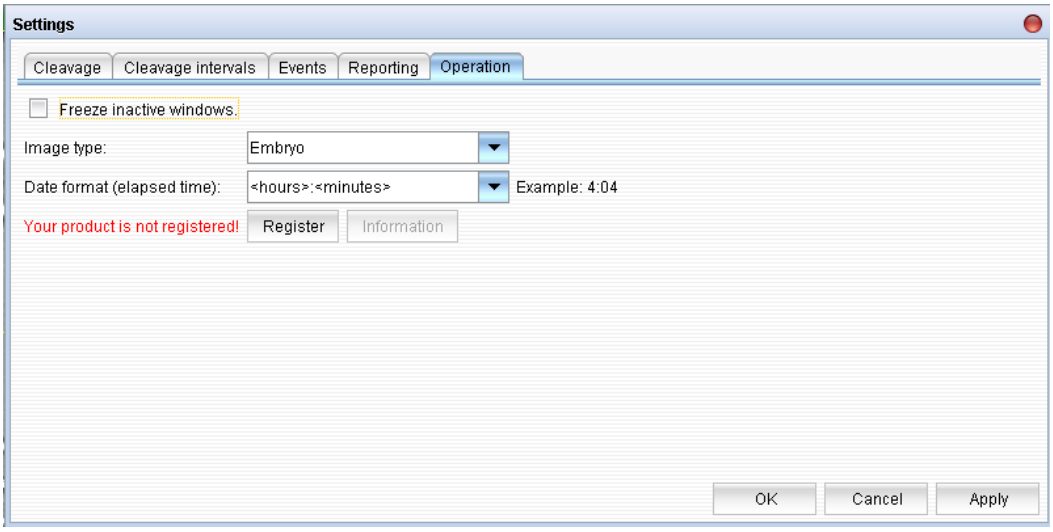
3.3.8.4. Reporting

Header data of the created reports can be set here: Institution name, Institution address and Institution logo.



3.3.8.5. Operation

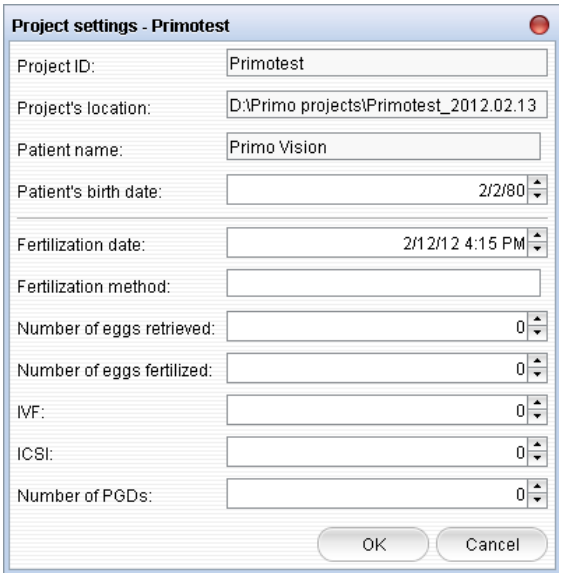
Some features of the operation can be set here.



- Freeze inactive windows: Simultaneous playing of different windows (Main window of all the embryos, Analyze, Compare…) can be set. If it is checked, only the active window is playing the image sequence, all the other windows will be in the last position until becoming active again. If it is not checked, all the windows play image sequence simultaneously. Please note, that this latter feature is recommended only on high performance hardware configuration.
- Image type: Type of the displayed images (cropped for the Wells or cropped for the Embryos) can be set here. „Embryo" is the recommended settings. Use „Well" if embryos cannot be seen in the „Embryo" type images, or more than one embryo are placed into a single well.
- Date format: The format of elapsed time can be set here. It can be displayed in minutes, hours:minutes or days and hours:minutes.
- Registration: The standalone Analysis software requires a registration that is available with the provided Primo Vision Activation Key only. The registration can be made online. Software runs for 10 days without a valid registration.

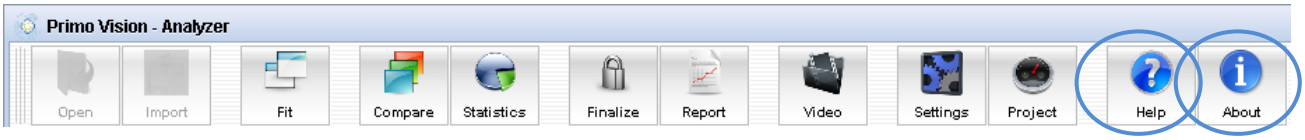
3.3.9. Project settings

With the help of this function the following details of the project (except the first three lines) can be changed or specified.



Once the project is started with the Capture software, project ID, Project's location and Patient name fields cannot be changed.

3.3.10. Help and About



After clicking on the „Help” button, a pop-up window with the Use and Maintenance Manual of the Primo Vision system will appear.

If clicking on the „About” button, the details of the producer and the version number of the software can be checked.

3.4. Exiting the software

To exit the Capture software, click on the exit tab of the program. In case of taking the Primo Vision Microscope out of the incubator and transporting to another place, tick in the checkbox of the Microscope Unit in order to place the optics into Parking Mode. Parking of the inner mechanics is important, in order to avoid any possibility of damaging the optical system, caused by the transportation shocks. Transportation with the optics not set in „Parking Mode” may lead to loss of warranty.

To exit the Analyzer software, click on the exit tab of the program. The program requires confirmation before closing. In order to exit, press button „Yes”, otherwise press „No”. Partly or completely analyzed projects can be closed without losing the results of measurements, status marks, etc.; these data will be saved and can be viewed after reopening the project.

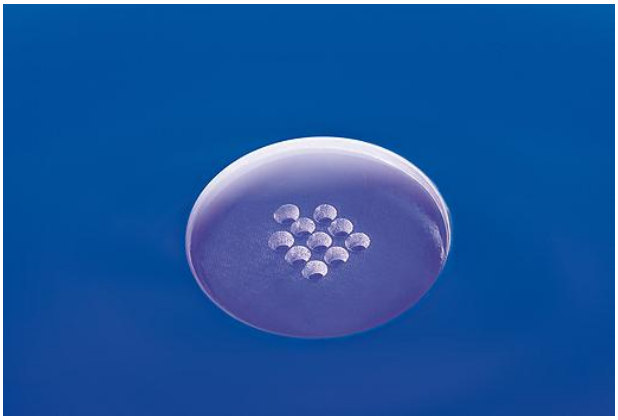
4. Primo Vision in practice

4.1. Loading the WOW dish

As air bubbles may reduce image quality, proper loading of the WOW embryo culture dish is of capital importance. The easiest way of loading the WOW dish without air bubbles is to load the microwells with culture media well by well, using a fine and flexible pipet which possesses an outer diameter not wider than 0.3 mm. An Eppendorf gel loading tip fits this purpose excellently.

On the day prior to starting the time-lapse sequence, load the WOW dish with culture media. For this purpose place the nib of the gel loading tip onto the bottom of the first microwell under a stereomicroscope and fill up with an amount of media which will not emerge from the microwell. Repeat this step with each microwell one by one. Avoid of loading bigger amount of culture media to the given microwell because in this case it will flow into the neighboring microwells, and air bubbles which stick to the surface of the dish will remain in the bottom.

The drop then can be enlarged to the desired volume and size by putting the given amount of culture media onto the middle of the WOW dish, over the wells. The WOW dish is to be used with 40-60 µl embryo culture media, depending on the spreading attributes of the media and the laboratory rules and regulations. The dome of the drop should be as flat as possible: the rim around the wells helps to achieve this. Practically, when one prepares the micro-drop she/he should start to place the drop next to the rim around the wells, and help the drop to spread over the rim. As there might be differences in types or batches of media, the amount can of course be changed and adjusted. Try to use the least amount that spreads evenly in the recession, containing the wells.



After this, approximately 2.5 - 3 ml oil shall be overlaid, to cover the whole droplet. This is important, as it will not only affect image quality, but culture conditions as well. Now the dish should pass an overnight equilibration.

On the next day, prior to placing the embryos into the WOW dish, check the dish for newly appearing bubbles. These new bubbles shall be removed, otherwise they may grow larger during incubation, which may affect embryo analysis. These bubbles usually appear in the nooks of the microwells or close to the rim around them, and as they are not sticked to surface of the dish, they are very easy to remove with a pipet.

When the dish is free of air bubbles, the embryos are placed one by one into the wells by fine pipetting. It needs some seconds for them to settle in the bottom of the wells. Occurring oil droplets or bubbles shall be removed from the drop, because these can float into the field of view of the Microscope and reduce image quality.

Then the dish should be carefully taken to the incubator and placed onto the sample holder of the Microscope. By doing this, one needs to be careful; otherwise embryos might migrate between the wells. For stable carrying, firm hand, or a lid of a large Petri dish, or a pre-warmed plate is used. This step needs some practice. After getting the hang of it, embryo migration is not an issue.

4.2. Starting the time-lapse project – stepwise description

Once the Primo Vision System is installed in your incubator, follow the next steps for starting a time-lapse sequence:

1. Pre-arrangements
    a. Place the pre-cleaned Primo Vision Microscope with the connected USB cable into the incubator (with tightly screwed protective cap), and let it warm up for at least 6 hours.
    b. Prepare the WOW dish and place it into the incubator for an overnight equilibration.

2. After the prescribed equilibration of the culture media, load the WOW dish with the embryos.
    a. Under a stereomicroscope, gently pipet each selected embryo, and release them one by one above a well. The embryo will sink to the bottom of the well. If an embryo misses the well, gently move it into the corresponding well by pipetting and gently aspirating or moving media around the embryo.
    b. Once all of the embryos are in their place, wait for a couple of seconds till they all sink to the bottom of the wells.
    c. Clean off the air bubbles and oil drops from the top of the culture media drop by using fine hand or mouth pipet in order to have a clear field of view.
3. Place the WOW dish onto the Primo Vision Microscope.
    a. Click on the „Live mode" button of the Primo Vision Capture Software. The Primo Vision Microscope's lamp will turn on in ~ 20 seconds.
    b. Gently transport the WOW dish into the incubator, and very gently place the dish to the dish holder of the Microscope, with the orientation well pointing away from the lamp consol. This is done most easily by orientating this well to the proper direction, while taking it into the hand before transporting to the incubator. By doing this, one needs to be extra careful; otherwise embryos might migrate between the wells. For stable carrying, firm hand, or a lid of a large Petri dish, or a pre-warmed plate is used. This step needs some practice. After getting the hang of it, embryo migration is not an issue.
    c. Check the position of the WOW dish on the screen. If necessary, carefully adjust the position of the wells into the field of view by careful rotation of the dish. When finished, close the door of the incubator; from this point, all the operations (focusing, zooming, scanning, time-lapse sequence, etc.) can be controlled from outside, using the provided software.
4. Adjust the focus, and prepare imaging (executed in the „Live" mode).
    a. Do the focusing using your mouse or the optional 3D Mouse (Please select the „Use scroll knob" option) connected to the Controlling Unit. Find the best focal plane for the photo sequence.
    b. Zooming can be useful for proper focusing. For this purpose move the mouse cursor to the image, and by moving the mouse wheel forward, zooming can be performed. If clicking on the enlarged image and moving the mouse, the image can be moved in order to see each microwell. Clicking on the image again, the original image with all the nine or sixteen microwells in the field of view will be seen again.

c. When finished focusing, switch off the Live mode with the „Close live mode" command of the software.

5. Adjust scan parameters if scanning is necessary during the time-lapse sequence.
    a. Click on the „Settings" button in the right upper corner of the screen, and select „Scan parameters". Adjust the number of focal planes, the scanned range and set the scan timing.
    b. Click on „Save settings" if the scan parameters are adjusted.
6. Start the time-lapse sequence.
    a. Click on Start Project button of the Capture software.
    b. In the Start Capture window fill out the relevant information in the Project data field, select culture dish type, and set time-lapse and autoscan parameters.
    c. Check the storage capacity, and if using the Controlling Unit for the first time after delivery, check date and time properties to avoid problems arisen from different time zones.

    Use the „Approve" command on the computer screen to start the photo sequence. From this time on, the software will take pictures of the developing embryos in the given frequency and for the given duration.

Repeat the above steps with each connected Microscopes. The tabs of the connected Microscopes are shown on the left side of the screen. Click these tabs to switch among the images of the connected Microscopes. By default, the program always shows the image of the first Microsocope.

If changing the culture media is required during the culture period, click on the „Pause" button to avoid of capturing images without embryos, then remove the dish from the sample holder. Move the embryos under regular stereomicroscope keeping the same order, into another, pre-equilibrated dish, then place the dish back to position. With the use of the „Live mode", re-check and if necessary, re-adjust focus, and close Live mode. After clicking on „Resume" button the time-lapse sequence continues uninterruptedly.

Changing of the media is possible by following the next steps:

1. After clicking on „Pause" button, gently remove the WOW culture dish from the Primo Vision Microscope, from the incubator, and place it under a regular stereomicroscope. Place the pipet to the edge of the microdrop, under the mineral oil, and by gentle aspiration, remove the media from above the embryos.
2. Fill the pipet with the new, pre-equilibrated media, and refill the drop under the mineral oil by careful expelling. In this way, approximately 90% of the media is changed. The embryos stay in their wells, and the change of their microenvironment will be gradual this way.

3. Wait for a couple of seconds, until embryos sit safely in their well; if they moved away during the procedure, place them back.
4. Switch on the „Live mode” button in the Primo Vision Capture Software. Gently place the WOW dish with the embryos, with the changed culture media back to the microscopic stage of Primo Vision Microscope, do the positioning and focusing as described previously. Then click on the „Close live mode” button.
5. Click on „Resume” button, and the monitoring continues.

5. Problems and solutions

- The embryos are not properly focused on the computer screen.
  - Adjust the focus while checking the image on the screen. Always adjust the focus slowly, because the screen refresh rate is also slow at high resolutions and low light intensity.
  - Check if vapor has condensed on the glass plate of the objective of the Primo Vision Microscope. If yes, wipe it dry with a soft cleaning cloth.
  - Check if the dish sits fitting well in the dish holder of the microscopic stage. If it doesn't, the dish is not in a horizontal position.
  - Focus setting can be performed more effectively after zooming in.
  - If the image of the embryos seems to be cloudy or dim, there may be some air bubbles or oil drops on the top of the culture media drop. Remove these impurities by gentle pipetting.
- The computer program does not show any image from the incubator.
  - Check if the Primo Vision Microscope that you are trying to adjust or focus complies with the number of the active Microscope adjusted in the Primo Vision Software on the screen.
- The Microscope does not show any illumination.
  - The light intensity value may be too low which practically means no active illumination; increase the light intensity, if this is case.
- Some very dark or black images occur during the time-lapse sequence.
  - The Microscopes can produce very dark or even black images when there is USB communication at the same time. This is not deterministic, depends on file size, computer load and Microscope capturing frequency but it is strongly recommended to use network connection for file transfer - for further instructions please ask your IT department.
  - If using the Microscope immediately after placing it into the incubator with no time to warm up, it may cause some black images. Leave the Microscope in the incubator for at least 6 hours before starting a time-lapse sequence.
  - The light intensity value may be too low which practically means no active illumination; increase the light intensity in this is case.
- The computer does not recognize the connected Microscope Unit.
  - Check if the number of the connected Microscope agrees with the selected one, and check if it is connected correctly.

- If the number agrees, restart the Controlling Unit, and start the Capture software again.

- The computer has stopped the time-lapse project.
  - The Controller is in sleep or hibernated status. Please do NOT change the power options of your Primo Vision Controlling Unit - the computer should never go to sleep or hibernated phase, never shut down the hard drive. When the computer goes to sleep or hibernated status it stops capturing images!
  - The Capture software was shut down. In case of exiting the Capture software, the time-lapse project will be stopped as well.

- The computer has stopped running due to blackout.
  - You can simply restart the program, but note that the photo sequence you have started previously will be continued in another directory. While the computer is not running, no images are created by the unit.

- The Controlling Unit does not see other computers after connecting to the local network.
  - Primo Vision controllers are shipped with standard Windows setting, regarding the network profile. It means that the computer is instructed to get IP address automatically from a DHCP server. When you attach the controller to your local network and it doesn't see other computers it means that fix IP addresses are used or there are special restrictions in your IT policy - in this case please ask your IT department.

6. Technical parameters

Configuration – Primo Vision system	
Main parts	Microscope Unit(s) Controlling Unit 3D mouse (optional) (Monitor is not included)
Maximum Microscope configuration	6 digital Microscope Units
Technical Specification – Microscope Unit	
Net external dimensions	220 x 80 x 110 mm
Net weight	2,25 kg
Field of view	Approx. 2600 x 1900 µm
Microscope	BW; 5 MP (2560 x 1920 pixel)
Optics	Custom made optics, (cca. 1 pixel / µm)
Illumination	Adjustable green LED (550 nm)
Technical Specification – Controlling Unit	
Type	Dell OptiPlex 390 SF N-series
Net external dimensions	290 x 95 x 330 mm
Net weight	5,45 kg
Processor	Intel Core i3-2100 (3.10 GHz, 3MB)
Memory	4 GB (2x2GB) 1333 MHz DDR3 Non-ECC
Storage capacity	500 GB – images and data of cca. 50-100 cycles
Platform	Windows XP Embedded POS-Ready
Data formats	Images PNG Reports PDF Data XLS, JPG Videos AVI, MOV
Inputs and outputs	6x USB 1x HDMI 1x VGA LAN

Operation – Primo Vision System	
Focusing method	Motorized – from outside the incubator
Focus plane(s)	Multiple (min. 3, max. 11 planes)
Image taking frequency	Configurable by the user (from 5 min till 60 min)
Microscope Unit – Controlling Unit distance	Up to 3m
User Interface /controller	Provided Dell computer with SW preinstalled
Display	Not included (required: 1920x1080 (HD) or higher screen resolution; typical 24”+)
Keyboard and mouse	Included
Remote access	Yes
Operating temperature	Microscope Unit: takes up the temperature of the incubator Controlling Unit: 20-30 °C
Sterilizable parts	Enclosure external surface, cable
Packaging	
Primo Vision starting kit package size	625 x 370 x 525 mm
Primo Vision starting kit package weight	13,5 kg
Primo Vision additional Microscope package size	520 x 365 x 365 mm
Primo Vision additional Microscope package weight	4,85 kg
WOW dish package size	95 x 70 x 92 mm
WOW dish package weight	0,1 kg

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