3DHISTECH

Pannoramic 250 FLASH III

2.0

User's Guide

June 4, 2018

Rev. 1

Contents

For research and education uses only, not for use in diagnostic procedures.

Disclaimer

Intellectual property related to the 3DHISTECH products are as follows:

EP 1,789,831; US 7,663,078; EP 2,024,772; JP 2009-512685; US 12/301,781; EP 1,644,767; JP 2007-516451; US7,327,515; EP 1,994,398; JP 2008-557835; US 12/281,566; U0700210; US 6,246,785; HU0900142; HU0700404; PCT/HU2007/000065; EP 08762668.5; US 12/663,537; PCT/AT2006/000492; JP 2008-542552; EP 06817469; US 12/095,545; PCT/AT2006/000493; EP 06817470.5; US 12/095,596; HU0900741; US 61/264,732; US 11/826,752; P0401870; PCT/IB2005/050344; US 11/662,976; HU0700409; US 12/042,050; US 77/636,144 and 007502032.

The above mentioned granted patents, granted utility model, registered trademarks, pending international and national patent applications and the other corresponding IP are the sole properties of the 3DHISTECH Ltd.

CAUTION

For research and education uses only, not for use in diagnostic procedures. This product has not been approved or cleared as a medical device by the U.S. Food and Drug Administration or the European Union.

Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these may interfere with the proper operation.

For research and education uses only, not for use in diagnostic procedures.

Further Information

For the latest information on 3DHISTECH products and services, please visit our website at the following URL: http://www.3dhistech.com.

Or contact the distributor:

Richard Allan Scientific Co.

Subsidiary of Thermo Fisher Scientific

4481 Campus Drive

Kalamazoo Mi 49008, USA

US Toll Free 1(800) 522-7270

Tudor Road, Manor Park

Runcorn, WA7 1TA

United Kingdom

+44 (0) 800 018 9396

+44 (0) 1928 534 000

Character Formats and Symbols

Example	Words or characters that appear on the screen. These include field names, screen and window titles, push-buttons and menu names, paths or options.
	Keys on the keyboard. For example, function keys (such as **F11**) or the **Ctrl+M** key combination.
Example	Cross-references within this document or to other documents.
⚠ **Warning!**	It might cause irreversible damage or harm to the user of the product or the product itself if this instruction is not followed.
⚠ **Caution!**	It might cause the product not to work properly if this instruction is not followed.
ⓘ **Note:**	Calls attention to some important features.

For research and education uses only, not for use in diagnostic procedures.

Declaration of Conformity

3DHISTECH Ltd. declares that the product Pannoramic P250F III slide scanner is designed and produced with consideration of specified requirements according to the IEC 61326-2-6:2006 standard, and conforms to the directives and other standards listed below:

EN 61000-3-2:2006 + Amendment 1, + Amendment 2, "Electromagnetic compatibility (EMC) - Part 3-2: Limits - Limits for harmonic current emissions (equipment input current ≤ 16 A per phase)"

EN 61000-3-3: 2008, "Electromagnetic compatibility (EMC) - Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤16 A per phase and not subject to conditional connection"

IEC 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 1: General Requirements"

IEC 61010-2-101:2002, " Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use

UL 61010-1:2004 R10.08, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use - Part 1: General Requirements"

EN 55011:2009, Group 1, Class A, + Amendment 1 "Limits and methods of measurement of radio disturbance characteristics of industrial, scientific, and medical (ISM) radio-frequency equipment"

$\mathbb{C}_1$ This product has been tested to the requirements of CAN/CSA-C22.2 NO.61010-1-04+GI1 (R2009) and CAN/CSA-C22.2 NO.61010.2.101-04 (R2009).

Further information may be obtained from the manufacturer:

Richard Allan Scientific Co.

Subsidiary of Thermo Fisher Scientific

4481 Campus Drive

Kalamazoo MI 49008, USA

US Toll Free 1(800) 522-7270

Tudor Road, Manor Park

Runcorn, WA7 1TA

United Kingdom

+44 (0) 800 018 9396

+44 (0) 1928 534 000

Notes Regarding Operational Safety

The below section of this User's Guide contains information and warnings of a kind that must be followed by owner/operator personnel.

Warning and advisory symbols which are used on the base unit of the Pannoramic 250 FLASH III and in the section *1.1.3 Warning and Information Labels* have the following meanings:

	CAUTION Disconnect mains plug before any kind of intervention into the device!
	CAUTION Crush hazard!

The Pannoramic 250 FLASH III, including any of its original accessory components, should not be operated in any other way than described in section *1.1.1 Intended Use*.

Any of the following notes should be strictly observed:

Caution!	In the event of any non-conforming type of use, including non-conforming use of assemblies or single parts, no liability will be assumed by the Manufacturer. This shall also apply to service or repair work of any kind, which is performed by other than duly authorized service personnel. All claims for warranty and warranty services will be null and void in any such case.
Warning!	Insert the power cord plugs only into a grounding outlet. The protective action must not be rendered ineffective by extension cables without protective conductor. The Pannoramic 250 FLASH III has a built-in protection which must not be rendered ineffective by extension cables without protective (earth-ground) conductor.
Warning!	The operation of the instrument in explosion-risk environments is not allowed.
Warning!	If protective devices are found to be ineffective, the Pannoramic 250 FLASH III must be shut down and precautions must be taken to prevent unintended usage. For necessary repair work or work to restore proper operating condition, you should contact the Thermo Fisher Scientific Customer Service.
Note:	Electric power to the Pannoramic 250 FLASH III is supplied via an internal mains power supply unit. It allows for operation at line voltages in the range of 100 V to 240 V ±10%, 50/60 Hz, without any extra facilities required for voltage transformation.
Warning!	Check that your local line voltage is compatible with Pannoramic 250 FLASH III specifications before you turn power on.
Disconnect plug!	Remember to unplug the power cord before you open the Pannoramic 250 FLASH III or replace a fuse!

For research and education uses only, not for use in diagnostic procedures.

	Warning!	Use only electric fuses which are rated for the specified nominal current. You are prohibited from using makeshift fuses or short-circuiting of a fuse holder.
	Warning!	The Pannoramic 250 FLASH III does not include any special facilities to protect from samples with an etching, potentially infectious, toxic, radioactive or other health-damaging effect. You are under obligation to comply with all currently binding legislation, notably, national accident prevention rules when handling samples of this kind.
	Caution!	Dirt or dust may adversely impact the operating capability of the Pannoramic 250 FLASH III. For this reason, measures must be taken to eliminate such influences to a maximum possible degree. During periods of non-use, the Pannoramic 250 FLASH III must be protected with a dust cover. Check that power is turned off before you put the cover on.
	Crush hazard!	There is crush hazard in the movement area of the slide insert during operation. Please note that the digitizing process has to be interrupted and the insert moved via software to the output position before removing the insert.
	Warning!	Clogged or covered ventilation slots may result in a heat build-up with a damaging effect for the Pannoramic 250 FLASH III. In extreme cases, this may also cause fire. Keep the ventilation slots always unobstructed and refrain from inserting or dropping objects into a ventilation slot.
	Warning!	A defective Pannoramic 250 FLASH III is not classified as domestic waste. It must be properly disposed in accordance with currently valid legal requirements.
	Warning!	The Pannoramic 250 FLASH III may not be operated by other than properly instructed persons. Operating personnel must be fully aware of the potential dangers which a particular field of application involves. The Pannoramic 250 FLASH III is a precision instrument which may suffer a significant reduction in operational functions or even physical destruction following intervention performed in any nonconforming manner.

Notes on Warranty

We at Thermo Fisher Scientific are proud of our quality, reliability and of our after-sales service. We continuously strive to improve our service to our customers.

Please ask a Thermo Fisher Scientific representative about Service Contracts which can keep your purchase in peak condition for many years to come.

Warranty provisions necessarily vary to comply with differences in national and regional legislation. Specific details can be found in the delivery documentation or from your dealer or representative.

Please note that your warranty may be invalidated if:

• This instrument is modified in any way.

• Accessories and reagents which have not been approved by Thermo Fisher Scientific are used.

• The instrument is not operated or maintained in accordance with the instructions in the Operator Guide.

For research and education uses only, not for use in diagnostic procedures.

Terms and Abbreviations

CSV	Comma Separated Value
FOV	Field of View
NA	Numerical Aperture
Photobleaching	A phenomenon that leads to a dramatic loss of fluorescence emission intensity (a fluorophore permanently loses the ability to fluoresce) due to photon-induced chemical damage and covalent modification, therefore fluorescent signals cannot be properly detected.
SWAP	A file that is used as a temporary location on the computer to store information that is not currently used by the RAM.
Virtual Slide / Slide	A digital image of a thin glass plate on which specimens are mounted for microscopic study:
	a dynamic, interactive image that you can manage, save, magnify, zoom, name, evaluate, annotate, mark and comment, send to a colleague electronically for co-operation or advice, and so on.

1   Product Description

1.1   Product Overview

1.1.1   Intended Use

Pannoramic 250 FLASH III is designed to use transmitted light and reflected light fluorescence to digitize biological samples and specimens (typically histological sections).

> ⚠️ **Warning!**
>
> After each scanning make sure to inspect the digital slide for over-exposed areas. If the slide is scanned based on improperly set parameters, it can highly affect the results because of information loss.

> ⚠️ **Warning!**
>
> Any modification to a hardware or software component of the PC will highly affect the functionality of the scanner program.

1.1.2   Features and Benefits

The Pannoramic 250 FLASH III offers world class brightfield and fluorescent whole slide scanning on up to 9 channels. An outstanding 0.19 µm/pixel (in Brightfield) and 0.16 µm/pixel (in Fluorescent) resolution is achieved with a 40×/0.95 NA (equivalent to 52× magnification in Brightfield and 62× magnification in Fluorescent modes) Plan-Apochromat objective.

The key features of the Pannoramic 250 FLASH III Scanner are as follows:

- Extreme fast brightfield scanning mode:
    - Continuously moving stage during scanning
    - Brightfield camera with a frame rate of up to 180 fps
    - Xenon flash lamp as light source

For research and education uses only, not for use in diagnostic procedures.

- Fluorescent scanning mode:
  - High-end fluorescent illumination device – Lumencor SPECTRA Light Engine 6
  - 3 times faster and more silent filter change
  - Multiband filter support
  - FL scanning with a low noise, 16-bit scientific CMOS pco.edge 4.2 camera
  - Software-aimed flat-field correction compensation method to create homogeneous slide image
  - Magazine stack cover to protect fluorescent slides against photo bleaching

- Image quality:
  - Image sharpening (both for Fluorescence and Brightfield) feature which improve the visual appearance of digital slides
  - Focus algorithm finds the focus which is most appealing to the human eye

- Motorized camera changer for color camera for brightfield, and a cooled monochrome CCD camera or a scientific CMOS camera for fluorescence

- Motorized Objective changer for two objectives (Plan-Apochromat 0.8 NA or 0.95 NA)

- 3MP camera for barcode reading and preview image

- Compact design:
  - Cameras are placed inside the unit
  - Built-in power supply

- 250 slide automatic loading and scanning

This device is recommended for customers, who need high quality and high throughput granted by the biggest loading capacity of 250 slides.

1.1.3   Warning and Information Labels

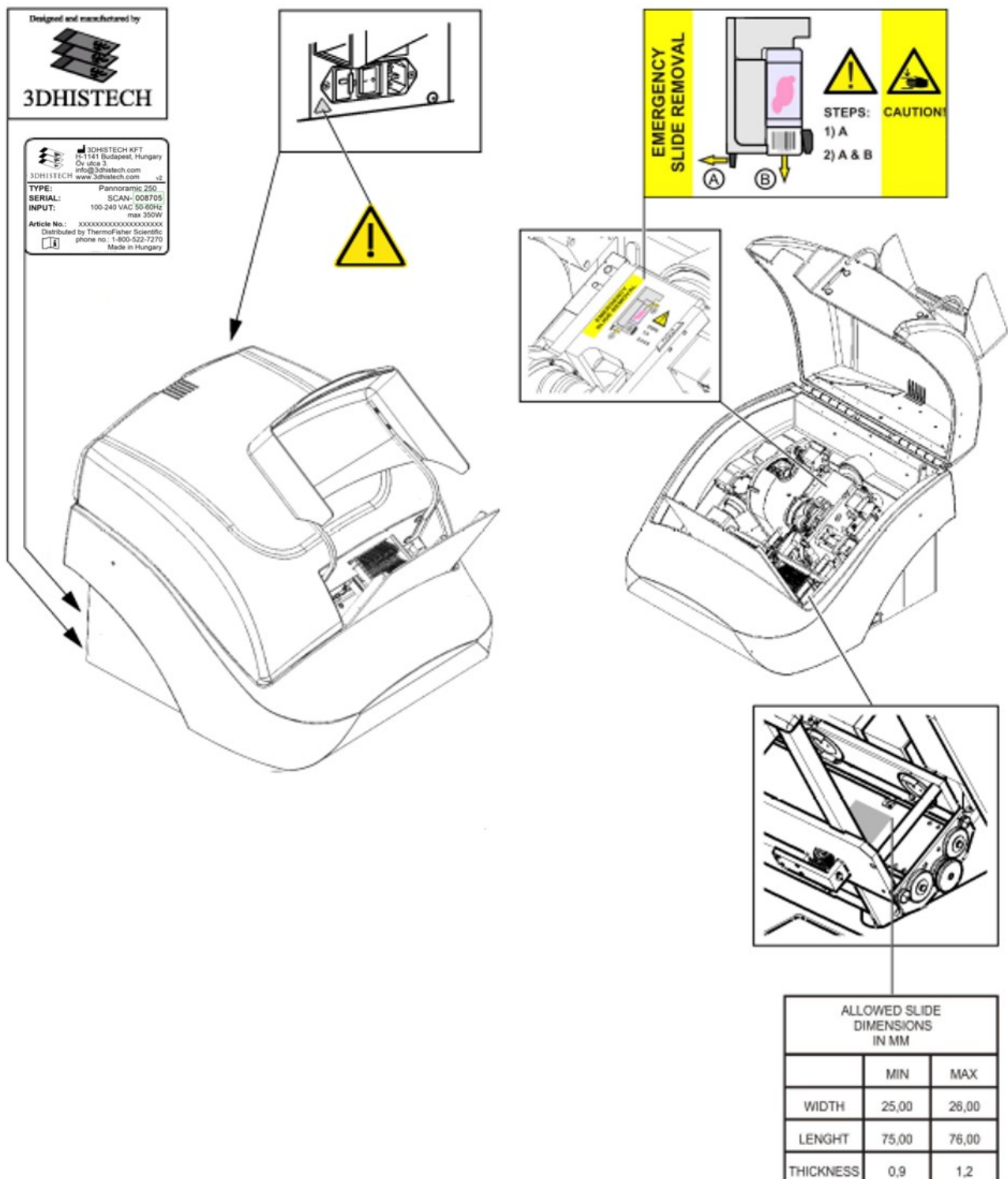The following figure contains the labels used on the Pannoramic 250 FLASH III main unit.

Figure 1 – Warning and information labels

1.1.4   Main parts of the Base Unit

The following figures show the main parts of the base unit.
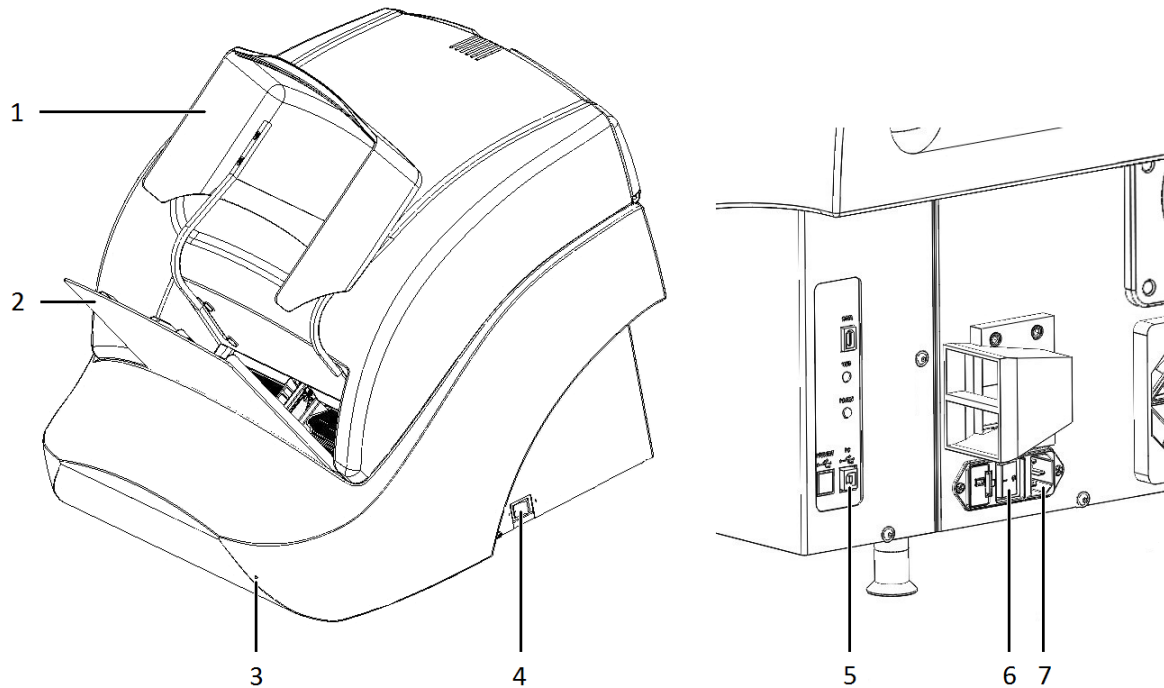

Figure 2 – Main parts of base unit

1. Protective FL cover

2. Input and output stack for magazines

3. Cover release hole

4. Power supply switch

5. USB port

6. Main power switch

7. Power supply cable connector

1.2   System Overview

The standard scope of delivery of the product includes the following hardware and software components.

1.2.1   Pannoramic 250 FLASH III system

The following figure shows the main parts of the Pannoramic 250 FLASH III system.
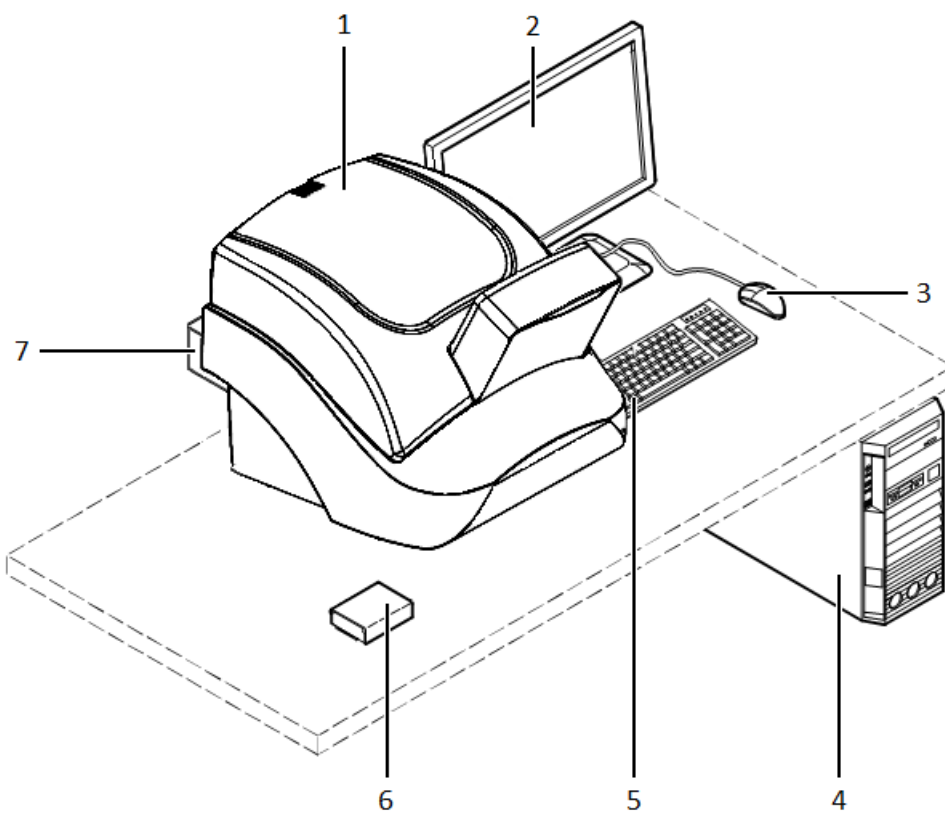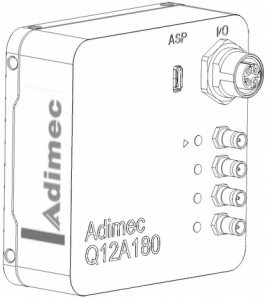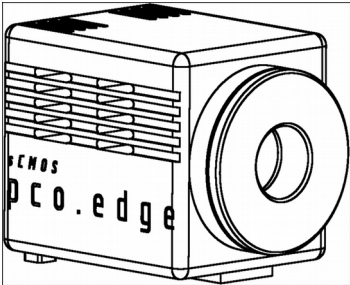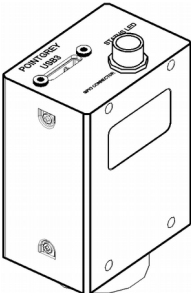

Figure 3 – Pannoramic 250 FLASH III system overview

1. Pannoramic 250 FLASH III base unit

2. Monitor

3. Mouse

4. Control computer

5. Keyboard

6. Slide magazine

7. Lumencor SPECTRA Light Engine illumination unit
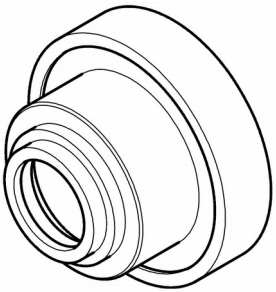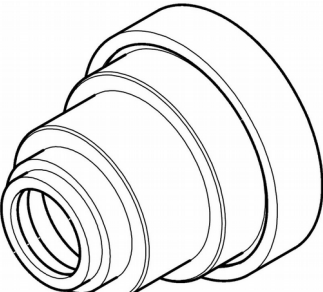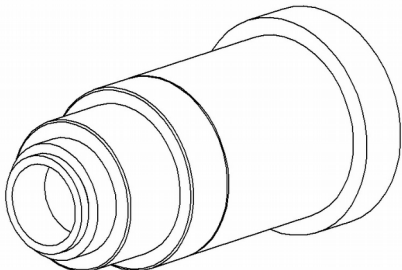
1.2.2 Hardware Options

The configuration of the control computer and monitor can vary depending on the product order.

Cameras

	Adimec QUARTZ Q-12A180 1 CMOS camera for high-quality brightfield applications allowing ultra-fast scanning up to 180 fps.
	pco.edge 4.2 Equipped with a high-quality sCMOS image sensor for fluorescent applications allowing fast scanning and an outstanding low read out noise. High quantum efficiency, cooled with Peltier element.
	Point Grey Grasshopper 3 Mono 1 CCD monochrome camera for high-quality sensitive fluorescent applications.

Camera adapters

0.63× adapter (for Point Grey Grasshopper	**1x adapter** (for Fluorescent only)	**1.6x adapter** (for Brightfield only)

3 camera only)		

Motorized camera changer
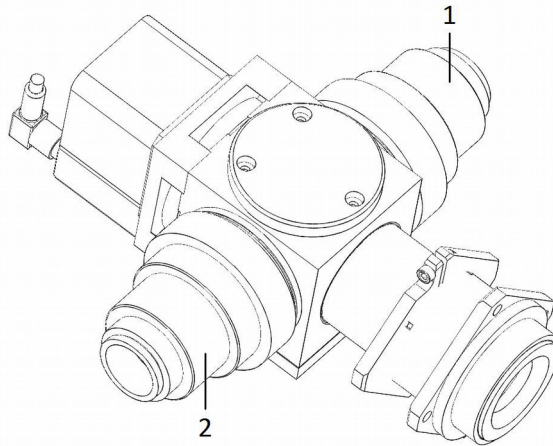
Figure 4 – Motorized camera changer

You can fit two cameras into the Pannoramic 250 FLASH III on the first adapter **(1)** (fluorescent position) and the second adapter **(2)** (brightfield position) at the same time. They are set and switched by the software automatically, without any human assistance.

This system also enables switching between brightfield and fluorescent scanning by one click.

> ⚠ **Warning!**
>
> The installation of the cameras in Pannoramic 250 FLASH III must be carried out by trained professionals!
>
> In any other case the warranty will be automatically voided. Supplier/Distributor is not liable for any damages caused by an installation not carried out by professional person.

Objectives
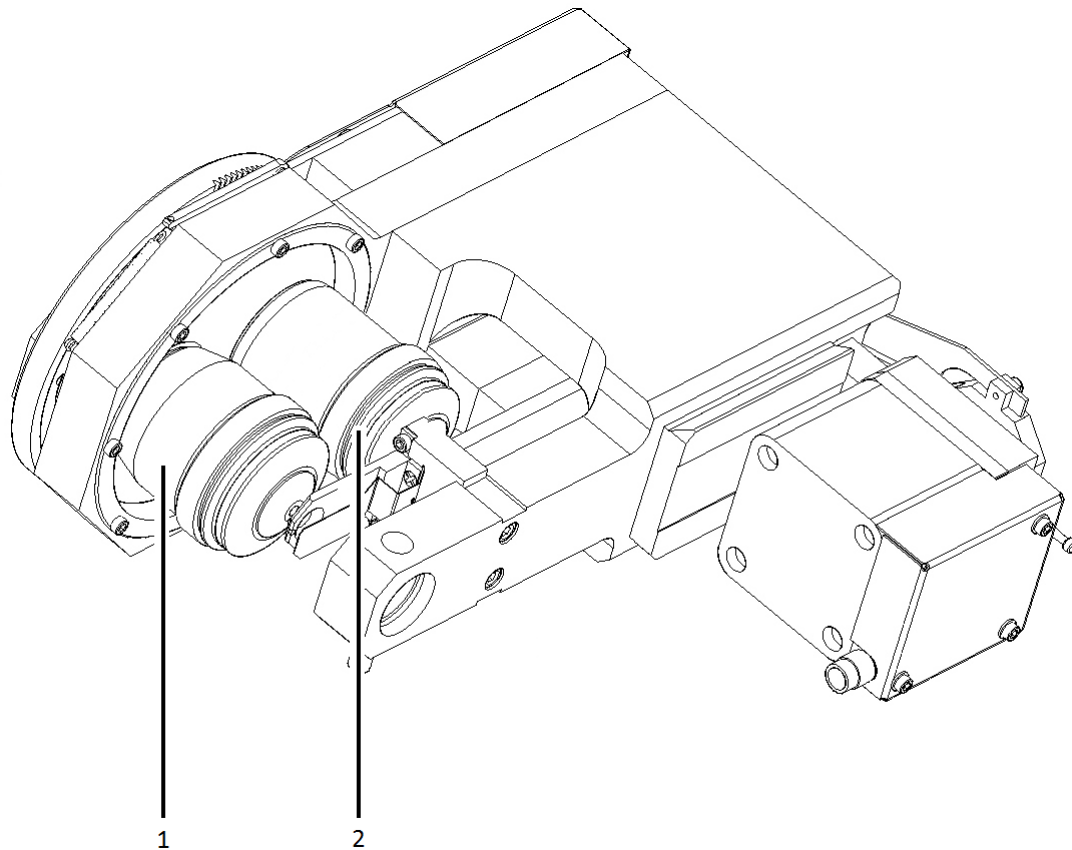
Figure 5 – Objective changer

You can select two different objectives to be equipped with the Pannoramic 250 FLASH III, the first objective **(1)** and the second objective **(2)**.

20x/NA 0.80 and 40x/NA 0.95 objectives can be used. You can change between the objectives with one click. This helps you to select the magnification and resolution that is the most suitable for your requirements.

⚠️ **Important!**

Note, that higher resolution results in slower digitization process.

For research and education uses only, not for use in diagnostic procedures.

Preview

Figure 6 – Preview camera (1), Brightfield preview lamp (2), and Dark-field preview lamp (3)

At fluorescent sections the dark-field preview makes the fluorescent samples detectable even for the user and for the automatic marking algorithm. By detecting the sample the fluorescent scanning will be much faster since the software will not waste time on empty FOVs.
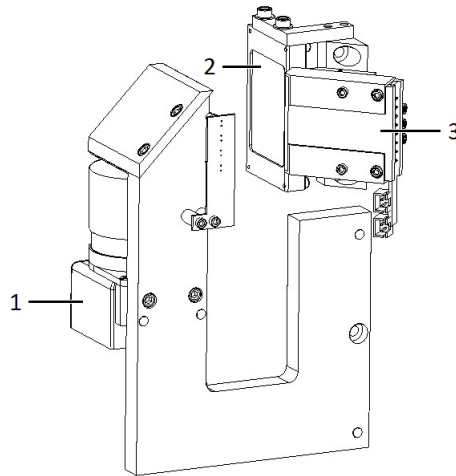
Unit options

- Pannoramic 250 FLASH III Basic Brightfield Option – base unit equipped with Adimec QUARTZ Q-12A180 camera for Brightfield scanning (no fluorescent accessories included)

- Pannoramic 250 FLASH III Basic Fluorescence Option – base unit completely equipped with Point Grey Grasshopper 3 camera and Lumencor SPECTRA 6 fluorescence illumination unit, trigger cable and filters for fluorescence scanning

- Pannoramic 250 FLASH III Advanced Fluorescence Option – base unit completely equipped with Adimec QUARTZ Q-12A180 camera for Brightfield scanning, and with pco.edge 4.2 camera and Lumencor SPECTRA 6 fluorescence illumination unit, trigger cable and filters for fluorescence scanning
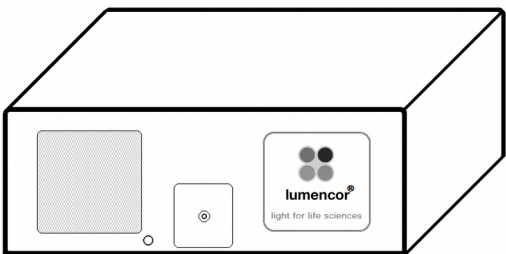
NOTE: If Lumencor SPECTRA 6 unit is ordered, the custom light source and filter modules can be variable. For more information, please contact Thermo Fisher Scientific customer service.

Objectives

- Plan-Apochromat 20× / NA 0.8

- Plan-Apochromat 40× Corr/ NA 0.95

<table>
<tr><td>⚠️ Warning!

Do not change correction collar settings on 40x objective, if you need it to be set, contact customer service.</td></tr>
</table>

Fluorescent Option for Pannoramic 250 FLASH III

	Fluorescent unit (internal part)
	Lumencor SPECTRA 6 Light Engine illumination unit

1.2.3   Control Software

- Pannoramic Scanner control software

    Optional:

    - Fluorescence
    - Extended focus
    - Z-stack
    - Barcode reader (licensed feature)

- Pannoramic Viewer software (fully functional)

    Optional:

    - CaseCenter
    - Pannoramic Viewer TMA module
    - 3D Reconstruction module
    - QuantCenter, HistoQuant, NuclearQuant, MembraneQuant, FISHQuant, CISHQuant, and DensitoQuant modules
    - TumorBoard module
    - E-School Encoder

For research and education uses only, not for use in diagnostic procedures.

2   Installation

> ⚠ **Warning!**
>
> The installation of Pannoramic 250 FLASH III must be carried out by trained professionals! In any other case the warranty will be automatically voided.
>
> Supplier/Distributor is not liable for any damages caused by an installation that is not carried out by a professional person.

Only qualified service assistants are allowed to install the hardware and software. The product is ready for use after installation.

2.1   Control computer ports

The following figure shows the location of the connectors in order to enable easier transportation of the product for a short distance, by disconnecting cables and detaching hardware components from each other.
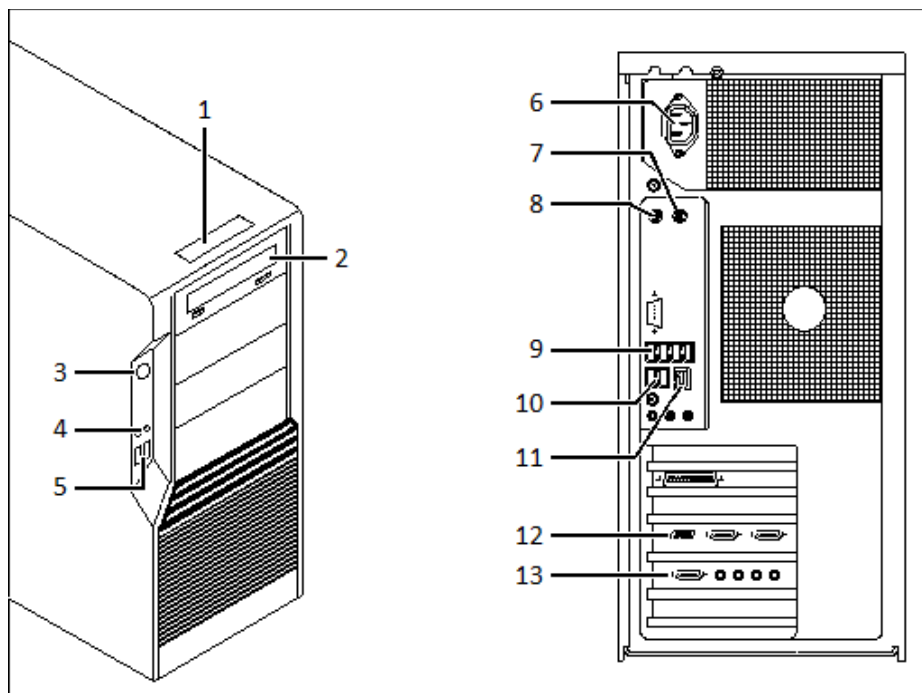

Figure 7 – Ports on control computer

1. Status display

2. DVD drive (RW)

3. Power on/off button

4. Earphone and microphone 3.5mm Jack ports

5. 2x USB port

6. Power supply connector

7. PS/2 mouse port

8. PS/2 keyboard port

9. 4x USB port

10. 2x USB port

11. Network port

12. Matrox Radient eCL Camera Link ports (for pco.edge 4.2 camera) or 4x USB 3.0 ports for Point Grey Grasshopper 3 camera

13. CoaXPress Coaxlink Quad (for Adimec camera)

2.2   Connectors and cables

• Control – USB (between scanner and control computer)

• Preview camera – USB cable (between scanner and control computer)

• Adimec – CoaXPress cables (between Adimec camera and control computer)

• pco.edge 4.2 – a pair of CameraLink cables (between pco.edge 4.2 camera and control computer)

• Point Grey Grasshopper 3 – USB 3.0 cable (between Point Goint Grey Grasshopper 3 camera and control computer)

• Video cable (between monitor and control computer)

• Power supply cable (between 230V/110V and monitor)

• Power supply cable (between 230V/110V and control computer)

• Power supply cable (between 230V/110V and Pannoramic 250 FLASH III main unit)

• Trigger cable (between pco.edge 4.2 and Lumencor SPECTRA 6 fluorescent illumination unit)

• USB-to-RS232 serial communication cable (between Lumencor SPECTRA 6 fluorescent illumination unit and control computer)

> ⚠️ **Warning!**
>
> Only connect external devices that are safety extra low voltage rated to the instrument to avoid the risk of electrical shock.

2.3   Mounting the Lumencor SPECTRA 6 unit

To operate the Lumencor SPECTRA 6 unit, it has to be attached to the Pannoramic 250 FLASH III base unit.

1. Switch off the main power supply on the rear panel of the Pannoramic 250 FLASH III and disconnect the power cables (see section ***1.1.4 Main parts of the Base Unit***).

2. Open protective cover (see section ***5.3.1 Opening the protective cover***).

3. Find the mounting hole (**1**) at the back side of the Pannoramic 250 FLASH III unit and rotate the Lumencor SPECTRA 6 unit (**2**) upside down.
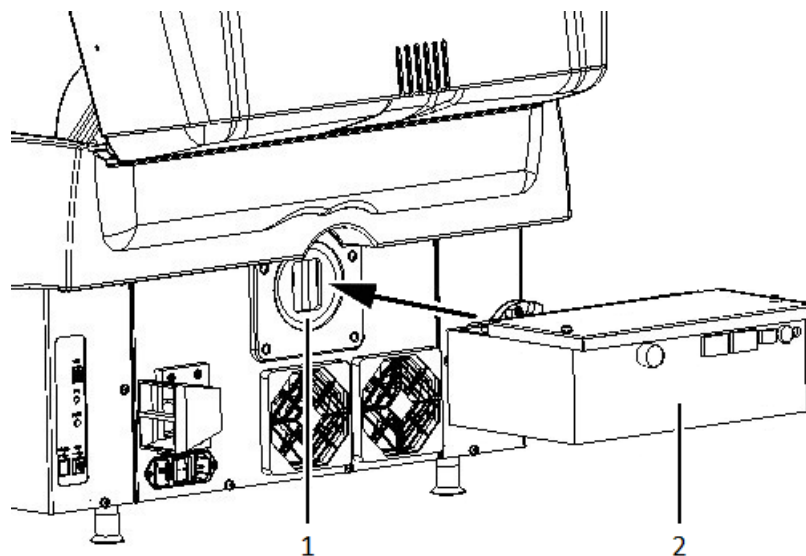

Figure 8 – Mounting position

4. Move the lever (**1**) to the open position, which is to the right when facing the front of the unit (see *Figure 9*).
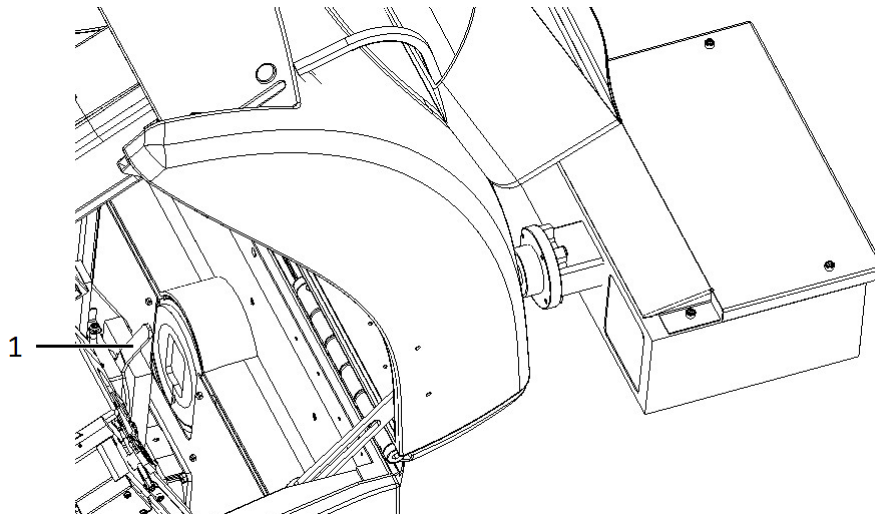

Figure 9 – Positioning locking lever

5. Attach the Lumencor SPECTRA 6 unit so the contact surfaces at the mounting point are tightly compressed (see *Figure 10 – Step 1*).

6. Secure the locking lever by moving it to the left (see *Figure 10 – Step 2*). If the lever stucks at around the center position, apply a bit more pressure to the Lumencor SPECTRA 6 unit.
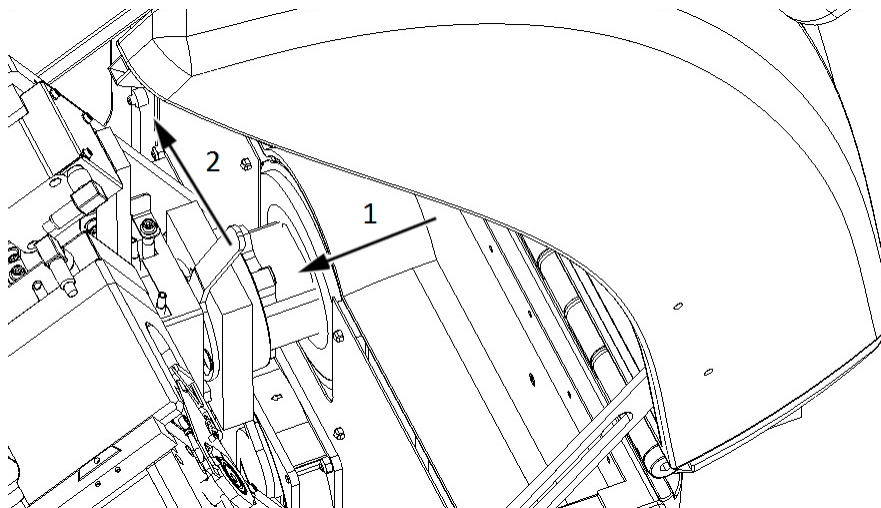

Figure 10 – Mounting the Lumencor SPECTRA 6 unit

7. Close the protective cover of the Pannoramic 250 FLASH III.

8. Connect power supply cables both of the scanner and the illumination unit.

9. Connect TTL trigger cable between pco.edge 4.2 camera (out 4) and the Lumencor SPECTRA 6 unit (TTL), and USB-to-RS232 communication cable between the Lumencor unit and the computer.
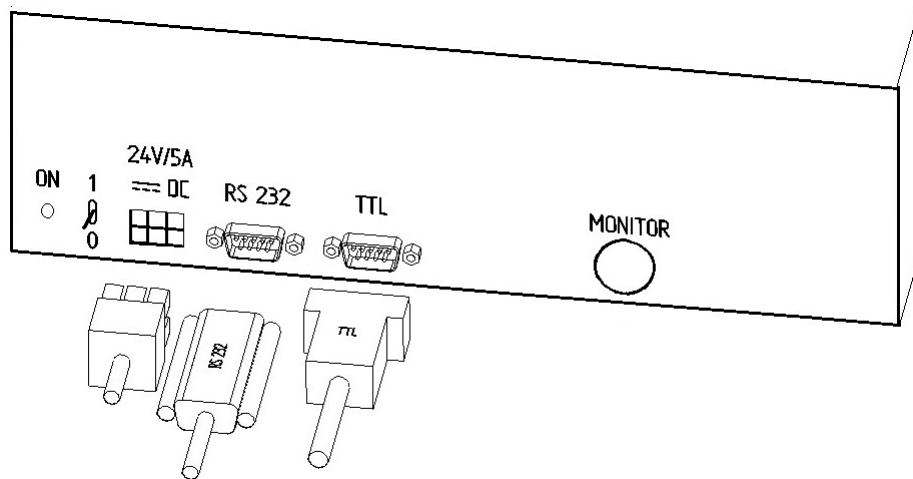

Figure 11 – Lumencor SPECTRA 6 light engine connecting ports

3 Operating Slides and Magazines

3.1 Preparing Slides and Magazines

This section demonstrates how to prepare slides and magazines.

You can use all slides and cover slips that meet the following specifications:

	Slides	**Cover slips**
Length	75.0 to 76.0mm	max. 50 mm
Width	25.0 to 26.0 mm	max. 24 mm (recommended: 22 mm)
Thickness	0.90 to 1.2 mm	No. 1 and No. 1.5 (0.13 to 0.16 mm and 0.16 to 0.19 mm)
Corners	45° beveled or 90° cut corners	
Edges	Ground or cut	

> (!) **Note:**
>
> The distance between slide edges and the edges of the mounted coverslip should be 1-2 mm, and they should be parallel with each other.

> ⚠ **Caution!**
>
> Ensure that no part of the mounted coverslip protrudes from the edges of the slide. Use slides only with completely dried embedding medium. If not so, the coverslip can be shifted that makes slide loading less reliable. Not completely dried embedding might also influence the operation of objectives and other device parts.

> ⚠ **Caution!**
>
> Do **NOT** scratch any circular marker onto the glass surface of a slide, use a soft tip pen instead for marking area of interest.

> ⓘ **Note:**
>
> Fluorescent signals become more easily detectable for the scanner, therefore resulting in a brighter and better image, by treating the slide with a formula that prevents rapid photobleaching of fluorescent reagents (e.g. VECTASHIELD® Fluorescent Mounting Medium by **Vector Laboratories** and ProLong® Gold and *SlowFade*® Gold antifade reagents by **Invitrogen**) during the FISH protocol.
>
> For further information, see the homepages of the manufacturers: http://www.vectorlabs.com/ and http://www.invitrogen.com.

3.2   Affixing Barcode Stickers to Slides

This section describes how to affix barcode stickers to slides. Barcodes enable virtual slide identification.
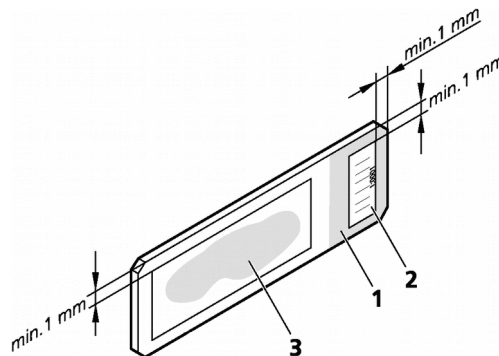

Figure 12 – Slide with a barcode sticker

The label area **(1)** is recorded by the preview camera, and can be displayed by the control and viewer software.

To affix barcode stickers to slides, do the following:

1. Peel off the barcode sticker **(2)** from the roll.

2. Stick it on the colored label area of the slide **(1)**, on the specimen side.

> ⚠️ **Caution!**
>
> Ensure that a marginal space of 1-2 mm remains on all sides between the sticker and the label area outer limits. It is vital for barcode identification.
>
> Do not let barcode sticker protrude from slide edges, or stick on the coverslip surface **(3)**.
>
> Do not stick more than 4 barcode stickers over each other and the overall thickness must must be kept below 1.65 mm (including the barcodes and the slide).

3.3 Inserting and Removing Slides

3.3.1 Insert slides into a magazine
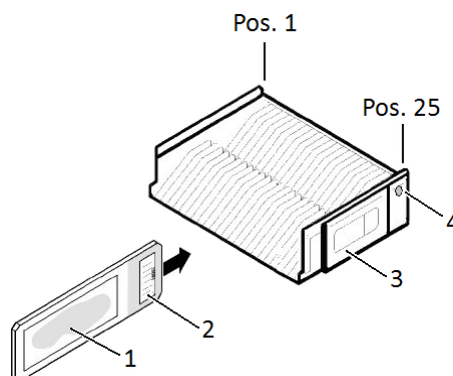
Figure 13 – Inserting slides into a magazine

> ⚠️ **Caution!**
>
> Only original 3DHISTECH P250 magazines are allowed for use with Pannoramic 250 FLASH III!

The magazines can contain a maximum of 25 slides.

- Push slide **(1)** into the magazine **(3)** with its barcode end **(2)** first. The symbols on the side of the magazine demonstrate the proper way of inserting slides.

- The magazine and its proper insertion is identified by a sensor in the main unit upon actuating it by the magnet in the side of the magazine **(4)**.

(!) Note:

You should insert several slides into a magazine without empty slots. Begin the insertion on position 1 of the magazine.

⚠ Caution!

To insert or remove a filled magazine from the device, slightly tilt it to its closed side to prevent the slides from accidentally falling out. Magazines should always be kept clean from dust, broken glass and other mounting media residues. Slide loader will only operate safely and properly, if magazines are clean.

3.3.2   Insert and remove magazines
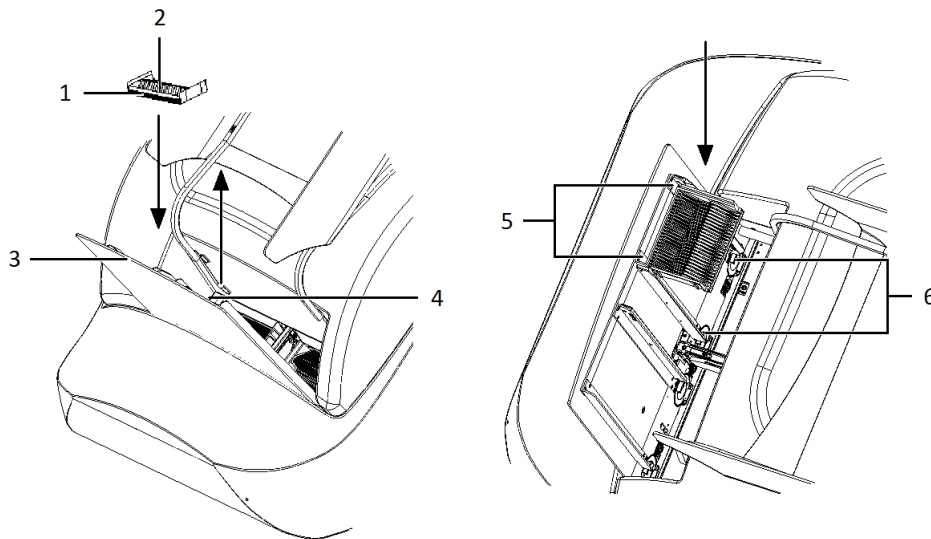
Figure 14 – Inserting a magazine

Pannoramic 250 FLASH III has two pairs of guide rails **(5)** to manage the loading and unloading of magazines.

⚠️ **Warning!**

The protective cover must always be closed while the microscope is loading and/or scanning!

🛇 **Note:**

The guide rails have built-in sensors that detect whether there are magazines inserted in the input stack, or whether the output stack is full.

If the system does not detect the inserted magazine in the input stack, check whether you have inserted it correctly. If the magazine is placed correctly, there may be a problem with the sensor. In this case, contact Thermo Fisher Scientific Service Support.

1. Remove magazine cover, before you insert magazines.

2. Put a magazine **(2)** into the input stack **(3)**.

    The magazine's rack (the back side of the magazine) **(1)** must contact with the guide rails **(5)**, with its open side towards the scanner.

3. Slide down the magazine on the input guide rails until it rests on the gear wheels **(6)** or magazines.
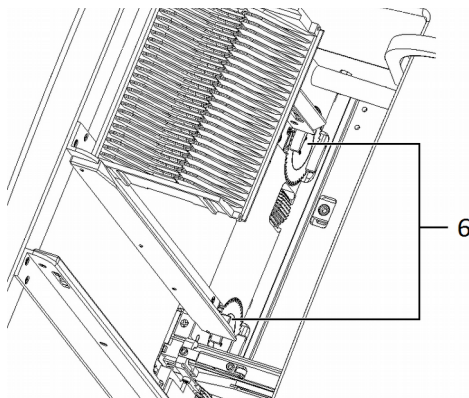
Figure 15 – Gear wheels

⚠ **Caution!**

Make sure that the magazines are properly placed between the rails, because it can cause serious damages in the feeding mechanism.
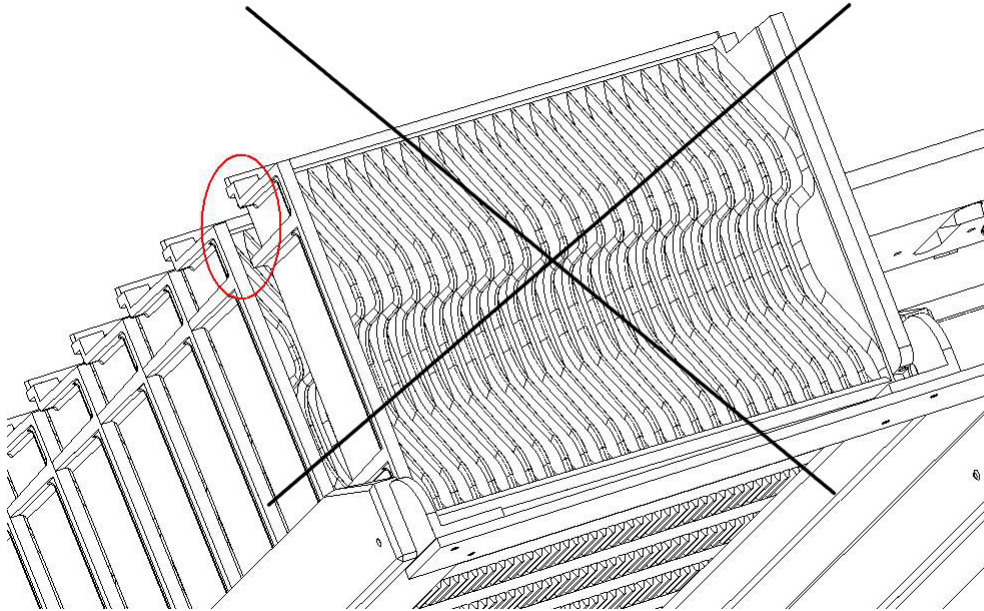

Figure 16 – Misplaced magazine

🛈 **Note:**

The input stack can be filled with a maximum of 9 magazines in addition to the one in the magazine loader.

⚠ **Caution!**

Do not place magazines into the output stack **(4)** or remove magazines with already digitized slides from there during the digitization process. Only remove magazines from the output stack when you have finished digitization of all slides.

3.3.3   Opening/Closing the FL protective cover
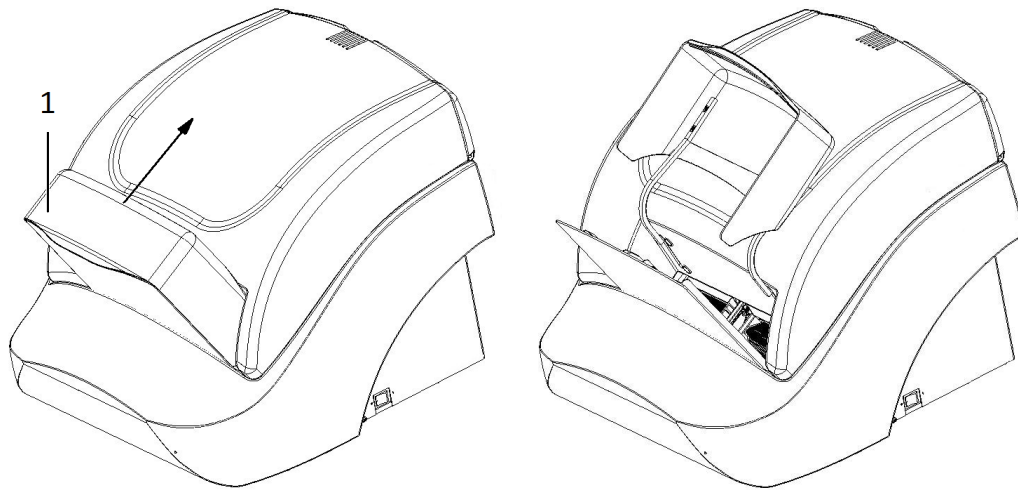
Figure 17 – Opening the FL protective cover

Open the protective cover by moving it upwards and lay it down to the upper cover of the unit, then insert magazines.

When scanning slides, close the FL protective cover **(1)** of the base unit after inserting the magazine(s) into the input stack.

1. Mount the protective cover properly.

2. After finishing the digitization process, open the protective cover.

3. Remove the magazine(s) from the output stack and put the magazine cover on them.

4   Software Description

4.1   Starting / Exiting Pannoramic Scanner Software

4.1.1   Starting the software

To start the program, double-click on the program icon on the desktop or start it from **Start \ Program Files \ 3DHISTECH \ Pannoramic Scanner**

For the scanning software you can choose from different scanning modes at the startup of the program.

After software initialization the user interface is opened and the mode selection icons are displayed.
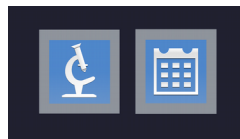
These two mode buttons represent **Microscope** and **Planner** scanner modes:

Microscope mode

- for digitization of individual slide or individual rack

- set scanning profile of the slide

- run Live teleconsultation

- use Live microscope

Planner mode

- plan the entire scanning procedure

- assign one or more profiles to each slide

- define Brightfield and Fluorescence or mixed scanning mode within a run

- define scanning profile to slides included in racks or magazines

- automated scanning mode

After selecting the required mode, the main window is displayed.

4.2   Main window functions
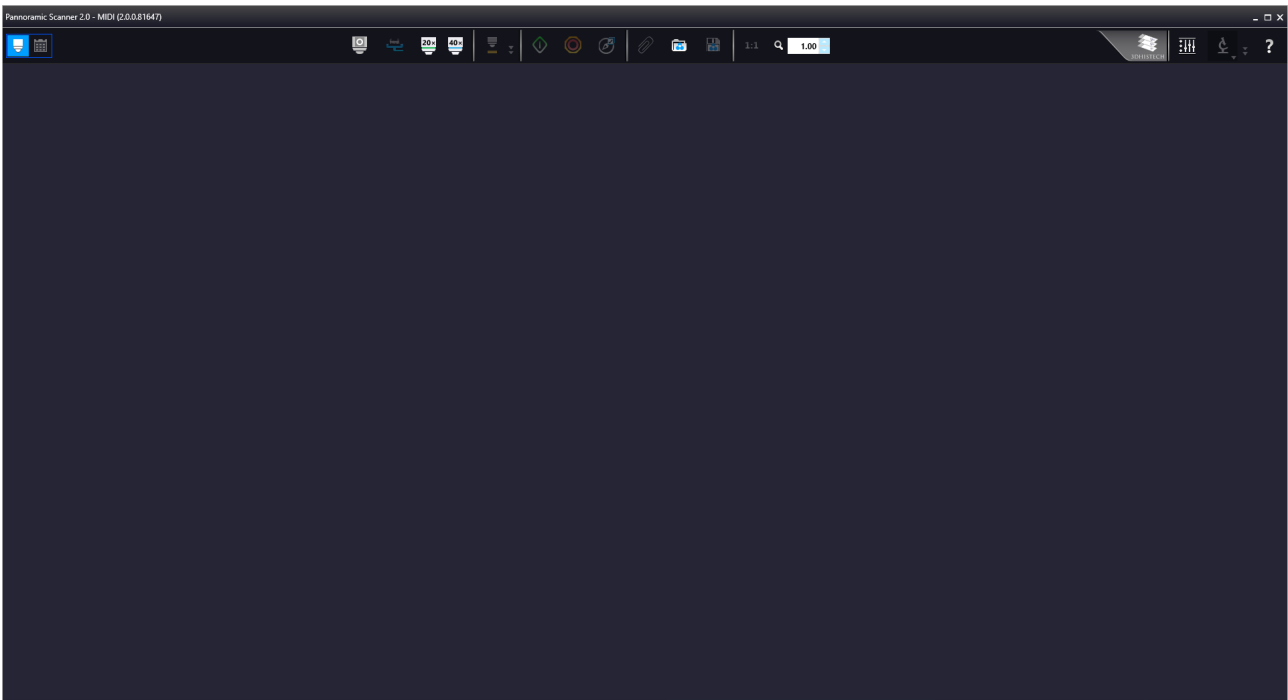
Figure 18 – Pannoramic Scanner 2.0 main window

The following functions are available for you to select from at the main menu bar:

 – Switch between Microscope and Planner modes
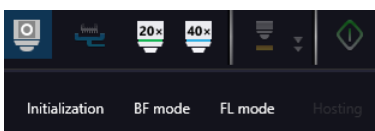
 – Microscope initialization

 Initialization – All motors and the preview camera during start-up

 BF mode – In BF mode: the camera for brightfield scanning is initialized

 FL mode – In FL mode: the camera for fluorescence scanning is initialized

 Hosting – Start and host a live Teleconsultation session

 – magazine/ Slide loading

Upon clicking the slide icon or entering slide position number at the right side, the slide can be loaded.

Unload the slide by clicking **Unload** next to slide image, and unload the magazine by clicking **Unload** next to the rack image.

If there is a lens marking at the bottom of the position preview in the magazine image, the preview image of that slide has already been created, and if a question mark is displayed the slide was not yet loaded. A hatch and lens marking represent a slide that is loaded at the moment.

Emergency Position: If a slide has been jammed in, the unloading position where the magazine should be moved can be defined.

Slide removed: Click this button to inform the control software that the slide has been removed manually.

 – Objective changer

 – Live view is on

 – Live view is on with the shutter closed

 – Toolbar for live images

- The toolbar is only available for the displayed live image, but not available for camera images during digitization.

- One pixel on the monitor corresponds to one pixel on the camera

- The live image completely fills the display area

- Displays or hides the vertical and horizontal scale bars

- Displays the magnifier window

- Activates red marking on overexposed pixels

- Saves the current image as BMP or JPEG file

Fast live mode – live images are displayed with a frequency of at least 10 images per second. If an exposure time is set to more than 100 ms, the pixel values are multiplied accordingly (for example, if

an exposure time of 200 ms is set, the pixel values are multiplied by 2, and the exposure time is reset to 100 ms). Therefore the resulting images will have a higher noise level.

This function is useful for fluorescent live view where in spite of the higher noise level, the resulting image will be refreshed as in normal mode.

Normal live mode – images are taken with the user-specified exposure time.

- Fast Mode

- Screenshot

- Magnifier Window

- Ruler

Image Processor Plugin functions:

- : Convolute gray-scale image by custom kernel

- : Multi-channel histogram with normalization

- : Invert image (for testing purposes)

- : Mark overexposed pixels

Scan Properties (included as well on the profile cards added to the slide in Planner mode) unique settings can be defined even for each separate scanning.

- **Scan to Local**

  ○ Select the Slide save Path

  ○ Folder from Barcode

  ○ Free space: Free space capacity of selected local drive

  ○ Use Barcode

  ○ Slide name: Can be defined by manual or generate automatically with **Generate name** setting.

  ○ Scan separately: The FOV areas of the preview image will be scanned and saved separately as discrete digital slides.
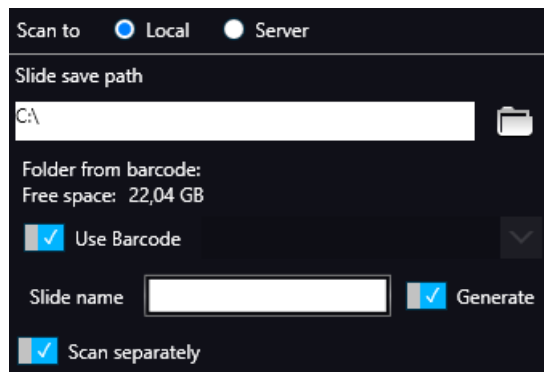
Figure 19 – Scan Properties window

- **Scan to Server**

    ◦ **Server:** the drop-down list includes all the saved servers. Click **Add new item ...** to create another server connection.
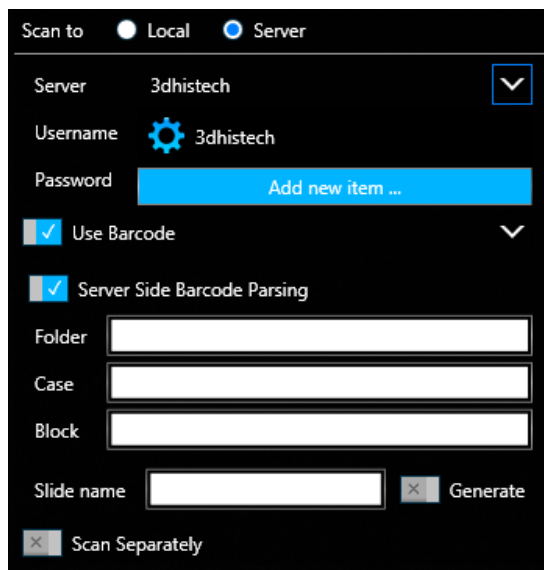

Figure 20 – Scan to server settings

- **Server Editor**: The user define here the following details of the Server:

    ◦ **Display Name**

    ◦ **Host Name**

    ◦ **Protocol**

    Server Editor stores each of the created server in a list. List entries can be removed or a new entry can be added if required. With the **Filters** function you can narrow down the results by searching for a specific server data. By double-clicking a list item, server data will be loaded in the **Scan to Server** panel.
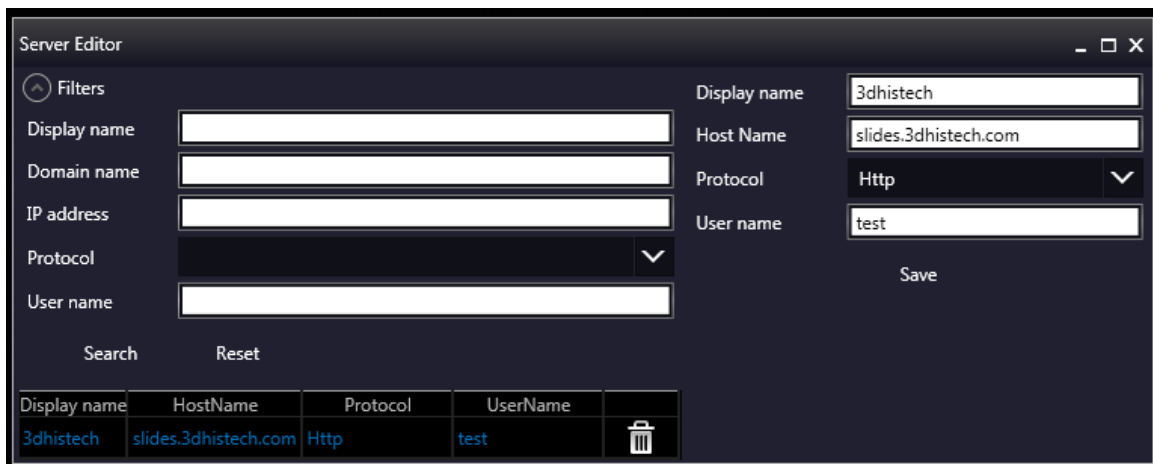
Figure 21 – Window of server editor

After loading server data, user name and password is required to be added for authentication and accessing the server. If connection is successful, a green checkmark is displayed in the window.
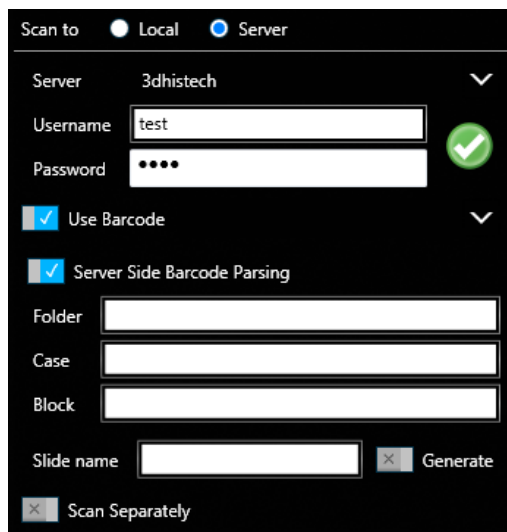
- **Use Barcode:** Barcode settings can be modified in the Barcode Editor window. By predefining barcode type and other settings.

- **Server Side Barcode Parsing** option can be selected if CaseCenter is connected with the supported HIS/LIS. This way the barcode read by the software is being identified, and the predefined location of that specific case in CaseCenter is automatically selected as scanning destination based on the CaseID (containing slide and patient data stored on HIS/LIS). Therefore, **Folder**, **Case**, **Block**, and **Slide name** field data cannot be modified, only a profile can be selected.
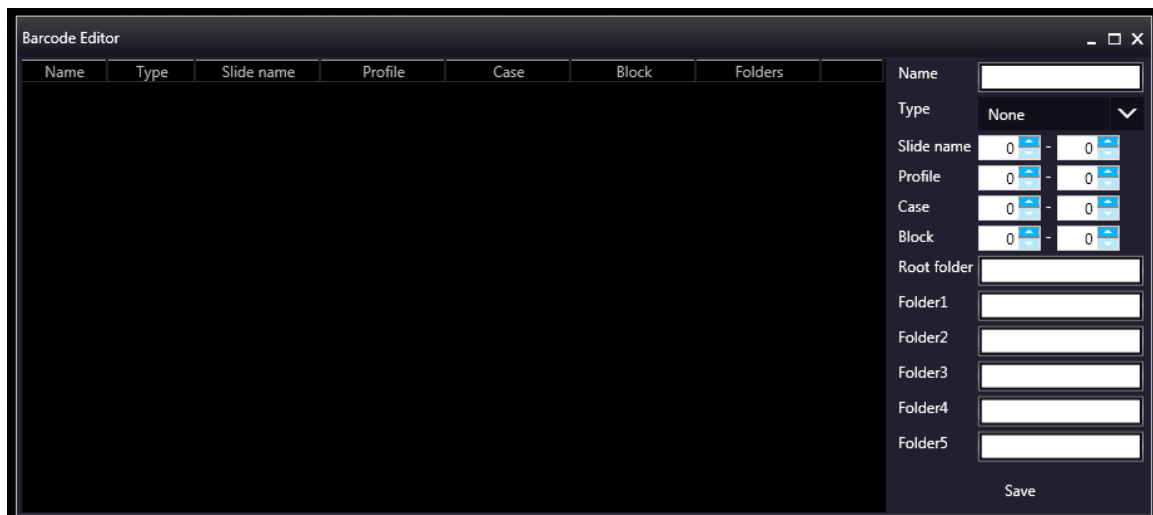
Figure 22 – Window of Barcode Editor

You can select the following barcode types:

1D barcode types

- ◦ *Code 39
- ◦ Code 128
- ◦ Code 16K
- ◦ *Interleaved 2 of 5
- ◦ Standard 2 of 5
- ◦ IATA 2 of 5
- ◦ Matrix 2 of 5
- ◦ Telepen
- ◦ Code 93
- ◦ Code 11
- ◦ MSI
- ◦ Codabar
- ◦ Patch Code
- ◦ Pharmacode
- ◦ EAN-13
- ◦ EAN-8
- ◦ UPC-A
- ◦ UPC-E

2D barcode types

- ○ Aztec

- ○ Data Matrix

- ○ PDF417/Compact

- ○ Micro PDF417

- ○ QR

- ○ Micro QR

- ○ MaxiCode

– Start Scan

Scanning of the actual slide or performing the whole previously planned scanning procedure can be initiated.

– Stop Scan

– Stop actual progress

– Take All Preview

Each preview of slides in the magazine is taken automatically. Preview types are the following:

- • Brightfield

- • Darkfield

- • Pre-Scan (FL)

- • Pre-Scan High-Resolution (FL)

- • Pre-Scan + High-Resolution (FL)
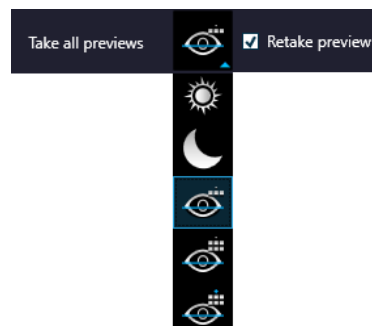
Figure 23 – Take all preview selector

– Auto attach

Actual profile is assigned to the loaded slide automatically.

 – **Open profile**

The Profile Browser window containing a list of previously saved profiles is opened. Based on the Illumination type, profiles are grouped into two clusters: Brightfield and Fluorescence. Additional profiles can be created here as well, and you can also search for a specific profile by filtering based on the following data:

- Objective type

- Multilayer mode

- Illumination

- Filters

- Modify date

- Last use date
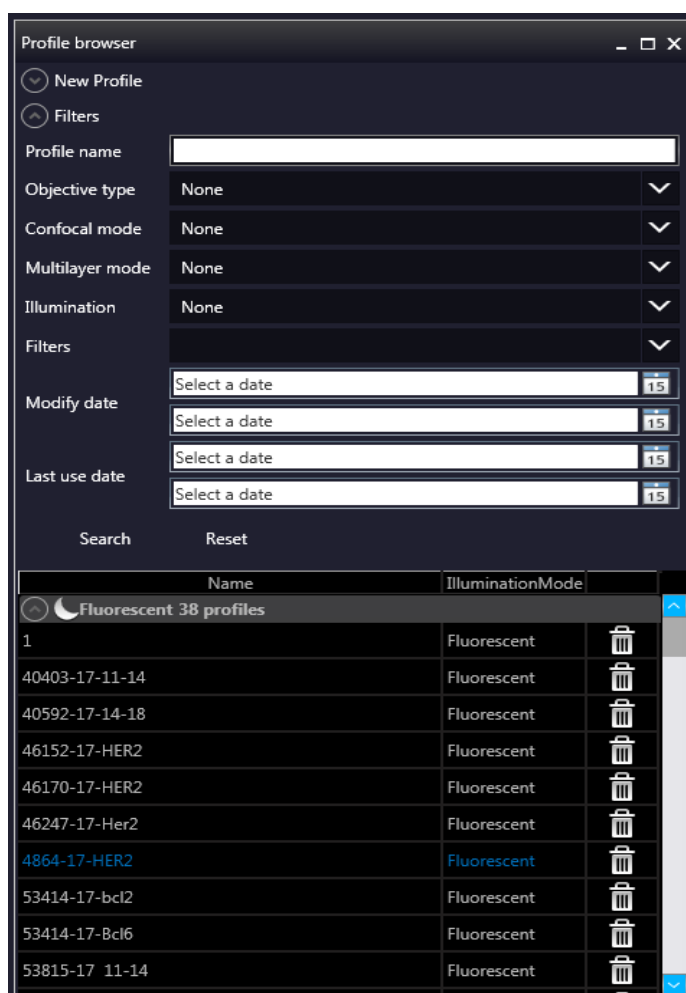
Figure 24 – Profile browser window

⊟ – Save Profile

The selected profile can be saved in this window after typing the profile name into the text field and clicking **Save as**.

1:1 – Reset Zoom

This function is active only in Live view at 1:1 magnification.

🔍 1,00 – Live image magnification

⫴ – Preferences

This panel contains user settings that have global effect on the GUI and some of its functions.

Appearance settings

- **View Mode Selector**

  - Text mode

  - Icon mode

  - Float Text mode

  - Float Icon mode

- **Style selector**

  - Black Jelly

  - Flat style

  - Black Jelly P1000

  - Flat style P1000

- **Planner style editor**

  - Slides with card

  - Table

  - Card Collection

Live mode

- **Rendered** – live image is displayed in an embedded window

- **External legacy** – live image is displayed in an external window

Profile settings

- Use profile history save – With this function each profile modification is saved in Profile History from which the different stages can be reloaded.

- Save interval – Profile content is automatically saved at the specified time-frames.

- Enable setting of focus time – Parameters (**Short / Normal / Long**) related to focus time will be enabled on the profile settings panel. **Normal** is set as default.

Slide settings

- ○ Take preview automatically – This function is designed to take preview images of slides automatically, once they are loaded.

- ○ Unload slide after FL grabbing

- ○ Slide label anonymity – Slide label area is displayed as a clear white image during scanning, patient data will not be visible in CaseViewer.

- ○ Do not save label to slide – The original label area is not saved to the slide, and will not be visible in CaseViewer.

- ○ Calculate exposure time for barcode grabbing – Three images are recorded for barcode image creation. Exposure times will be recalculated each time, thus creating prefect light conditions for barcode reading, so to make sure that barcode identification is successful.

Scanning History

- ○ Hardware log

    Scanner hardware operations are contained in this log. To enable this function, first the database from the following folder must be loaded: C:\ProgramData\3DHISTECH\ SlideScanner\HWLogDataBase
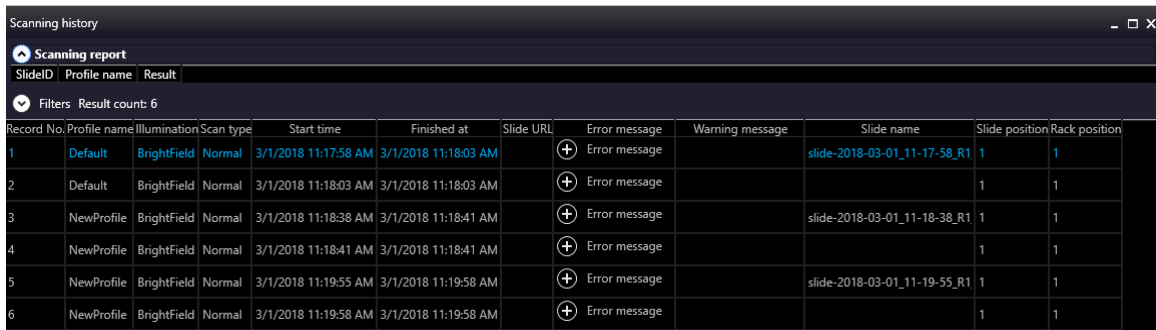
    The following motor movements are recorded in the log list:

    - ▪ Camera Changer
    - ▪ DFP Light
    - ▪ Filter Wheel
    - ▪ Flash Light
    - ▪ General
    - ▪ Halogen Light
    - ▪ Microscope Part
    - ▪ ND
    - ▪ Objective Changer
    - ▪ Preview Light
    - ▪ RGB Light
    - ▪ Relay
    - ▪ Scan Loader
    - ▪ Service
    - ▪ Shutter
    - ▪ Slide Scanner

- Stage

○ Software log

All of the circumstances and states of the slide scanning procedure is saved. LogID is the sequence number of scanning performed on the scanner in use. (Profile Name, Illumination mode, Scan type, Scan Start, Scan Finished, URL, Error)

Filtering for the following parameters is available:

- Scanning date

- Profile name

- Illumination mode

- URL

- Slide name

- Slide index

- Rack index

Email Settings

An e-mail can be sent automatically about every failure and error that is occurred during scanning to the specified address. The log files are compressed into a ZIP file, that is attached to the mail.

 – Help

5   Scanning modes and settings

After microscope initialization, if FL HW option is provided, you can select from the following two modes:

- **Brightfield scanning mode** (see section *5.1 Brightfield Scanning Mode for more information)*

- **Fluorescence scanning mode** (see section *5.2 Fluorescence Scanning Mode for more information)*

- **Option modes**

    ○ ![icon] **Off**

    ○ ![icon] **Services** (see section *5.6 Services and Settings* for more information)

    ○ ![icon] **Settings** (see section *5.6 Services and Settings* for more information)

In accordance with the selected mode, the **Profile Settings Panel** and the **Microscope Control Panel** views are modified.

5.1   Brightfield Scanning Mode

Microscope View consist of three different sections:

- **Main functions**: Located at below the window header, these functions are identical in both modes.

- **Profile Settings panel**: Profile settings containing parameters used for scanning.

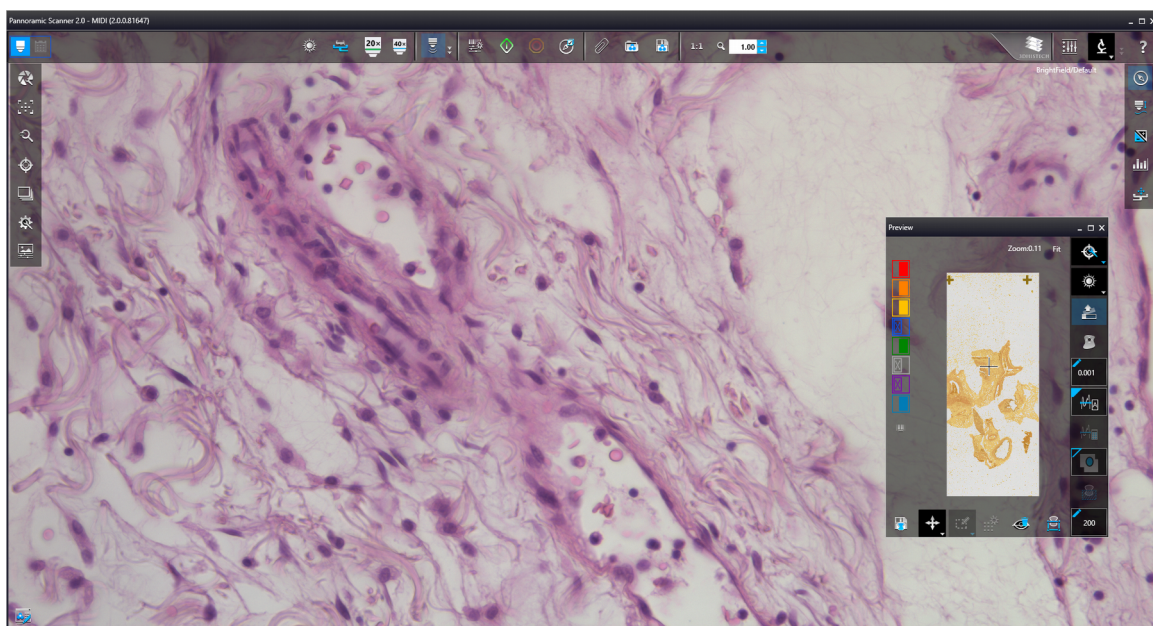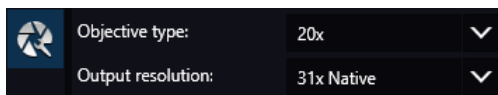- **Microscope Control Panel**: Live microscope movement functions

Figure 25 – Main window of Brightfield Microscope mode

For research and education uses only, not for use in diagnostic procedures.

5.1.1   Profile Settings Panel

Objective settings

This field displays the objective used for scanning. Objective type: 20x; 40x

Focus settings for the scan profile

Figure 26 – Focus settings for scan profile

- **Limit the range**: You can define focus limits by selecting **Limit the range** for focusing. You can set the upper and lower limits by selecting the desired value in the selection boxes. This is useful if focusing was not targeted on the specimen, but on a spot on the coverslip.

- **Focus distance:** the distance of focal points can be set to create a focus map, based on which the software calculates interpolated focus values (one step in distance means one FOV).

Sample detection

This panel offers options for threshold setting and selection of the area to be scanned.
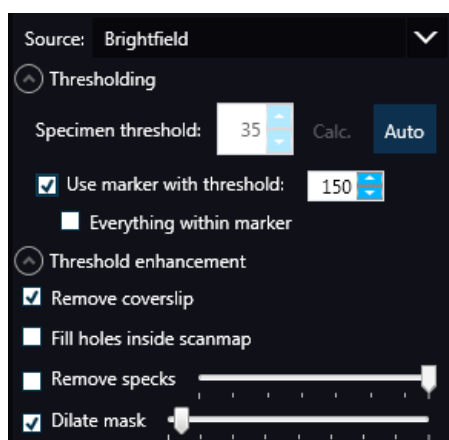
- **Source:** Brightfield preview – a normal type of illumination in the microscope for scanning the slide. This option is recommended when scanning in Brightfield mode.

- **Thresholding:** The manually specified value is used for thresholding during scanning. Preview image of the loaded slide is displayed in the Preview window, and when modifying any of the parameter values, it will have effect on the preview image. If the threshold value presented on the Microscope Controller preview window is modified, this value is automatically applied by the profile in the Profile Settings panel.

  ○ **Specimen threshold**

    This value is definable manually: This sets the sensitivity of the specimen detection. To set the desired threshold value manually, change the setting in the **Specimen threshold level** selection box in the range of 0-255. The default value is 220. Smaller values result in a more sensitive scanning, meaning that more areas will be digitized, however, this may also include specks or other imperfections of the slide. Greater values result in a less sensitive scanning, but it is possible that some of the smaller specimen areas will not be digitized.

  ○ **Auto Threshold**

    By clicking the specimen will automatically be detected in the preview image.

    > ⚠ **Caution!**
    >
    > Make sure that the detection result is acceptable for you. Otherwise, set the threshold value manually or append missing areas with the help of appropriate functions in the **Preview** window.

  ○ **Use marker with threshold**

    If the specimen cannot be identified as required for digitization, (for example, weak contrast), it can help to encircle completely the specimen with a black marker pen. You have to draw a full circle with the pen to enable the detection. Marker pen detection will make specimen detection easier by limiting the scanned area. If this option is selected, only the area encircled with a marker pen will be scanned. You can define the threshold level by changing the threshold value in the **Use marker with threshold** selection box in the range of 0-255. This sets the sensitivity of the specimen detection inside the marker area. To set the desired threshold value manually, change the setting in the **Specimen threshold** selection box in the range of 0-255. The default value is 105.

○ **Everything within marker**

To scan everything within the marked area, check the **Everything within marker** selection box.

Alternatively, click [icon] in the preview window to activate this function.

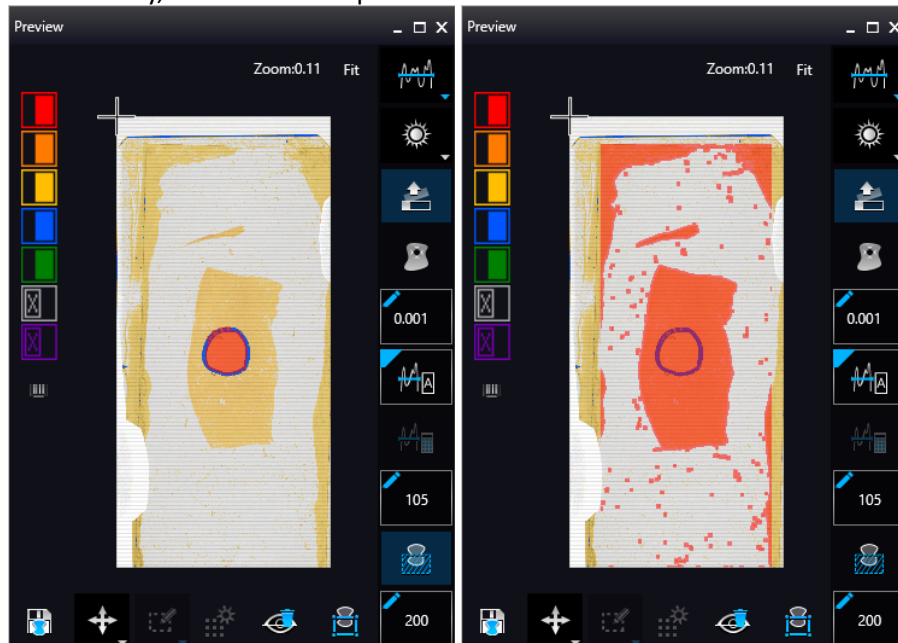**Figure 27 – Scan everything within the marked area (selected / not selected)**

• **Threshold enhancement**

○ **Remove coverslip –** If selected, the edges of the coverslip are not detected as specimen to be scanned. Depending on the threshold level, it is possible that the edges of the coverslip are detected as areas to be digitized.
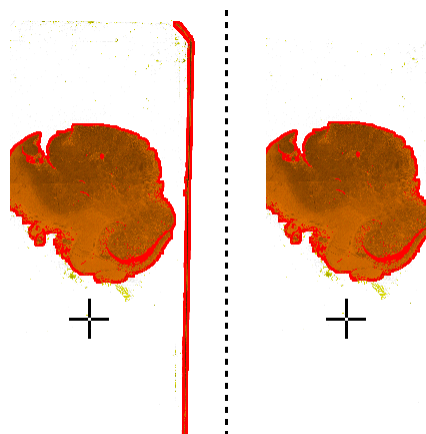

Figure 28 – Preview image with and without coverslip edge

> ⚠️ **Caution!**
>
> It requires lots of time (since it cannot find focus neither on the edges, nor on specks) to digitize coverslip edges. Select **Remove coverslip** function to eliminate those areas.

> ⚠️ **Warning**!
>
> Do **NOT** use **Remove coverslip** function on TMA slides! The layout of TMA slides may mislead the detecting algorithm.

- ○ **Fill holes inside the scanmap –** If selected, the holes inside the specimen will also be scanned. Often these holes contain small sample objects that otherwise would not be scanned.

- ○ **Remove specks –** If selected, the scanner does not digitize objects smaller than the predetermined size. This helps filtering specks and other uninteresting objects out. Set the maximum size of specks not to be digitized by adjusting the slider.

- ○ **Dilate mask –** Adjust the slider to enlarge or decrease the scanned area. The edge is extended discretely by 100 μm each time.

⊕ Scanmap

A previously saved scanmap can be loaded and applied.

- • **Use Threshold:** Preview settings defined in the Microscope Controller Panel / Preview window are applied as the basis of scanmap.

- • **Load scanmap:** This function is useful if the slides contain areas of interest that cannot be detected by the preview camera. This function along with saving profiles helps to scan all slides with the most adequate settings.

- • To load a mask, click **Load**. It will display a drop-down menu. Select whether to load preview mask or TMA grid.

- • **Custom scanmap:** The scanmap draws manually on the preview image is used for scanning.

Use selection box restriction

If selected, only the area inside the box is scanned. It is useful if you only want to scan the area that is equivalent with the size of the coverslip, so external specks will not be scanned.

Multilayer mode

- **Single layer mode:** Select **Single layer** option if you want to scan a single layer of the slide.

- **Extended focus:** By choosing the **Extended focus** option, the software selects the sharpest image from each focus level for each image field, and combines them into one single image. Therefore those parts that were blurry in auto/manual focus will also be sharp. This method guarantees the maximum depth of sharpness, however, it reduces digitization speed.
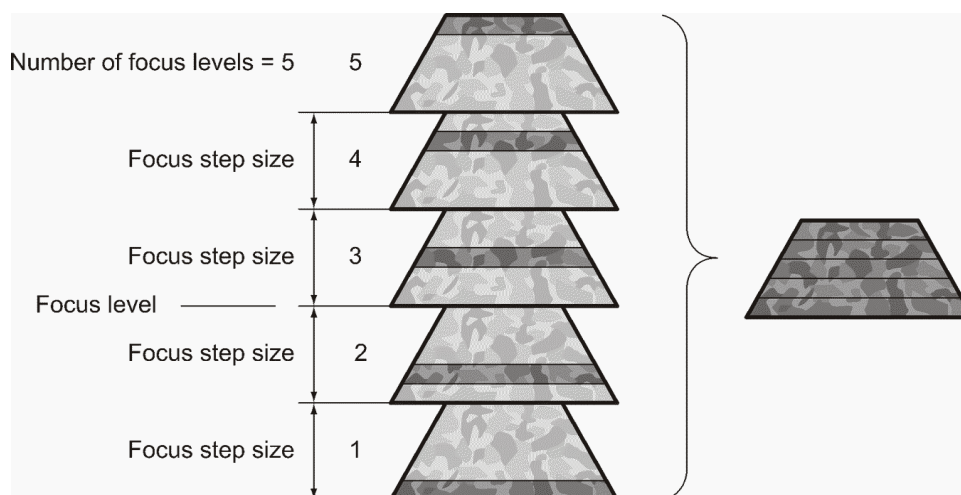

Figure 29 – Focus level and step size

- **Z-stack scan mode:** Both for Extended focus and **Z-Stack** scanning methods, you can determine the desired number of focus levels with the **Levels** selection box. The current focus value is the starting point. If you set *5* for example, one current, two focus levels above and below FOVs are captured. Therefore, it is advised to choose an odd number for the focus levels. Separate layers are created and stored this way, and can be opened with Pannoramic Viewer. The Z-stack slide contains the extended focus layer.
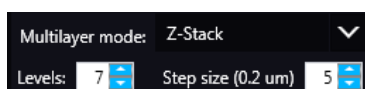
Step size determines the spacing between focus levels. One step is around 0.2 μm.

Default settings are: **Levels**: 7; **Step size**: 5.

⚙ **Scanning Settings**

Includes the **Stop and Go** scanning mode.

Use Color matrix: Select an applicable preset color profile to be used for Brightfield scanning from the drop-down menu. Settings according to the profile has effect on the color set, color temperature, and gamma value of the scanned image.

Separate color matrix can be defined and used for each profile and scanning.

Preset profiles are the following:

- **Calibrated Linear**: Color scheme is defined by calibration (Gamma value is not included)

- **sRGB standard**: Color scheme is defined by calibration (standard Gamma value -2.2- is applied)

- **sRGB standard Microscope Like**: Color scheme is defined by calibration (standard Gamma value / White Point: D50)

- **Legacy**: a Color scheme calibrated for the previous software version is applied

- **Custom:** Color profile set in Service is applicable if needed.

🖼 **Image settings**

You can choose the following predefined image formats (and a subsequent custom option) from the **Image Quality** drop-down list in the **Image Encoding** section of the window:

- Excellent (JPEG: 90)

- Good (JPEG: 80)

- Medium (JPEG: 60)

- Low (JPEG: 30)

- Custom...

    If choosing this option, the Image compression setting is displayed on which you can select from different image formats. Accordingly, further adjustments can be made, except if PNG or BMP image format is selected.
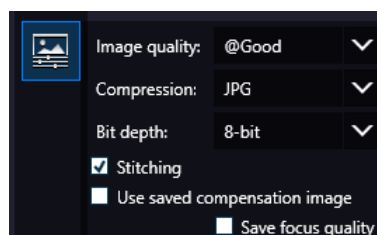

Figure 30 – Image encoding window

- **JPEG** – The quality can be adjusted with the **JPEG quality factor** slider. Greater values result in better quality, but larger files. The default setting is 80%.

- **PNG** – Lossless compressed image format.

- **BMP** – Lossless uncompressed image format, but larger files due to less effective compression. It is a raw data file.

- **Stitching:** Use Stitching function enables the seamless stitching of digital tiles.

- **Use save compensation image:** The **Use saved compensation image** function enables the use of previously recorded reference images for all slides. Use this function when the slide is completely covered by the specimen (for example, blood smear) thereby making compensation image capture impossible. For such slides, use an empty slide with coverslip to grab a compensation image. If you select this, the software will not create new compensation images for each slide. Compensation images are reference images used for white balancing and shading.

- **Save focus quality**: The measurement of focus quality (correctness of average focus on layers) is saved. This option is inactive when **Extended focus** option is activated.

- **Save image in original Bayer size** function is only available if pco.edge 4.2 camera is installed on the scanner. It is not accessible for configurations installed with Point Grey Grasshopper 3 camera. Image fields will be saved with native pixel resolution. If this function is disabled, you will work with reduced pixel resolution. These resolution differences are not really visible with the human eye, what is important in this feature is the resulting smaller file size.

5.1.2   Microscope Control Panel

The Microscope Control panel includes the main control features of the microscope.

5.1.2.1   ⊚ Preview Window

The Preview panel offers options for threshold setting and selection of the area to be scanned.
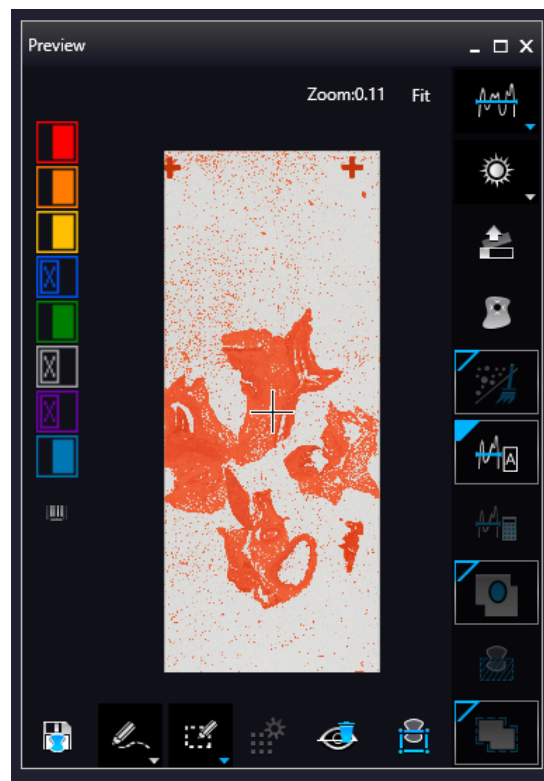

Figure 31 – Preview panel

Threshold options

This section is located at right side of the preview window. **Scanmap Source** is designed to set which type of scanmap is used on the preview image. This scanmap is displayed automatically in the **Profile Scanmap Source** setting. Selectable scanmap types are the following:

- Use threshold ⎇

- Load previously saved scanmap from file 📁

- Custom scan map 🔍

Preview Type setting determines the type of illumination. In Brightfield mode, the following preview types are available:

- **Brightfield preview** ⚙ – a normal type of illumination in the microscope for scanning the slide. This option is recommended when scanning in Brightfield mode.

Remove Coverslip ⏏ – If selected, the edges of the coverslip are not detected as specimen to be scanned. Depending on the threshold level, it is possible that the edges of the coverslip are detected as areas to be digitized.
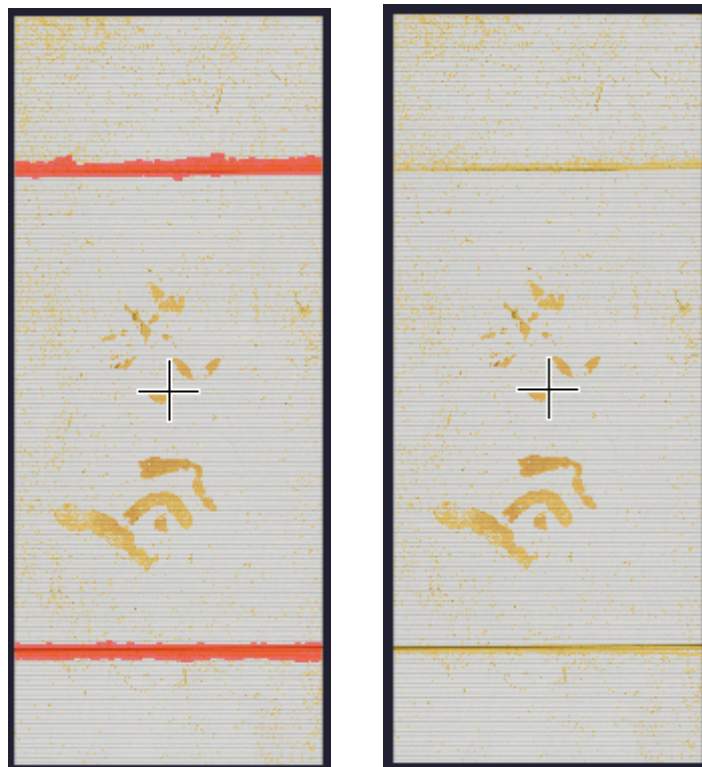

Figure 32 – Preview image with and without coverslip edge

⚠ **Caution!**

It requires lots of time (since it cannot find focus neither on the edges, nor on specks) to digitize coverslip edges. Select **Remove coverslip** function to eliminate those areas.


> **Warning**!
>
> Do **NOT** use **Remove coverslip** function on TMA slides! The layout of TMA slides may mislead the detecting algorithm.

Fill holes inside scanmap : If selected, the holes inside the specimen will also be scanned. Often these holes contain small sample objects that otherwise would not be scanned.

Remove specks : If selected, the scanner does not digitize objects smaller than the predetermined size. This helps filtering specks and other uninteresting objects out.

Set the maximum volume of specks manually in the setting window .

Threshold settings have two different statuses:

- **Auto threshold** : It automatically detects the specimen in the preview image.

> **Caution!**
>
> Make sure that the detection result is acceptable for you. Otherwise set the threshold value manually.

Make sure that the detection result is acceptable for you. Otherwise set the threshold value manually.

- **Manual threshold** : Set threshold value manually, or calculate value on the actual

  preview with **Calculate Threshold** function which is active only if setting the value manually. This sets the sensitivity of the specimen detection. To set the desired threshold value manually, change the setting in the **Specimen threshold** selection box in the range of 0-255. The default value is 212. Smaller values result in a more sensitive scanning, meaning that more areas will be digitized, however, this may also include specks or other imperfections of the slide. Greater values result in a less sensitive scanning, but it is possible that some of the smaller specimen areas will not be digitized.

Use marker with threshold **:** If the specimen cannot be identified as required for digitization, (for example, weak contrast), it can help to encircle completely the specimen with a black marker pen. You have to draw a full circle with the pen to enable the detection. Marker pen detection will make specimen detection easier by limiting the scanned area.

Manual setting of **Use marker with threshold** **:** If this option is selected, only the area encircled with a marker pen will be scanned. You can define the threshold level by changing the threshold value in the **Marker threshold level** selection box in the range of 0-255. This sets the sensitivity of the specimen detection inside the marker area. To set the desired threshold value manually, change the setting in the **Threshold** selection box in the range of 0-255. The default value is 212.

Everything within marker **:** This function is available only to select if the **Use marker with threshold** function is activated. To scan everything within the marked area, check the **Everything within marker** selection box.
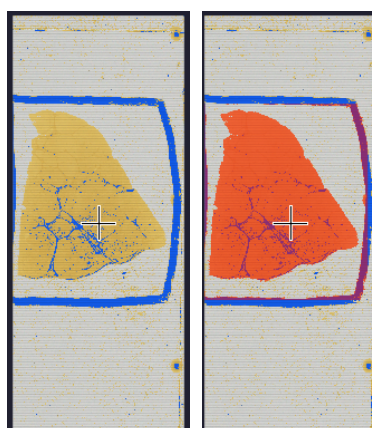

**Figure 33 – Scan everything within the marker selected / not selected**

Dilate mask **:** Adjust the slider to enlarge or decrease the scanned area. The edge is extended discretely by entering a new value each time. To switch to manual mode click the top left corner of the button and the required value can be entered in the box .

Use selection box restriction **:** If selected, only the area inside the box is scanned. It is useful if you only want to scan the area that is equivalent with the size of the coverslip, so external specks will not be scanned.

Preview Toolbar

This section is located at the bottom of the preview image.

- **Save scanmap** – Click this icon to save the currently selected areas to scanmap.

- **Position** : To position the specimen holder that holds the current slide, click **Position sample** and then click on the area of interest on the preview image. This will define the Live image that you can access from the **Live view** .

- **Union** : Add the selected areas to the preview. Mark area for scanning based on the shape selected in the Preview Toolbar. This area will not be scanned.

- **Erase** : Delete selected areas from the preview image. Deselection is made based on the selected shape from the Preview Toolbar. This area will not be scanned.
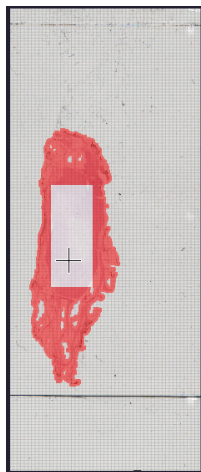

Figure 34 – Not scanned selection

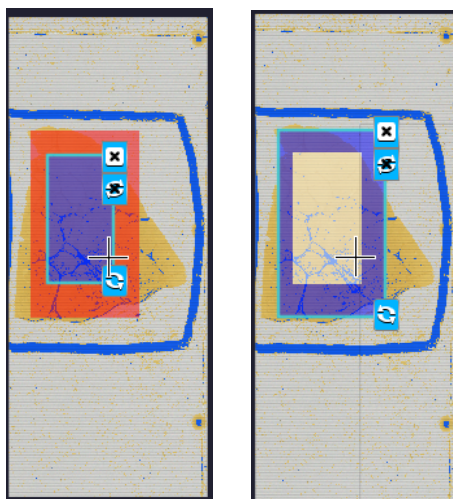- **Edit** : The size of selected area (positive or negative) can be modified at the borderlines or corners.

Figure 35 – Edited negative and positive selection masks.

- **Crop** **:** Crop the area of the selected area.

- **Place Circle** **:** You can select the area to be scanned with the different tools on the **Preview toolbar.**

- **Rectangle tool** : Draw rectangle shaped area.

- **Ellipse tool** : Draw ellipse selection.

- **Polygon tool** : Area selection by defining a polygon shape. Finish area selection by double-clicking with the mouse at or near the starting point.

- **TMA tool** **:** TMA tool is used for selecting TMA spots on TMA slides. You can set the selection settings on the following four tabs:

- **TMA Grid Setting** **:** This function is enabled only if a TMA grid has already been placed on the preview image. Set grid parameters (row and column numbers) and spot shape (round or rectangle).

Figure 36 – TMA tool: Grid selector

Delete TMA grid by clicking the **X** at the top right corner, and rotate grid by clicking the icon at the bottom right corner. Enter the number of rows and columns in the **Rows** and **Columns** fields or select the appropriate values with the arrows.

Enter the distance between two spots on the X and the Y axis in μm (in the **Spot distance X (μm)** and **Spot distance Y (μm)** fields or select the appropriate values with the arrows. Enter the average diameter of the spots in the **Diameter of spots (μm)** field or select the appropriate value with the arrows.

For research and education uses only, not for use in diagnostic procedures.

5.1.2.2   Preview area scanned by preview camera

The preview image shows the area that will be digitized with a red and/or an orange mask. If the **Use selection box** is enabled, and the crop function is selected, a black frame displayed in the image.
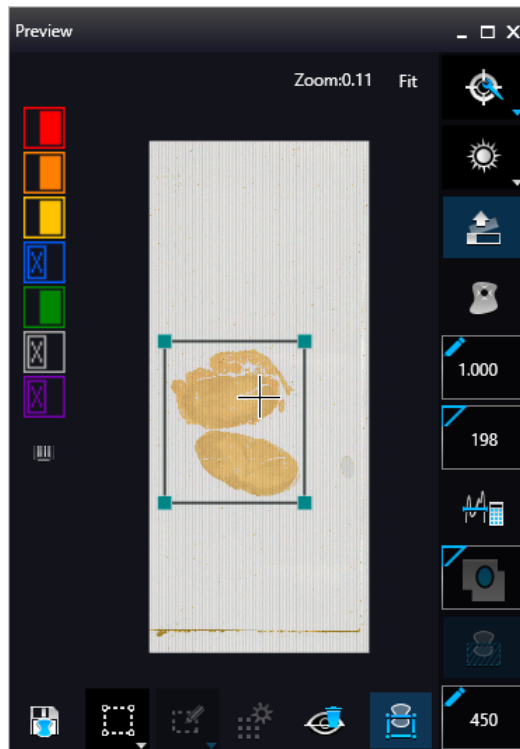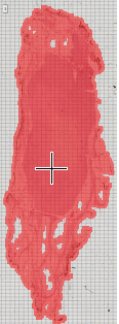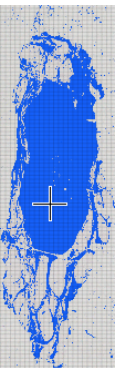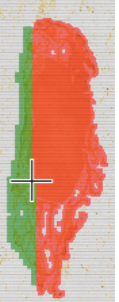
Figure 37 –   Preview image

The following four colors are used in the preview image to demonstrate digitization and selection settings:

Figure 38 – Colors in preview image and Label area switching button

Areas that will be scanned

These areas are digitized on the basis of user settings (for example, **Fill holes in scan area**) and not detected by the selected threshold value.

Detected sample areas that will be scanned

These areas are digitized. They are detected on the basis of the selected threshold value.

Detected sample areas that will not be scanned

These areas are not digitized on the basis of their position (for example, they are outside the selection box). They are detected by the selected threshold value.

Marker

If **Scan area inside the marker** is selected in the Preview panel, the preview camera detects the marker pen. The inside areas appear in red color and will be digitized.

Areas scanned in

This color is only displayed during scanning.

Hide all layers

All the layers will be hidden / existing but not visible.

Separate scanning

Discrete sample areas will be scanned separately as individual slides. If there are two samples on a slide, two separate virtual slides will be created.

Click the ▥ button to display barcode label area. To rotate the label area by 180°, click ↻.

Figure 39 – Barcode label area window

5.1.2.3   Focus Indicator

Click 🔬 to activate the **Focus indicator** function. Search for the sharpest focus on the current field of view automatically by clicking ⊡ - **Auto focus** button.

To define focus limits, select **Limit the range**. Set the upper and lower limits by selecting the desired value in the selection boxes. By using **Set +-Range**, the **Lower limit** value is set to -32 of the current focus value and the **Upper limit** value is set to +32 of the current focus value. This is useful if **Auto Focus** does not find the focus on the specimen, but on the coverslip. Also, this makes the focusing sequence faster.

For manual focusing define the manual focus step size by adjusting the **Step size** slider and use the two arrow buttons to focus manually. This is useful when you are interested in an area that remained blurry with **Auto Focus** (for example, the specimen is thicker in that area). This does not influence the way slides are digitized in Auto focus mode.

Figure 40 – Focus indicator panel

5.1.2.4   Exposure Indicator

Activate Exposure indicator function by clicking ▨. Click ▦ to calculate exposure time for the actual Live image. In Brightfield mode there is no option for manually setting the values. Exposure time can be displayed in microsecond, millisecond, and also in second.
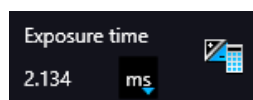
Figure 41 – Window of Exposure Indicator in Brightfield scan mode

For research and education uses only, not for use in diagnostic procedures.

5.1.2.5   Image Intensity

Click ![icon] to open Image intensity settings window. An **image** is defined as a two-dimensional function f(x, y) the amplitude of at any pair of coordinates (x, y) is called the **intensity** or gray level of the **image** at that point. The pixel intensity at the coordinate pointing at with the cursor is displayed in the Image Intensity window.
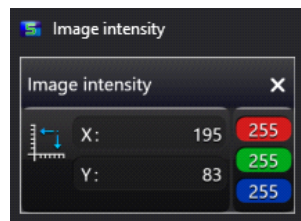
Figure 42 – Window of Image Intensity

5.1.2.6   Stage positioning

Click ![icon] to activate **Stage Positioning Tool** to navigate on the slide, so to perform microscope movements on the Live image with this control function. The number at the center of the controller defines the step size by which the stage is moved. Value can be entered by hand or with the slider.
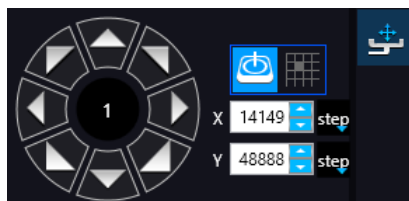
Figure 43 – Stage positioning tool

This tool offers easy and punctual slide navigation in distinctive steps. Set step size (FoV) – **1**, **½**, or **¼** to determine the size of movement in proportion of one FOV. Click an arrow to move to the required direction with the set step size.

5.1.2.7 Quick preview functions

Use **Ctrl+mouse wheel** to zoom in or out to the preview area or the Live image.

A quick preview function tool pops up by right-clicking to the preview area. It allows a quick access to main preview-related functions:

- Drag

- Draw

- Erase

- Retake previews

- Settings
    - Toggle deletion always on top
    - Reset zoom
    - Window auto-size
    - Clean scanmap
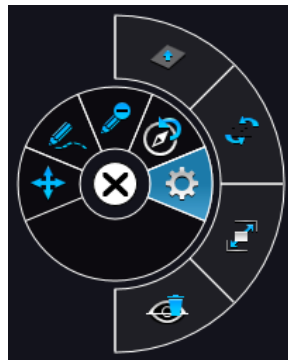
Figure 44 – Quick toolbar

5.1.3 Quick microscope functions

A quick microscope functions tool can be displayed upon right-clicking on the background of the main window (outside the preview window). It includes quick-access-buttons to main functions:

Figure 45 – Quick toolset for microscope

For research and education uses only, not for use in diagnostic procedures.

- **Focus**

Figure 46 – Focus

- ○ Autofocus

- ○ Set coarse focus point

- ○ Set interpolated focus point 1

- ○ Set interpolated focus point 2

- ○ Set interpolated focus point 3

- **Live tools**

- ○ Fast mode

- ○ Screenshot

- ○ Magnifier

- ○ Ruler

- ○ Image processor plugins

- ○ Add scanmap

- **Auto exposure**

For research and education uses only, not for use in diagnostic procedures.

5.2   Fluorescence Microscope Mode

Fluorescence Microscope View consist of three different sections:

- **Profile Settings panel**: Profile settings containing parameters used for scanning.

- **Microscope Control Panel**: Live microscope movement functions

- **Main functions**: Located at below the window header, these functions are identical in both modes.

5.2.1   Profile Settings panel

Set Parameters to auto

Every parameter is set automatically during scanning.

Filter Settings

Compact table view of microscope filters used for Fluorescence scanning.

Legend of the table:

- **A**: Actually used filter

- **Use**: Filters used for viewing Live image and for scanning.

- **Auto Exp**: When activated, the exposure time of the selected filter is calculated automatically during scanning.

- **Exp. Time**: If **Auto Exp** is inactive, exposure time of each filter can be defined manually. Values such as **Calc Exposure time** in Microscope Control panel and those that are specified manually are loaded in these fields. Value units can be the following: microsec, millisec, sec.

- **Exc %**: The given value specifies the excitation intensity in percentage of the illumination unit.

- **DG** (Digital Gain): This is camera-dependent, with Point Grey Grasshopper 3 the index value ranges from 0 to 4, and with pco.edge 4.2 from 0 to 6. Give **Index** value by entering it in the box or by selecting the value with the arrow buttons. If you raise the **Index** value, the exposure time is halved by every step, and the image becomes noisier. To move between the filters, click **Previous Filter** or **Next Filter**.

- **TW %** : You can set the **Exposure time weight (%)** here (displayed under **TW** in the **Filter Selection** frame).

- **F** : Focus channel under the scanning

- **S** : The selected channel will be the stitching channel under the scanning.

- **E** : Multilayer option: Select Extended focus or Z-stack scanning for the required channels in accordance with the scanning mode set in the profile.

Figure 47 – Filter table

Objective Settings

This field displays the objective used for scanning. Objective type: 20x; 40x

Focus

Focus settings for the scanning. Focus modes can be the following:

- **Coarse focus:** By selecting the **Use manual focus** box, you can preset the coarse focus value. By activating the **Coarse focus** function, the lower limit value is set to -64, and the upper limit value is set to +64 of the current focus value.

> ⊘ **Note:**
>
> Before entering coarse focus value, turn live image on and set a suitable focus position.

You can accept the current Z-value by clicking **Assign Current**.

- **Interpolated focus:** If you select the **Interpolated focus** option, the coarse focus level is calculated from three different focus points of the specimen. Carry out a manual focusing sequence in the preview image on those three points. Set the correct focus value of the desired preview position by clicking **Set** in the table of interpolated focus. If you want to go back to any of the previously set focus points, click the appropriate **Goto** button.

  To delete the stored values, click the other proper focus point or turn off the function. The software calculates a coarse focus level from the three values determined this way as a basis for the fine focusing routine. You can only define the start parameter here. This will, however,

speed up the focusing process. If you deselect the **Use fine focus** checkbox, the software immediately starts scanning on the basis of the interpolated values, without pre-fine-focusing.
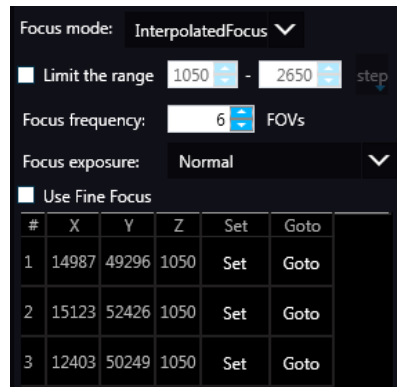
- **Auto focus:** Focus value is set automatically to match sample characteristics.

- **Limit the range**: You can define focus limits by selecting **Limit the range** for focusing. You can set the upper and lower limits by selecting the desired value in the selection boxes. This is useful if focusing was not targeted on the specimen, but on a spot on the coverslip.

- **Focus frequency :** This value determines how many field of views will pass before the sample is refocused. This value should be selected depending on the unevenness and size of the specimen.

- **Focus Exposure:** The exposure time during focusing, it can have three states:

  ◦ Short

  ◦ Normal

  ◦ Long

Sample detection

The Sample detection panel offers options for setting threshold and the selection of scannable areas.

- **Source**

  ◦ **Brightfield preview** – a normal type of illumination in the microscope for scanning the slide. This option is recommended when scanning in Brightfield mode.

  ◦ **Fluorescent PreScan -** creates a fast FL preview image that is displayed in the Profile Wizard window. Scanning process takes a couple of minutes only, and it cannot be interrupted.

  ◦ **Fluorescent PreScan (HR) -** creates a High-Resolution fast FL preview image of every FOV within the selected area (in single layer DAPI, with an illumination time of 20ms) that is displayed in the Profile Wizard window. Scanning process takes a couple of minutes only, and cannot be interrupted.

- **Fluorescent PreScan (+HR) -** creates a High-Resolution fast FL preview image of every FOV within the selected area (in single layer DAPI, with an illumination time of 20ms) that is displayed in the Profile Wizard window. Scanning process is initiated with a simple PreScan, the located sample with auto threshold is selected, and finally, an HR PreScan is launched.

- **Thresholding**

  The manually set threshold value is applied during scanning. The preview of the loaded slide is displayed in the Preview window. The set value has effect on the preview image. If threshold value is modified in the Microscope Controller preview window, that value is automatically applied in the profile in the Profile Settings panel.

  - **Specimen threshold**

  - **Manually set value:** This sets the sensitivity of the specimen detection. To set the desired threshold value manually, change the setting in the **Specimen threshold level** selection box in the range of 0-255. The default value is 212. Smaller values result in a more sensitive scanning, meaning that more areas will be digitized, however, this may also include specks or other imperfections of the slide. Greater values result in a less sensitive scanning, but it is possible that some of the smaller specimen areas will not be digitized.

  - **Auto Threshold:** By clicking [Auto] the specimen will automatically be detected in the preview image.

  > ⚠️ **Caution!**
  >
  > Make sure that the detection result is acceptable for you. Otherwise, set the threshold value manually or append missing areas with the help of appropriate functions in the **Preview** window.

  - **Use marker with threshold**

    If the specimen cannot be identified as required for digitization, (for example, weak contrast), it can help to encircle completely the specimen with a black marker pen. You have to draw a full circle with the pen to enable the detection. Marker pen detection will make specimen detection easier by limiting the scanned area. If this option is selected, only the area encircled with a marker pen will be scanned. You can define the threshold level by changing the threshold value in the **Use marker with threshold** selection box in the range of 0-255. This sets the sensitivity of the specimen detection inside the marker area. To set the desired threshold value manually, change the setting in the **Specimen threshold** selection box in the range of 0-255. The default value is 105.

  - **Everything within marker**

    To scan everything within the marked area, check the **Everything within marker** selection box.

Alternatively, click in the preview window to activate this function.
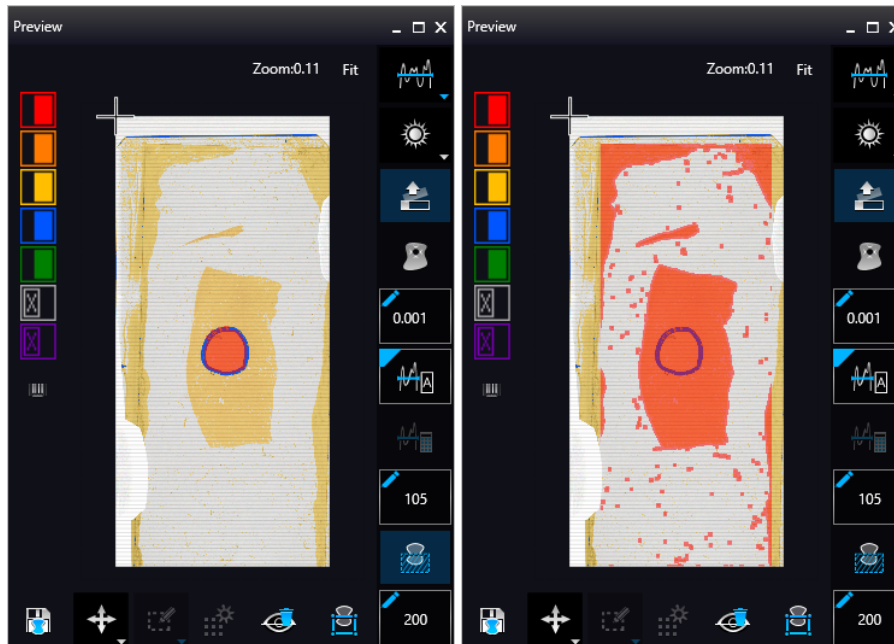
Figure 48 – Scan everything within the marked area (selected / not selected)

• **Threshold enhancement**

  ◦ **Remove coverslip –** If selected, the edges of the coverslip are not detected as specimen to be scanned. Depending on the threshold level, it is possible that the edges of the coverslip are detected as areas to be digitized.
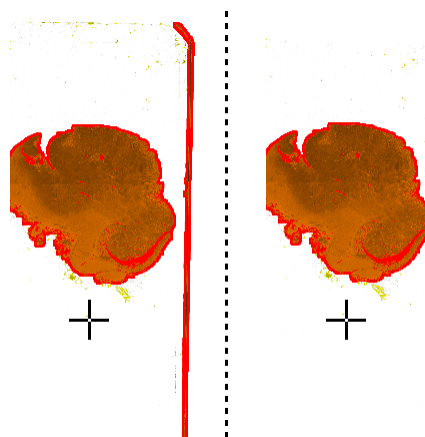
Figure 49 – Preview image with and without coverslip edge

For research and education uses only, not for use in diagnostic procedures.

> ⚠️ **Caution!**
>
> It requires lots of time (since it cannot find focus neither on the edges, nor on specks) to digitize coverslip edges. Select **Remove coverslip** function to eliminate those areas.

> ⚠️ **Warning**!
>
> Do **not** use **Remove coverslip** function on TMA slides! The layout of TMA slides may mislead the detecting algorithm.

- ○ **Fill holes inside the scanmap –** If selected, the holes inside the specimen will also be scanned. Often these holes contain small sample objects that otherwise would not be scanned.

- ○ **Remove specks –** If selected, the scanner does not digitize objects smaller than the predetermined size. This helps filtering specks and other uninteresting objects out. Set the maximum size of specks not to be digitized by adjusting the **Speck size** slider.

- ○ **Dilate mask –** Adjust the slider to enlarge or decrease the scanned area. The edge is extended discretely by 100 μm each time.

Scanmap option

The previously saved scanmap can be loaded for use.

- • **Use Threshold:** Preview settings set in Microscope Controller Panel / Preview window are applied to create a scanmap.

- • **Loaded scanmap:** This function is useful if the slides contain areas of interest that cannot be detected by the preview camera. This function along with saving profiles helps to scan all slides with the most adequate settings.

  To load a mask, click 📁 first, then browse for the scanmap to be loaded.

- • **Custom scanmap:** The scanmap drawn on the preview image is used for scanning.

- • **Use selection box restriction**

  If selected, only the area inside the box is scanned. It is useful if you only want to scan the area that is equivalent with the size of the coverslip, so external specks will not be scanned.

Multilayer mode

- **Single layer mode:** If none of the filters are selected in the Filters table for multilayer scanning, this mode is active and can be selected, and Multilayer mode turns inactive.

- **Extended focus:** By choosing the **Extended focus** option, the software selects the sharpest image from each focus level for each image field, and combines them into one single image. Therefore those parts that were blurry in auto/manual focus will also be sharp. This method guarantees the maximum depth of sharpness, however, it reduces digitization speed.

- **Z-stack scan mode:** Both for Extended focus and **Z-Stack** scanning methods, you can determine the desired number of focus levels with the **Focus levels** selection box. The current focus value is the starting point. If you set *5* for example, one current, two focus levels above and below FOVs are captured. Therefore, it is advised to choose an odd number for the focus levels. Separate layers are created and stored this way, and can be opened with Pannoramic Viewer. The Z-stack slide contains the extended focus layer.
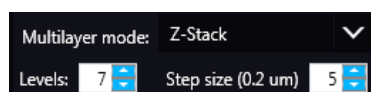
Focus step size determines the spacing between focus levels. One step is around 0.2 μm. Default settings are: **Number of focus levels**: 7; **Focus step size**: 5.

Figure 50 – Focus level and step size

Scan settings

- **Change filters after whole slide scanning**

  To optimize speed, filter change should take place after the digitization of the slide with one channel.

- **Change filters at each field-of-view**

  Filters should be changed for each field of view if very precise colocalization is required, but consider that this option is more time consuming.

> **⊘ Note:**
>
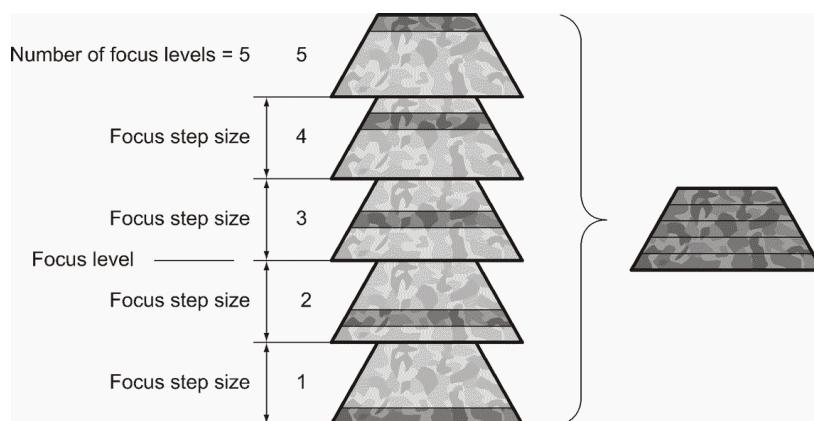> Make sure to select the **Change filters at each field of view** option if using multi band filter for multi-channel digitization.

> **⚠ Caution!**
>
> If the specimen area is barely visible, it is possible that the software will analyze that area as an empty one.

- **Multiple shots**

- **Maximum exposure time** – A limit value can be set to determine that the exposure time cannot be longer than the time of focusing and calculation of exposure. This is necessary because the exposure time would be too long when taking image of an empty field of view. Default value is 2000 ms.

Image Correction settings

- **Use Flatfield correction –** If this function is active the scanned slide compensates itself with a special method (light intensity statistical data, not to modify the average intensity of the slide. Quantitative measurements can be made on the slide. The following three options can be selected from the drop-down list:

  ◦ **Intensity statistics** – legacy compensation algorithm

  ◦ **Reduce intensity** – pixel-level analyzation of the acquired image during which the background of the image is reduced based on the comparison of the image recorded by the camera and the compensation image.

  ◦ **Intensity equalization** – pixel-level analyzation of the acquired image, a one-by-one type image compensation method is applied.

- **Extended focus** – Select the type of the extended focus algorithm used during scanning:

  ◦ **Legacy** – uses original extended focus algorithm, it is recommended for wide-field fluorescence scanning.

  ◦ **Maxima** – the final image is made up from all of the layers created, areas (pixel groups) with the highest intensity are combined together.

  ◦ **AVG** – an average of the layers is used for the final image.

○ **Max-AVG** – the final image is made up from all of the layers created, areas (pixel groups) with higher intensity than the average are combined together.

Image settings

Choose one of the following predefined image formats (and a subsequent custom option) from the **Image Quality** drop-down list:

• Excellent (JPEG: 90)

• Good (JPEG: 80)

• Medium (JPEG: 60)

• Low (JPEG: 30)

• Custom... – If choosing this option, the Image compression setting is displayed on which you can select from different image formats. Accordingly, further adjustments can be made, except if PNG or BMP image format is selected.

Figure 51 – Image encoding window

• **Compression**

○ **BMP** – Lossless uncompressed image format, but larger files due to less effective compression. It is a raw data file.

○ **PNG** – Lossless compressed image format.

○ **JPG** – The quality can be set in the value box. Greater values result in better quality, but larger files. The default setting is 80%.

• **Bit depth:** 8-bit

• **Stitching:** Use Stitching function enables the seamless stitching of digital tiles.

• **Sharpening**: Select this option to improve the visual appearance of digital slides.

5.2.2   Microscope Control Panel

5.2.2.1   Preview window

Click [icon] to open the Preview panel that offers options for threshold setting and selection of the area to be scanned.
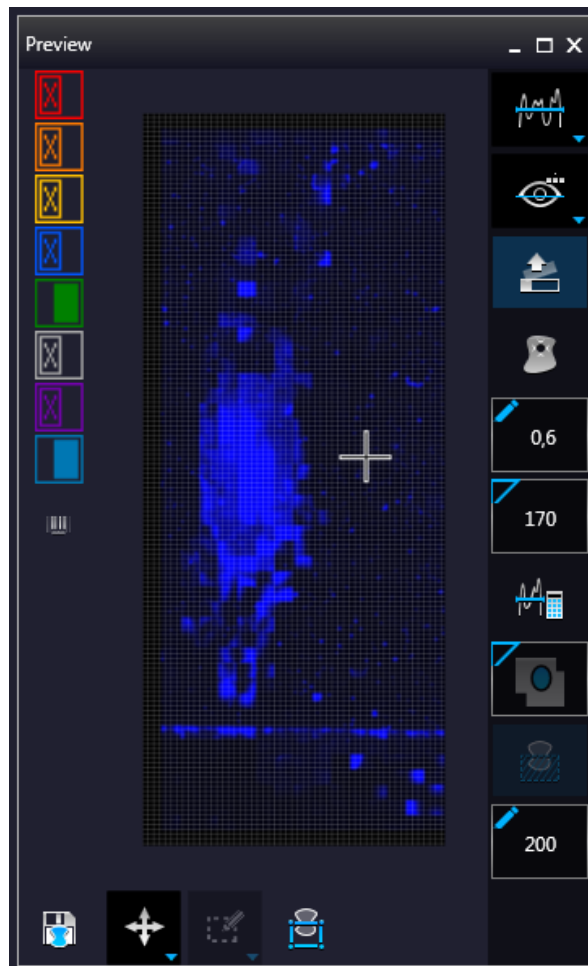
Figure 52 – Pre-scan preview image

Threshold options

This section is located at right side of the preview window. **Scanmap Source** is designed to set which type of scanmap is used on the preview image. This scanmap is displayed automatically in the **Profile Scanmap Source** setting. Selectable scanmap types are the following:

- Use threshold [icon]

- Load previously saved scanmap from file [icon]

- Custom scan map [icon]

Preview Types

In FL Microscope mode the following additional preview modes are available:

- **Brightfield preview** ☀ – a normal type of illumination for scanning. This option is recommended when scanning in Brightfield mode.

- **Darkfield preview** 🌙 – preview image is created with transmitted Darkfield illumination. Make sure to select this option in Fluorescent mode.

- **Pre-scan** 👁 – creates a fast FL preview image that is displayed in the Profile Wizard window. Scanning process takes a couple of minutes only, and it cannot be interrupted.

- **Pre-scan high-resolution** 👁 – creates a High-Resolution fast FL preview image of every FOV within the selected area (in single layer DAPI, with an illumination time of 20ms) that is displayed in the Profile Wizard window. Scanning process takes a couple of minutes only, and cannot be interrupted.

- **Pre-scan + high-resolution** 👁 – creates a High-Resolution fast FL preview image of every FOV within the selected area (in single layer DAPI, with an illumination time of 20ms) that is displayed in the Profile Wizard window. Scanning process is initiated with a simple PreScan, the located sample with auto threshold is selected, and finally, an HR PreScan is launched.

Remove coverslip ⏏

If selected, the edges of the coverslip are not detected as specimen to be scanned. Depending on the threshold level, it is possible that the edges of the coverslip are detected as areas to be digitized.
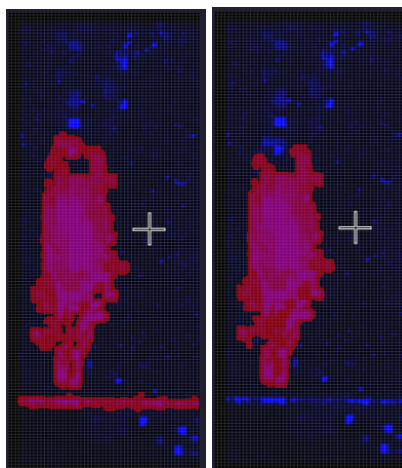
Figure 53 – Preview image with and without coverslip edge

> ⚠️ **Caution!**
>
> It requires lots of time (since it cannot find focus neither on the edges, nor on specks) to digitize coverslip edges. Select **Remove coverslip** function to eliminate those areas.

> ⚠️ **Warning**!
>
> Do **not** use **Remove coverslip** function on TMA slides! The layout of TMA slides may mislead the detecting algorithm.

Fill holes in scanmap

If selected, the holes inside the specimen will also be scanned. Often these holes contain small sample objects that otherwise would not be scanned.

Remove specks

If selected, the scanner does not digitize objects smaller than the predetermined size. This helps filtering specks and other uninteresting objects out.

Set the maximum volume of specks manually in the setting window.

Threshold

- **Auto threshold**

  Select this option to automatically detect the specimen in the preview image.

  > ⚠️ **Caution!**
  >
  > Make sure that the detection result is acceptable for you. Otherwise set the threshold value manually.

- **Manual threshold**

Set value manually or calculate value by using the **Calculate Threshold** function (can be activated only when manual thresholding is selected). This sets the sensitivity of the specimen detection. To set the desired threshold value manually, change the setting in the **Specimen threshold level** selection box in the range of 0-255. The default value is 212. Smaller values result in a more sensitive scanning, meaning that more areas will be digitized, however, this may also include specks or other imperfections of the slide. Greater values result in a less sensitive scanning, but it is possible that some of the smaller specimen areas will not be digitized.

Use marker with threshold

If the specimen cannot be identified as required for digitization, (for example, weak contrast), it can help to encircle completely the specimen with a black marker pen. You have to draw a full circle with the pen to enable the detection. Marker pen detection will make specimen detection easier by limiting the scanned area.

- Manual setting of **Use marker with threshold**

If this option is selected, only the area encircled with a marker pen will be scanned. You can define the threshold level by changing the threshold value in the **Marker threshold level** selection box in the range of 0-255. This sets the sensitivity of the specimen detection inside the marker area. To set the desired threshold value manually, change the setting in the **Threshold** selection box in the range of 0-255. The default value is 212.

Everything within marker

Available only if **Use marker with threshold** function is activated. To scan everything within the marked area, check the **Scan everything within marker** selection box.
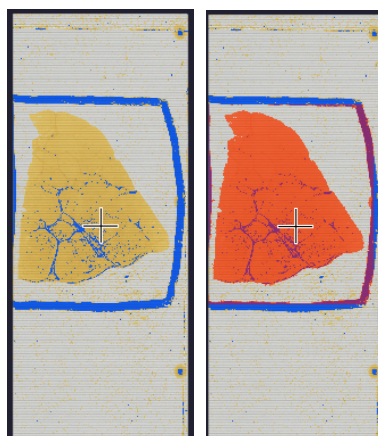

Figure 54 – Scan everything within the marker selected / not selected

Dilate mask

Adjust the slider to enlarge or decrease the scanned area. To switch to manual mode click the top left corner of the button and the required value can be entered in the box .

Preview Toolbar

This section is located at the bottom of the preview image.

- **Save scanmap**

    Click this icon to save the currently selected areas to scanmap.

- **Position**

    To position the specimen holder that holds the current slide, click **Position sample** and then click on the area of interest on the preview image. This will define the Live image that you can access from the **Live view** .

- **Union**

    Add the selected areas to the preview. Mark area for scanning based on the shape selected in the Preview Toolbar. This area will not be scanned.

- **Erase**

    Delete selected areas from the preview image. Deselection is made based on the selected shape from the Preview Toolbar. This area will not be scanned.

Figure 55 – Not scanned selection

- **Edit**

  The size of selected area (positive or negative) can be modified at the borderlines or corners.
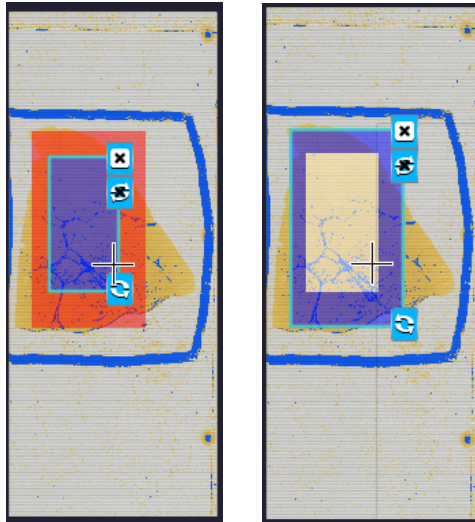
Figure 56 – Edited negative and positive selection masks

- **Crop**

  Crop the area of the selected area.

- **Place Circle**

  You can select the area to be scanned with the different tools on the **Preview toolbar.**

- **Rectangle tool**

  Draw rectangle shaped area.

- **Ellipse tool**

  Draw ellipse selection.

- **Polygon tool**

  Area selection by defining a polygon shape. Finish area selection by double-clicking with the mouse at or near the starting point.

- **TMA tool**

    TMA tool is used for selecting TMA spots on TMA slides. You can set the selection settings on the following four tabs:

- **TMA Grid Setting**

    This function is enabled only if a TMA grid has already been placed on the preview image. Set grid parameters (row and column numbers) and spot shape (round or rectangle).
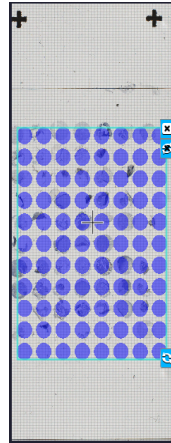

Figure 57 – TMA tool: Grid selector

Delete TMA grid by clicking the **X** at the top right corner, and rotate grid by clicking the icon at the bottom right corner. Enter the number of rows and columns in the **Rows** and **Columns** fields or select the appropriate values with the arrows.

Enter the distance between two spots on the X and the Y axis in µm in the **Core distance** fields or select the appropriate values with the arrows. Enter the average diameter of the spots in the **Core size** field or select the appropriate value with the arrows.

- **Clear scanmap**

    The entire scanmap area will be cleared and reset to default.

- **Use selection box restriction**

  The preview image shows the area that will be digitized with a red and/or an orange mask. If the **Use selection box** is enabled, and the crop function is selected, a black frame displayed in the image, and only the area inside the box is scanned. It is useful if you only want to scan the area that is equivalent with the size of the coverslip, so external specks will not be scanned.
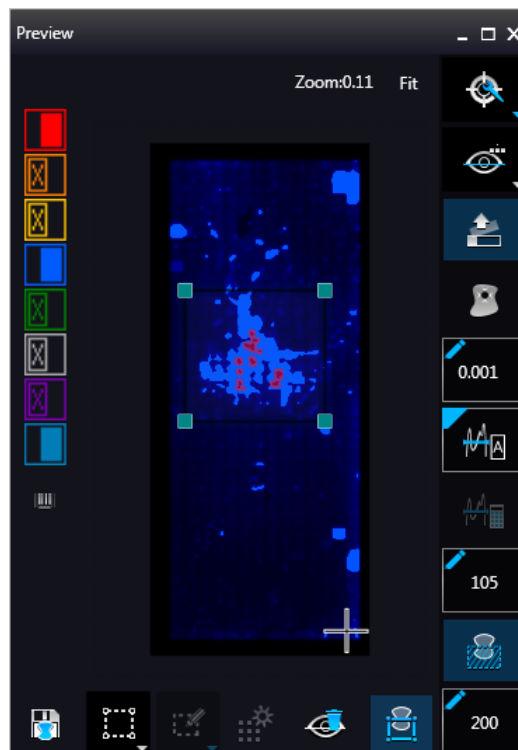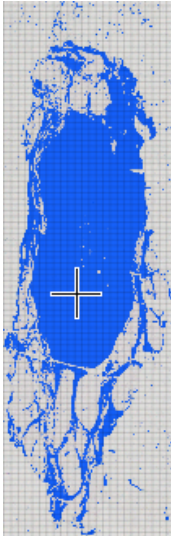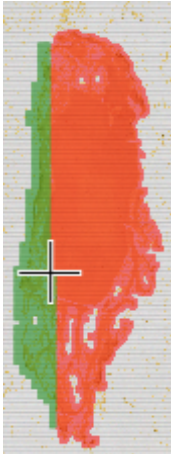

Figure 58 – Use selection box

For research and education uses only, not for use in diagnostic procedures.

The following colors are used in the preview image to demonstrate digitization and selection settings:

Figure 59 – Colors in preview image and Label area switching button

 Areas that will be scanned

These areas are digitized on the basis of user settings (for example, **Fill holes in scan area**) and not detected by the selected threshold value.

 Detected sample areas that will be scanned

These areas are digitized. They are detected on the basis of the selected threshold value.

 Detected sample areas that will not be scanned

These areas are not digitized on the basis of their position (for example, they are outside the selection box). They are detected by the selected threshold value.

 Marker

If **Scan area inside the marker** is selected in the Preview panel, the preview camera detects the marker pen. The inside areas appear in red color and will be digitized.

 Areas scanned in

This color is only displayed during scanning.

 Hide all layers

 Separate scanning

Discrete sample areas will be scanned separately as individual slides. If there are two samples on a slide, two separate virtual slides will be created.

 Show pre-scans pseudo-colorized

Click the button to display barcode label area. To rotate the label area by 180°, click .

Figure 60 – Barcode label area window

For research and education uses only, not for use in diagnostic procedures.

Figure 61 – Pseudo-colorized pre-scan image

5.2.2.2   Focus Indicator

Click ![icon] to activate the **Focus indicator** function. Search for the sharpest focus on the current field of view automatically by clicking ![icon] - **Auto focus** button.

To define focus limits, select **Limit the range**. Set the upper and lower limits by selecting the desired value in the selection boxes. By using **Set +-Range**, the **Lower limit** value is set to -32 of the current focus value and the **Upper limit** value is set to +32 of the current focus value. This is useful if **Auto Focus** does not find the focus on the specimen, but on the coverslip. Also, this makes the focusing sequence faster.

For manual focusing define the manual focus step size by adjusting the **Step size** slider and use the two arrow buttons to focus manually. This is useful when you are interested in an area that remained blurry with **Auto Focus** (for example, the specimen is thicker in that area). This does not influence the way slides are digitized in Auto focus mode.

Figure 62 – Focus indicator panel

For research and education uses only, not for use in diagnostic procedures.

5.2.2.3 Exposure Indicator

Exposure time for the live image can be calculated by using the **Calculated Exposure** function . It is available to switch between microsecond, millisecond, and second units for the reading of exposure time.
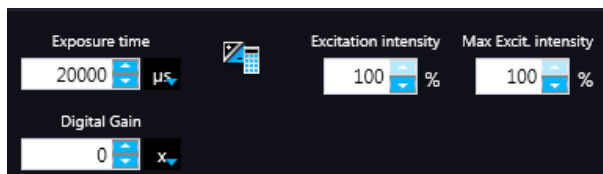
Figure 63 – Window of Exposure Indicator in Fluorescence scan mode

Select the types of filters you want to use during scanning. You can select the filter types and the number of channels to be used. The values in the **EXC** column define the excitation of the Lumencor illumination, in **TW** define exposure ti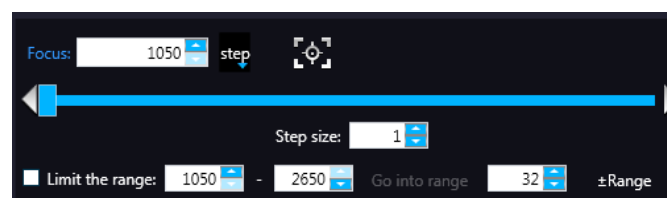me weight, and in the **DG** the amount of digital gain is displayed. It is also available for you to enable Extended Focus (**EF**) and/or Z-stack (**ZS**) functions in this field.

Exposure Settings

You can set the **Exposure time weight (%)** here (displayed under **TW** in the **Channel Settings** frame). Set Lumencor excitation intensity at the **Excitation intensity (%)** field. The defined value will be used as maximum when applying Auto Exposition time if you activate the **Max. excit. intensity** option.

> ⚠ **Caution!**
>
> To prevent over-exposure, the **Excitation intensity (%)** value can be modified by the software when calculating **Auto Exposure** time.

Image Intensity

Click to open Image intensity panel. An **image** is defined as a two-dimensional function f(x, y) the amplitude of at any pair of coordinates (x, y) is called the **intensity** or gray level of the **image** at that point. The pixel intensity at the coordinate pointing at with the cursor is displayed in the Image Intensity window.
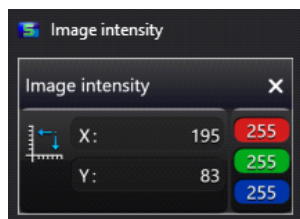
Figure 64 – Window of Image Intensity

5.2.2.4   Stage positioning

After clicking you can perform microscope movements on the Live image with this control function. The number at the center of the controller defines the step size by which the stage is moved. Value can be entered by hand or with the slider.
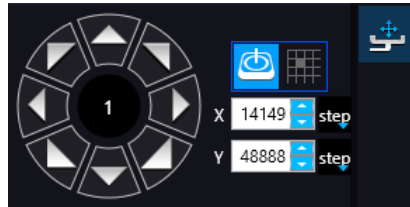
Figure 65 – Stage positioning panel

5.2.3   Quick preview functions

Use **Ctrl + mouse wheel** to zoom in or out to the preview area or the Live image.
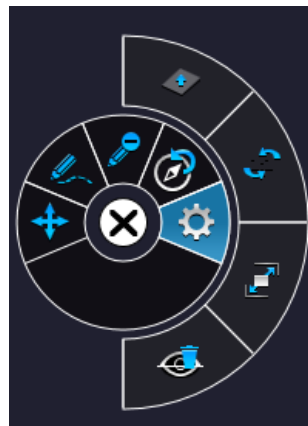
Figure 66 – Settings of preview quick toolbar

A **Quick Toolbar** pops up by right clicking any of the control menu items. It allows a quick access to main functions, and for FL these are the following:

- Drag

- Draw

- Erase

- Retake preview

- Settings

    ◦ Toggle delation always on top

    ◦ Reset Zoom

    ◦ Window auto-size

    ◦ Clear Scanmap

5.2.4   Quick Microscope functions

A quick microscope functions tool can be displayed upon right-clicking on the background of the main window (outside the preview window). It includes quick-access-buttons to main functions:

Figure 67 – Quick toolset for microscope

- **Focus**

Figure 68 – Focus

- ○   Autofocus
- ○   Set coarse focus point
- ○   Set interpolated focus point 1
- ○   Set interpolated focus point 2
- ○   Set interpolated focus point 3

- **Objective type**
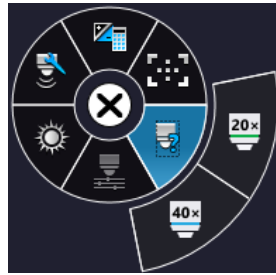
Figure 69 – Objective type

  - ○ 20x

  - ○ 40x

- **Filters**
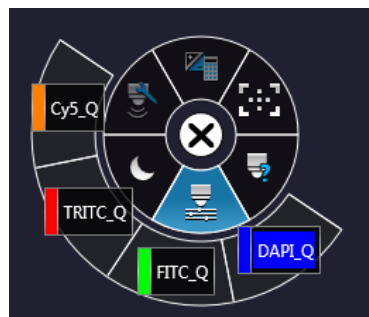
Figure 70 – Filters

- **Microscope mode**
  - ○ Brightfield
  - ○ Fluorescence

Figure 71 – Microscope mode

- **Live tools**
  - ◦ Fast mode
  - ◦ Screenshot
  - ◦ Magnifier
  - ◦ Ruler
  - ◦ Image processor plugins
  - ◦ Use pseudo-color
  - ◦ Add scanmap

Figure 72 – Live tools

- **Auto-exposure**

Figure 73 – Auto-exposure

5.2.5   Bottom menu bar of the main window

Profile History

This function can be activated by clicking [icon] in **Preferences**. During setting scanning profile all of the previously used parameter will be saved automatically.

Each modification in the profile creates a so-called Milestone unit. These stage units are linked in a row displayed at the bottom left corner of the main window.
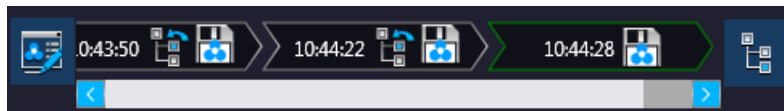
Figure 74 – Progress of the profile modification history

Once you are moving the mouse cursor over a unit all of the parameters will become visible in a pop-up window.
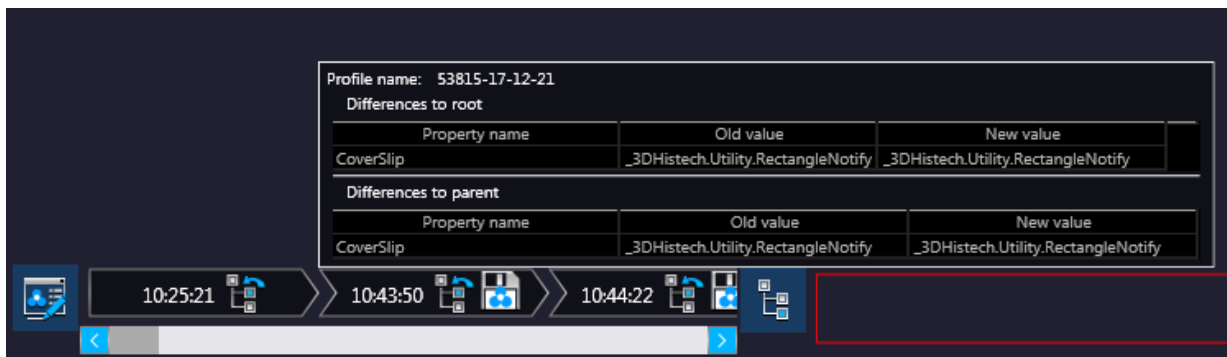
Figure 75 – Details of parameter modification

For the easier perspicuity, modifications can be observed in a pop-up window by clicking the [icon] button in which both the original and the modified values are presented.

Click [icon] to save parameters of the milestone unit into a new profile.

Click [icon] to restore a modification stage (load parameters of a milestone unit) if needed.

For research and education uses only, not for use in diagnostic procedures.

Figure 76 – Profile history tree

5.3   Planner mode

The whole scanning procedure can be planned in this mode. Assign profiles to slides, modify scan-maps without performing physical loading of slides. In Planner mode not only the scanning of the actually loaded magazine can be planned, but also profile assignment to slides of prepared virtual magazines is available to be performed. It is also possible to scan to different locations with each profile, and virtual slides can be created and stored in different folder or server.
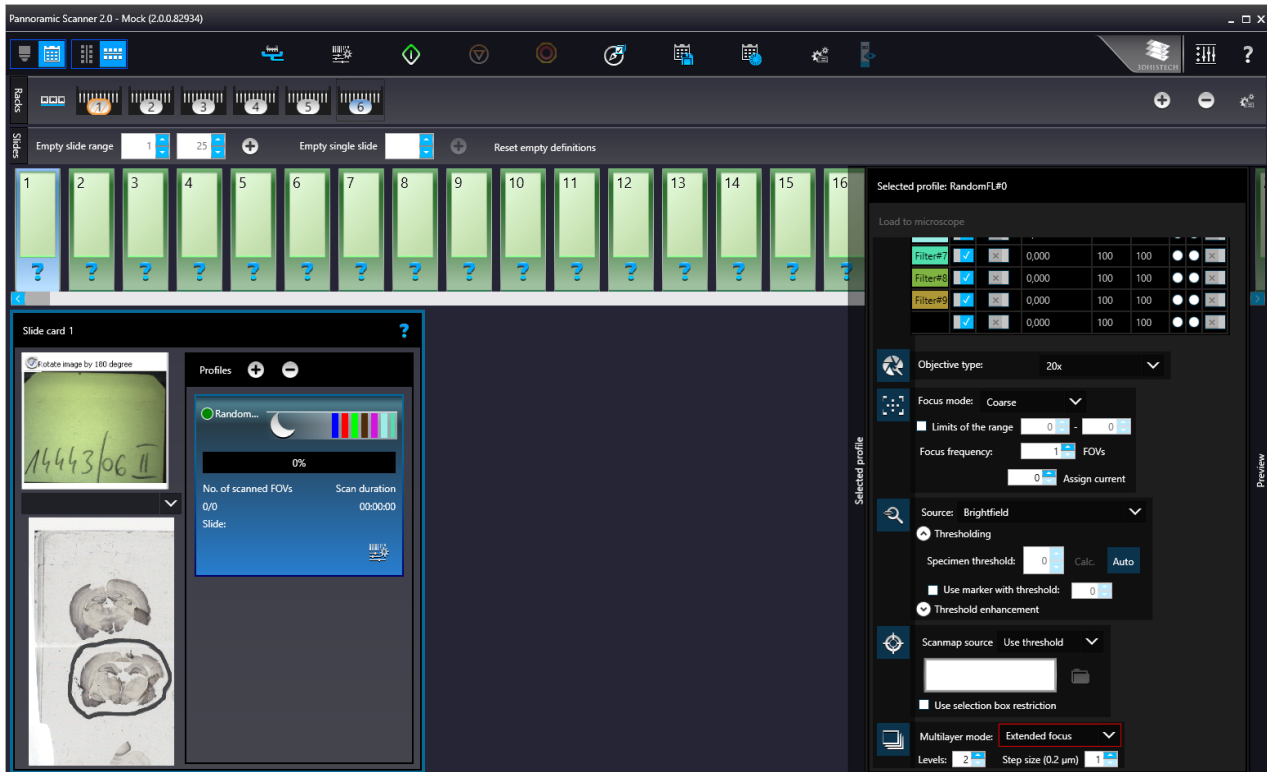
Planner module offers you three window layout types for planning scans, located under the **Preferences / Planner Layout Selector** menu:

- ▪   – Slide view with card

- ▪   – Table

- ▪   – Card collection

5.3.1   General functions at the main menu

Microscope/Planner selector

Horizontal/Vertical selector (Available in **Slide view with card** mode)

Stage Control – Available after initialization, upon clicking the slide icon or entering slide position number at the right side, the slide can be loaded.

Scan to – Available after loading procedure, unique settings can be defined even for each separate scanning.

Start scan – Scanning of the actually loaded slide, or if the planner has been defined, the whole scanning process can be initiated.

Stop scan

Stop actual process

Take all previews – Preview images of all the slides in the magazine are taken automatically. Select the required preview type from the following: Brightfield, Pre-scan, Pre-scan high-resolution, Pre-scan + high-resolution.
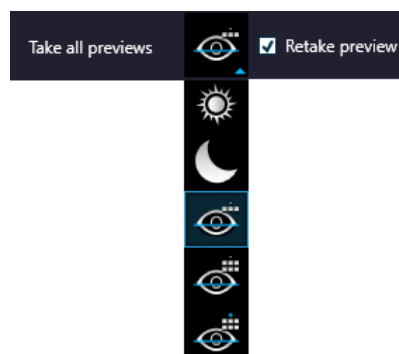
Figure 77 – Take all preview selector

Save planner – A scanning plan containing all parameters and profile assignment will be saved.

Load planner – The previously saved scanning plan can be loaded.

Planner menu – The following profile-related tasks applicable for the planner can be performed:

- Add current profile to all racks

- Open profile for all racks

- Detach current profile from all racks

- Detach profiles from racks

- Clear planner
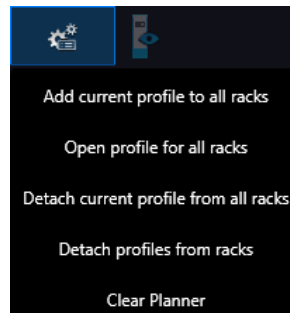
Figure 78 – Planner menu

Jump to scanned – Loads the planner view of the actual slide during scanning

Preferences

Help

For research and education uses only, not for use in diagnostic procedures.

5.3.2   General functions at the magazine menu

Rack view mode selector

Switch view by clicking this button. Click ⊕ to add a virtual magazine to the planner, and ⊖ to remove a magazine from the planner.

Rack menu includes the following global settings applicable to all magazines:

- Add current profile for all slides

- Open profile to all slides

- Detach current profile from all slides

- Detach profiles from slides

5.3.3   General functions at the slide menu

- Mark empty slide positions – Define range in the **Empty slide range** boxes to set multiple slide positions as empty. For single slides, enter / set slide position with the arrows in the **Empty single slide** box or right-click on a slide and select **Mark as empty**.

- Click **Reset empty definitions** to cancel slide selections as empty, or right-click the slide selected as empty and click **Reset**.
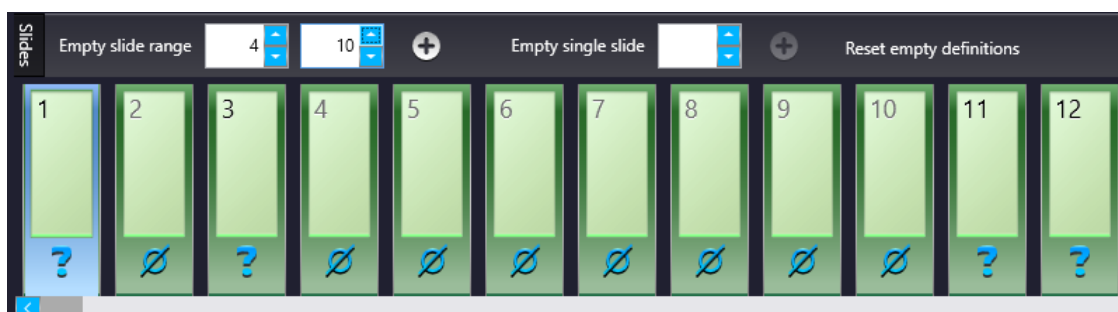
Figure 79 – General functions at the slide menu

For research and education uses only, not for use in diagnostic procedures.

5.3.4   Slide view with card

Select ![icon] from **Preferences / Appearance settings / Planner layout selector** to activate this view.

Depending on the actual view preference selected at ![icon], slide positions can have a horizontal or vertical layout. Slide preview and the list of the assigned profiles are displayed.
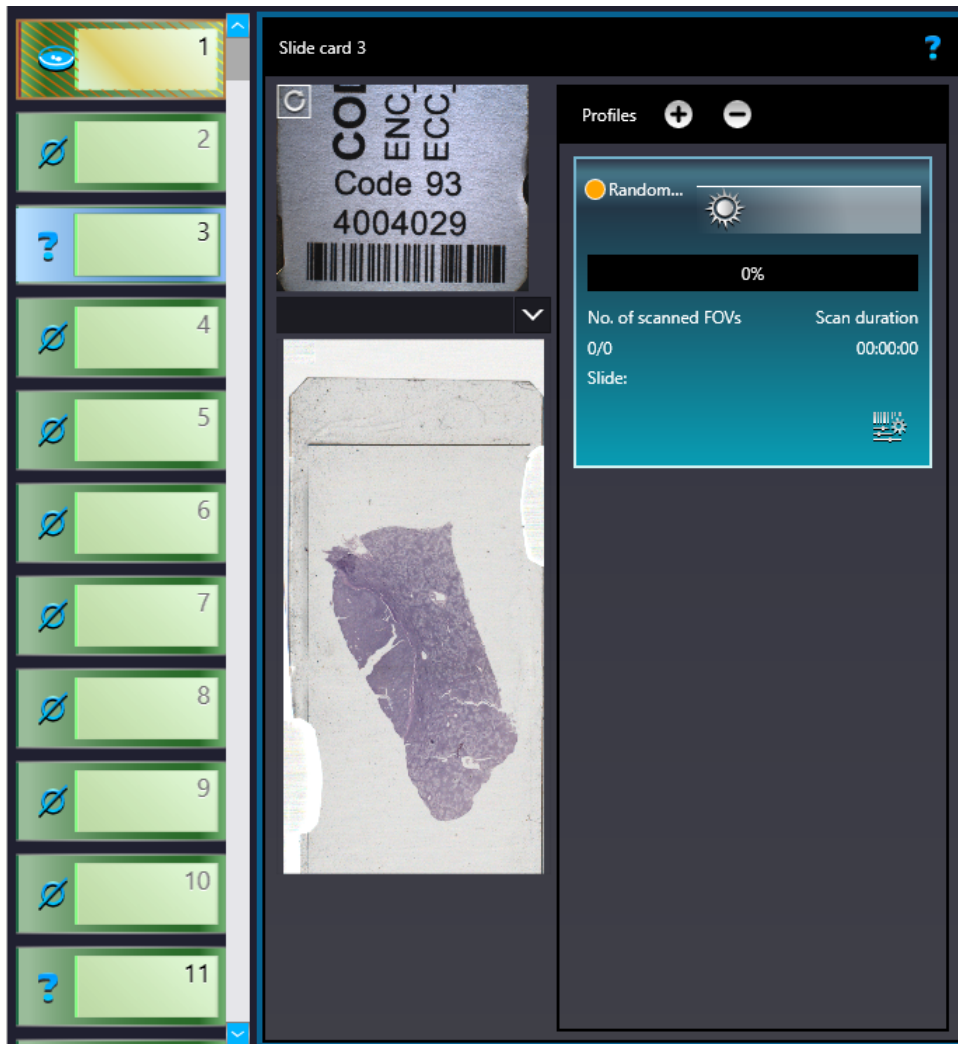
Figure 80 – Vertical slide view with card
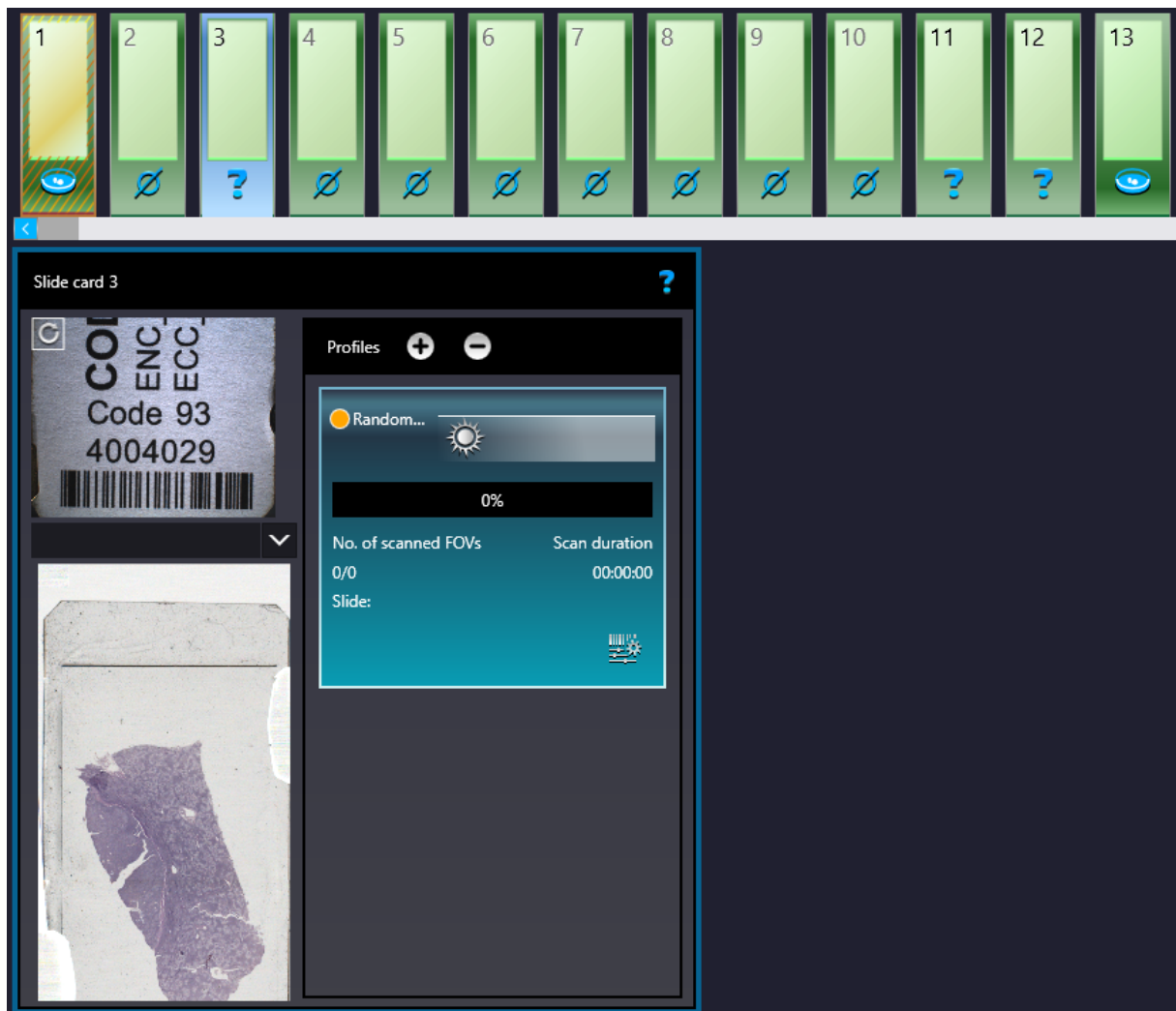
Figure 81 – Vertical slide view with card

By clicking ⊕ or ⊖ profiles can be added or removed for/from the slide, respectively. The parameters of the assigned profile is displayed in a card. The colors of a Brightfield and a Fluorescence profile cards are different.
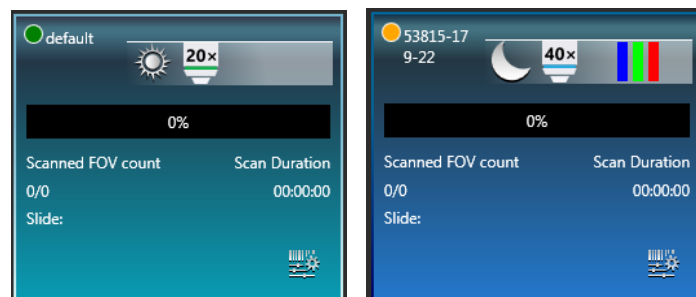
Figure 82 – Brightfield profile card and Fluorescence profile card

The following information are included in a profile card:

- Profile name in the top left corner

- Profile status is marked with spots of different color:

  ○ ⬤ – Stored

  ○ ⬤ – Edited

  ○ ⬤ – Draft

- Scanning mode icon representing Brightfield or Fluorescence mode

- Objective type

- Filter channel color bars (for FL mode only)

- Scan progress

- Scanned FOC count

- Scan duration

- Slide: actual status of the slide is presented here. If the slide is under scanning, the "Scanning", if scanning has been interrupted by some error, the "Failed" text is visible.

  NOTE: If slide status is marked as "Failed", delete status before scanning by clicking ⊠.

  If the virtual slide is created (the scanning is finished), the location path of the destination folder is displayed. To re-scan and thus recreate the slide, click ⊠ at the front of the displayed path to start the scanning process over.

- Scan properties 🗒

5.3.5   Table view mode

By clicking ▦ each slide will be displayed in a separate row.

Click ⊕ to assign profile to a slide, and ⊖ to detach a profile from a slide. A slide can have more than one profile assigned to it, and these profiles can either be BF or FL if the scanner is capable to operate in these two scanning modes.
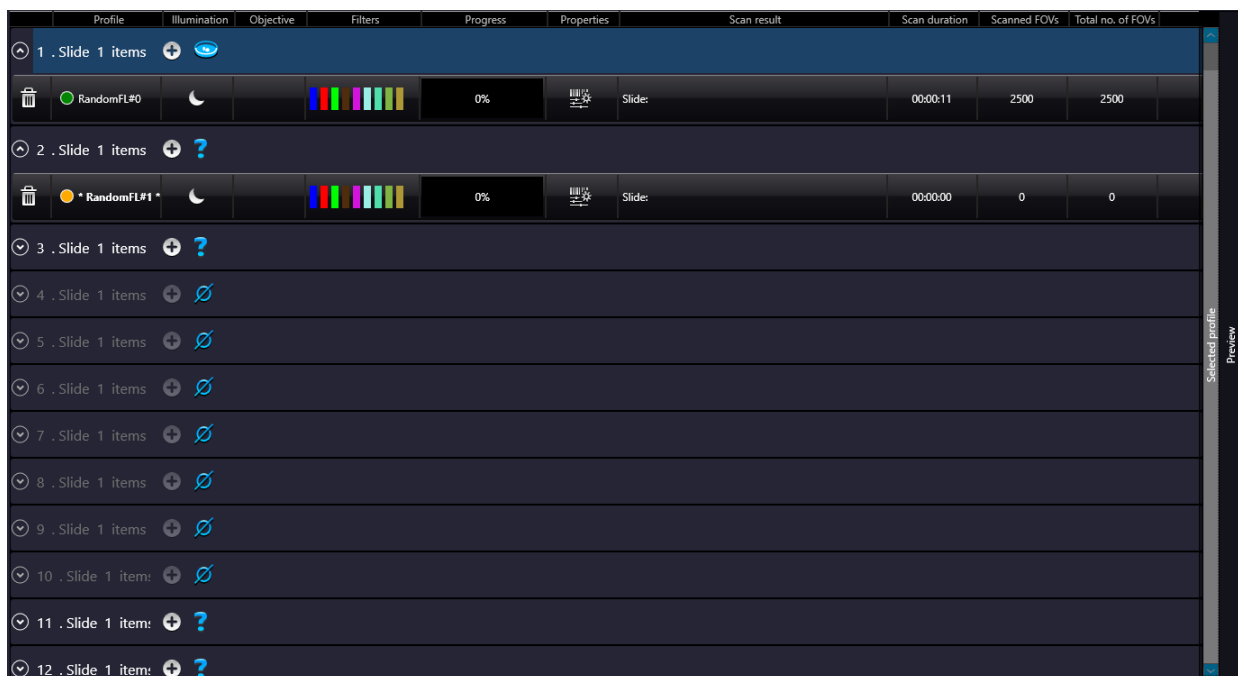
Figure 83 – Table view mode

In Table view the following information are displayed in the profile strip based on the loaded profile:

- Profile name

- Illumination

- Objective type

- Filters

- Progress

- Properties: The location of the virtual slide with the selected profile can be defined

  ○ Scan result

  ○ Scan duration

  ○ Scanned FOV'S

  ○ Total FOVs

5.3.6 Card collection view mode

If you click the [image] button, each of the slide previews are displayed in the window. Add profiles by clicking [image] and detach profiles by clicking [image]. Functions are the same as with the other modes.
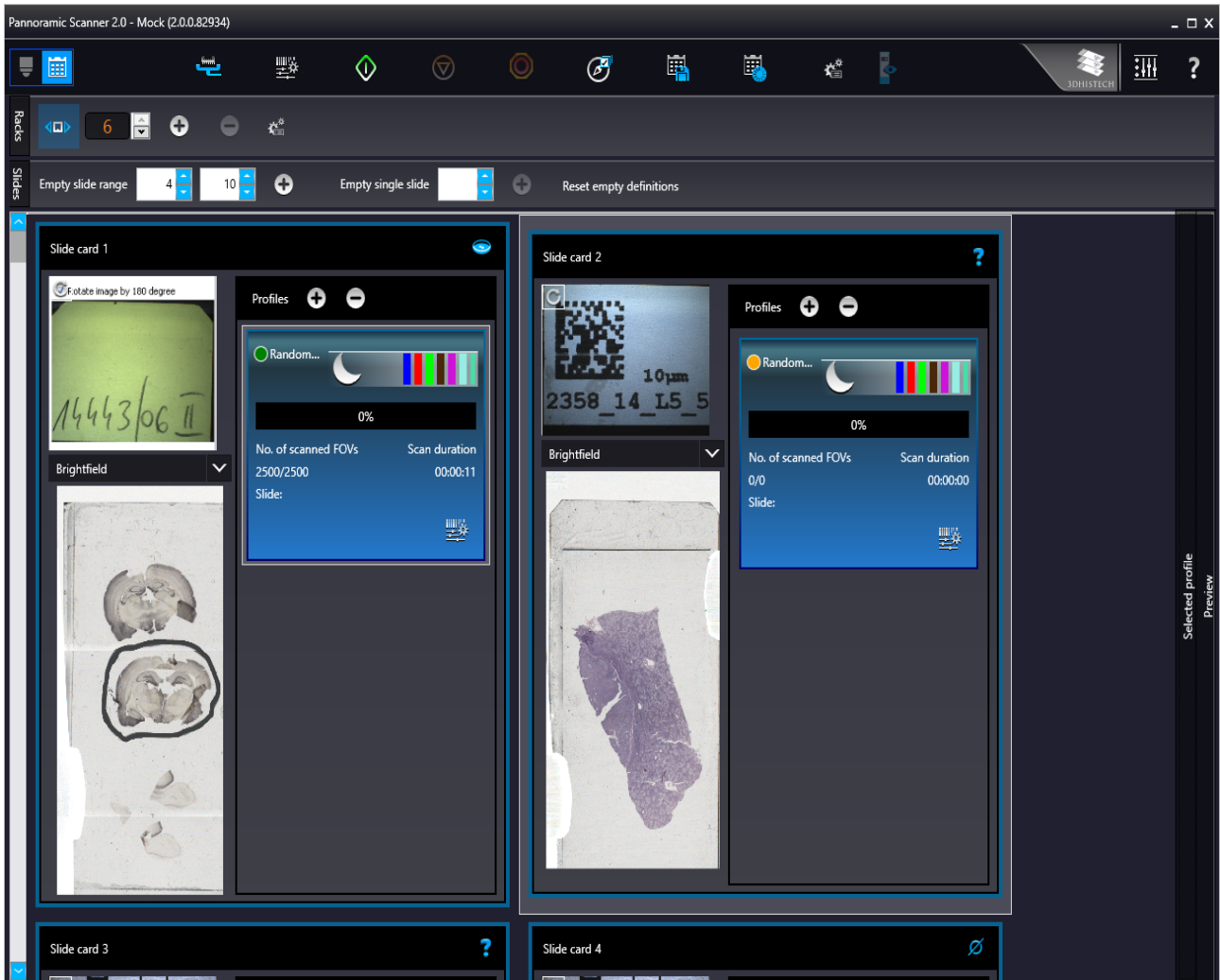
Figure 84 – Card collection view mode

For research and education uses only, not for use in diagnostic procedures.

5.4   Profile and Preview overview on the Planner

Each of the assigned profiles are checkable and modifiable. The expanding panel at the right side includes all of the profile settings, in coherence with the view type of the Profile Setting panel. Slide preview is displayed in the Preview window in which the scanmap can be modified until beginning the scanning process. Every aforementioned Profile setting can be modified depending on the scan profile type, being Brightfield or Fluorescence.
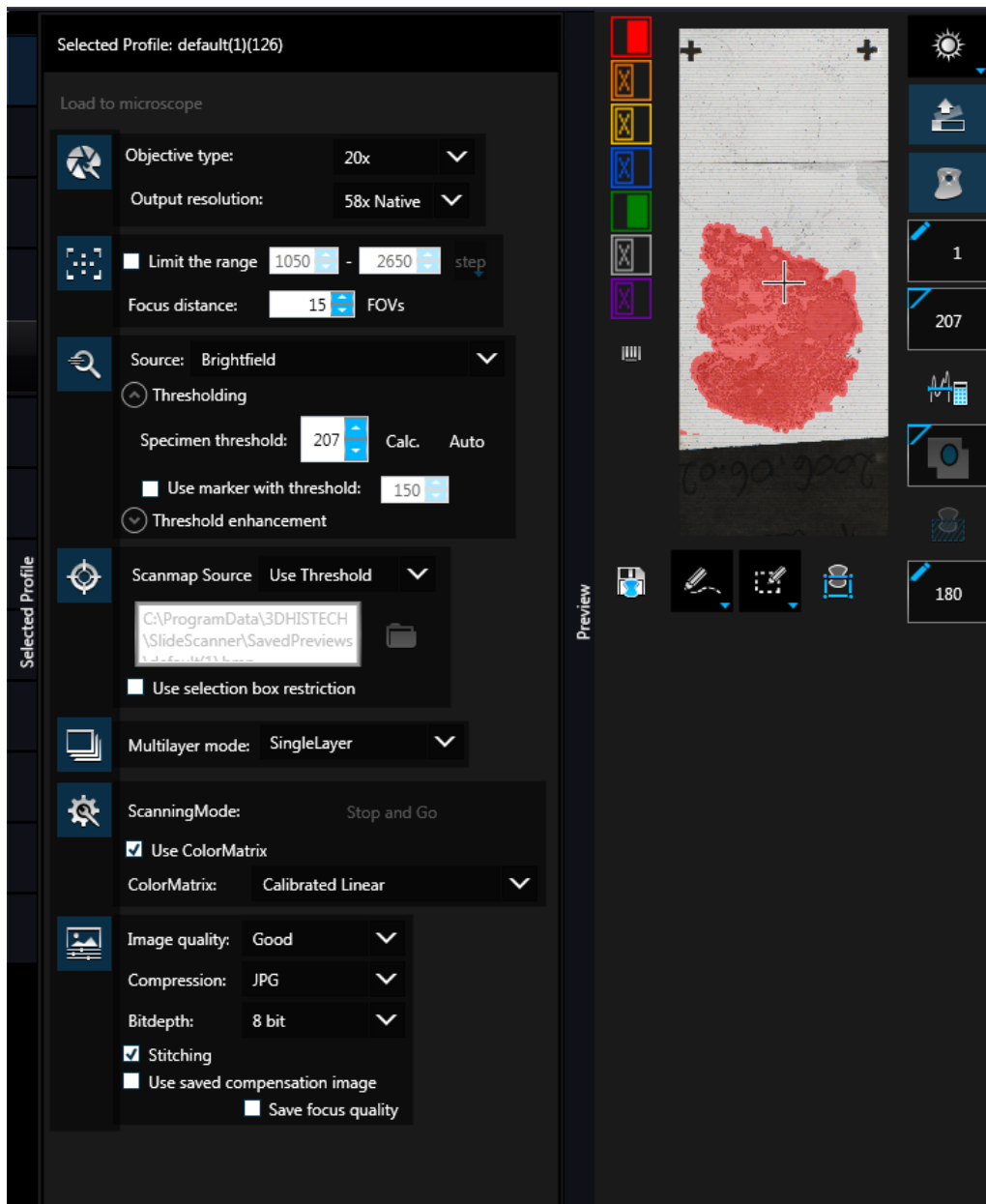
Figure 85 – Selected profile and expanded Preview window in Planner mode

For research and education uses only, not for use in diagnostic procedures.

5.5   Progress features

The progress is visible both in Microscope and Planner modes whenever there is a running hardware operation or scanning procedure. Progress content can be therefore different depending on the type of the running procedure.

The following information is included in the progress log list during scanning:

- Filter

- Elapsed time

- Location

- Slide name

- Free space

- FOV number

5.6   Services and Settings

Activate Services by clicking [icon] and Settings by clicking [icon] at the right side of the main menu bar.

5.6.1   Services

Activate **Services** by clicking [icon] at the right side of the main menu bar.

- [icon] – **Camera settings**

  ○ **Position**

  ○ **Objective type** – Select the type and the magnification level of the objective.

- ○ **Magnification**

- ○ **Use manual camera changer** – Changing the cameras is performed manually.

- ○ **Prescan objective protection enabled** – If activated, slide area near the edges will not be scanned to record fast preview image. If inactive, Fast Preview processing becomes slower.

- ○ **Cameras**

    Activate **BF** and/or **FL** mode for the camera. The software automatically loads the camera settings upon clicking the **Test and apply camera settings** button.
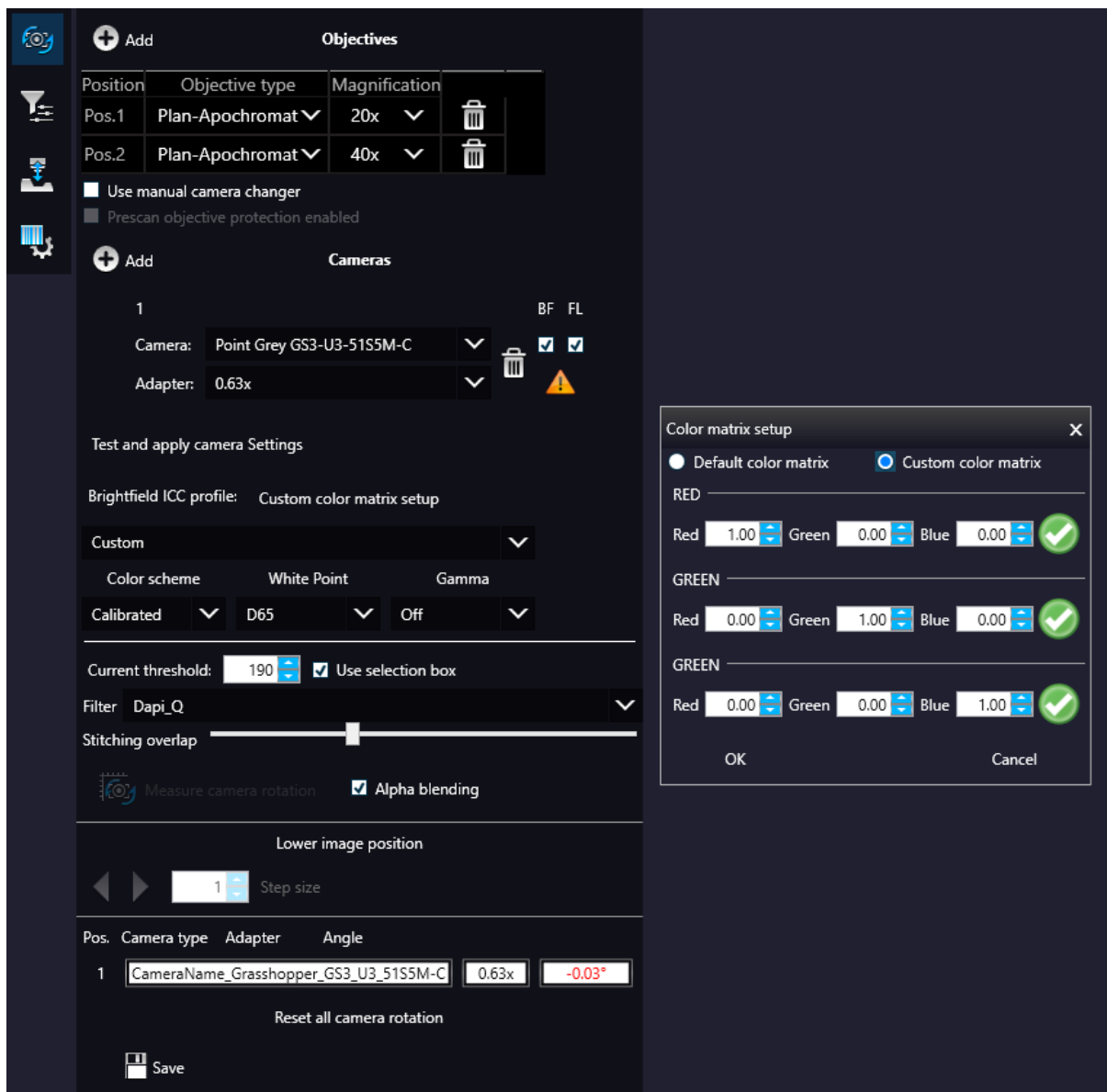
○ **Brightfield ICC Profile**

Select an applicable preset color profile to be used for Brightfield scanning from the drop-down menu. Settings according to the profile has effect on the color set, color temperature, and gamma value of the scanned image.

Preset profiles are the following:

- **Calibrated Linear**: Color scheme is defined by calibration (Gamma value is not included)

- **sRGB standard**: Color scheme is defined by calibration (standard Gamma value -2.2- is applied)

- **sRGB standard Microscope Like**: Color scheme is defined by calibration (standard Gamma value / White Point: D50)

- **Legacy**: a Color scheme calibrated for the previous software version is applied

- **Custom**: parameters are freely customizable as follows:

○ **Color Scheme**

- **Calibrated**: Scheme is defined by the color calibration slide

- **Legacy**: a Color scheme calibrated for the previous software version is applied

- **Raw**: the color information of the recorded raw image is applied

○ **White Point**

- **D65**: color temperature according to a standard illuminant D65 white point value is applied (6500K / daylight)

- **D50**: color temperature according to a standard illuminant D50 white point value is applied (5000K / horizontal daylight)

○ **Gamma**

- **Standard**: a value of 2.2 is used

- **Medium**: a value of 1.8 is used

- **Off**: a value of 1.0 is used
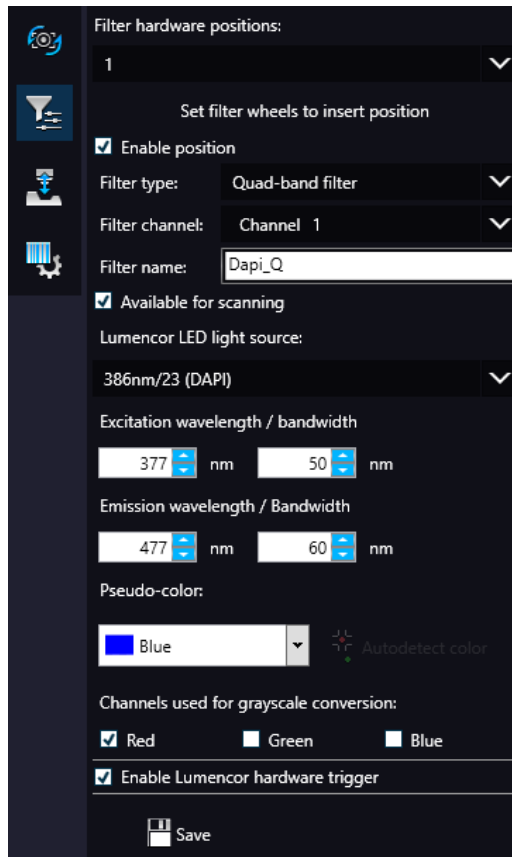
> (!) **Note:**
>
> If Lumencor SPECTRA 6 is configured as illumination unit, the corresponding **Enable Lumencor hardware trigger** option is displayed on the **Filter settings** form.

- ◦ **Current threshold** – Set the desired threshold level in the box.

  - ▪ **Use selection box** – If selected, only the area inside the box is scanned. It is useful if you only want to scan the area that is equivalent with the size of the coverslip, so external specks will not be scanned.

- ◦ **Stitching overlap** – Set the amount how much the FOVs should extend over

- ◦ **Measure camera rotation** – Camera rotation angle is measured

- ◦ **Alpha blending** – The two images are blended

- ◦ **Lower image position** – Moves the upper image sideways in distinctive steps

- ◦ **Reset all camera rotation** – Click this button to set camera rotation values to default.

  Save settings by clicking ▦ .

- • ▦ – **Filter settings**

On this panel you can define which filters occupy which turret positions.

- ◦ Select filter hardware positions from the drop-down list

- ◦ Move filter wheel to insert position

- ◦ **Enable position**

- ◦ Select **Filter type**, **Filter channel**, and **Filter name**

- Check **Available for scanning**

- Set **Lumencor LED light source**, set **Excitation** and **Emission wavelength / bandwidth** values for the selected filter.

- Set **Pseudo-color**

- Select channels used for grayscale conversion

- Select **Enable Lumencor hardware trigger**

Click **Save** to save settings.

- – **Z-difference**

    This function is available only for fluorescence settings; it enables you to set the basic focus differences between the filters. This facilitates more precise focusing during scanning.

- – **Barcode settings**

    By predefining barcode type, foreground color (black or white – the color of the barcode), and other settings, you enable the **Read Barcode** option when using the control software.

    To display an image of the barcode area, click ▭ . You can redefine the barcode reading area by dragging the edges of the blue square.

    To check whether the barcode reading settings are correct, display the barcode digits in the **Barcode** field by clicking ▭ . If the label area is not correctly illuminated, adjust **Barcode LED intensity** value.

    Click ▭ to check that barcode settings are correctly set.
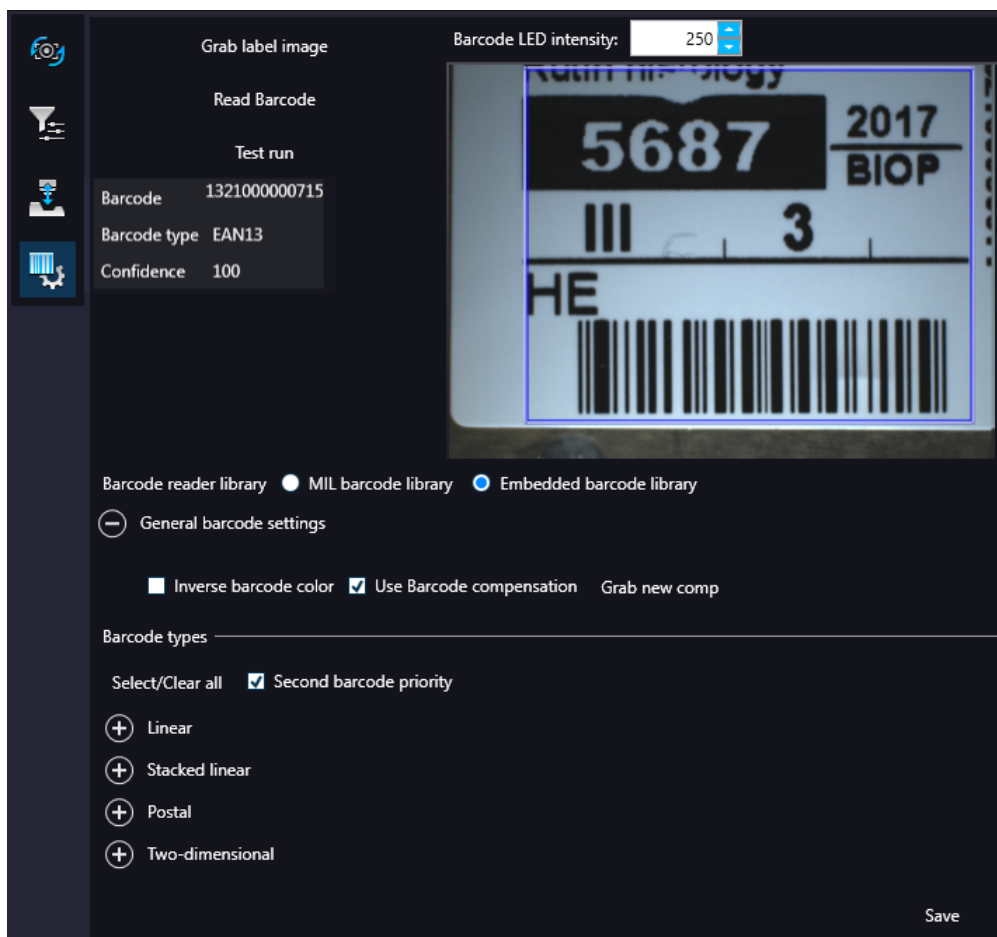
> ⊘ **Note:**
>
> If you did not set the barcode type correctly, or there is no barcode in the barcode area, the software will display the following error message: *"Barcode detection unsuccessful! There might be no barcode present."*

- ○ **Barcode reader library**
  - ▪ Select **MIL barcode library** or **Embedded barcode library**
- ○ **General Barcode settings**
  - ▪ **Inverse barcode color**

    Activate to invert foreground and background colors of the barcode.

  - ▪ **Use barcode compensation**

    A compensation image is used during barcode reading

▪ **Grab new comp**

Click this button to create a new compensation image for barcode.

NOTE: New compensation image for barcode can be recorded only if MIL is used for barcode identification.

• **Barcode types**

  ○ **Linear**

  ○ **Stacked linear**

  ○ **Postal**

  ○ **Two-dimensional**

  ○ **Second barcode priority**

  The software is capable to read two barcodes at the same time. If activated, the second barcode will be the barcode used for slide identification.

(!) **Note:**

When having two barcodes on the slide, barcode layout must be consequential.

5.6.2   Settings

Activate **Settings** by clicking ⚙ at the right side of the main menu bar.

- ✗ – **Basic settings**
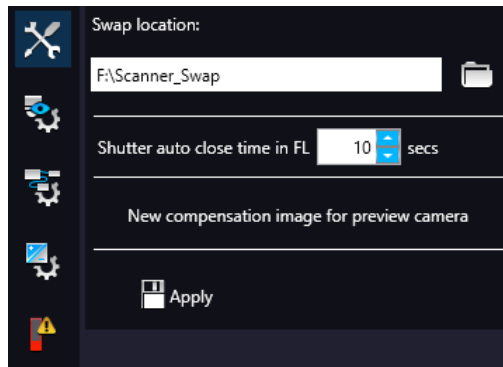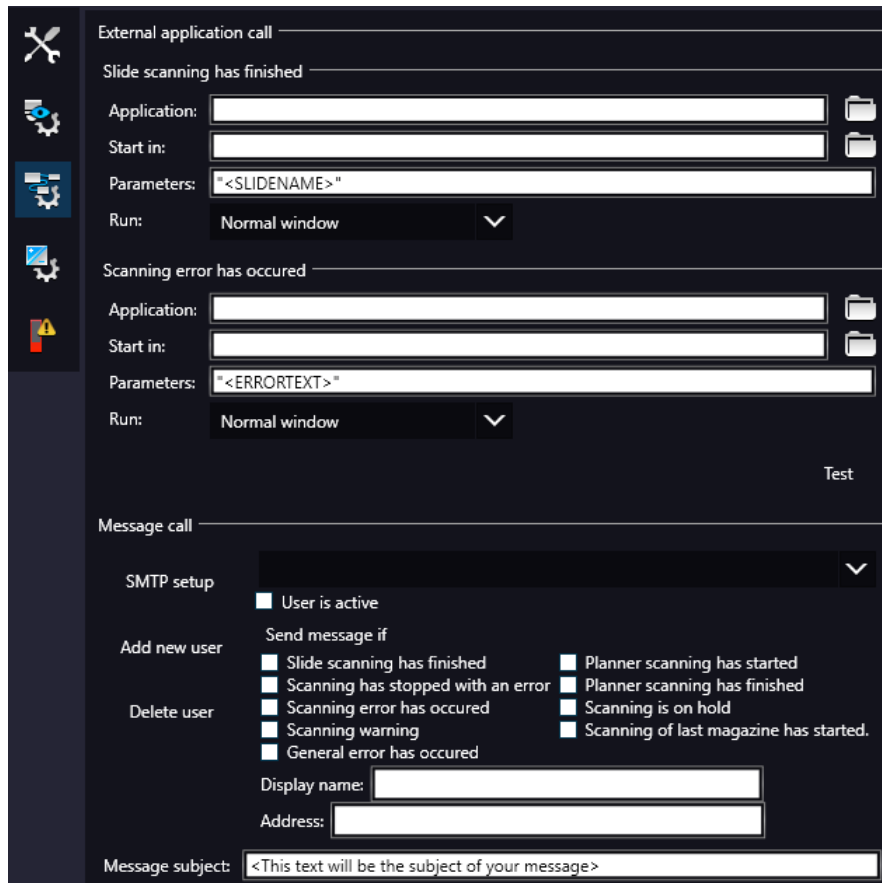
On this panel, you can define the swap file location, set shutter auto close time in FL, and create new compensation image for preview camera if needed. Click **Apply** to accept modifications.

- 🔵 – **Prescan settings**

For research and education uses only, not for use in diagnostic procedures.

- – **External application call**

Two of the three settings are responsible for opening external applications (.exe or .bat) as defined (for example, CaseViewer software can be opened after scanning is finished).

You can define external application call settings for the following cases:

- Slide Scanning Finished
- Scanning Error Occurred

For **Slide scanning has finished** and **Scanning error has occurred** you can define the external software by entering the file path in the **Application** box, or by browsing for it by clicking .

If you define parameters in the **Parameters** field, it will pass them to the software that it starts. If no parameter is defined, then the parameter will not be passed. The default parameters are the following:

- For **Slide scanning has finished**: "`<SLIDENAME>`" – transfers the slide name

For research and education uses only, not for use in diagnostic procedures.

- For **Scanning error has occurred**: "<ERRORTEXT>" – transfers the error message

Run defines the window mode that the external application will start in. The following window modes can be set:

- Normal window

- Minimized

- Maximized

You can test whether the **Scanning Error Occurred** settings work by clicking ⁝ .

In the **Message Call** section of the window complex messaging functions can be defined. Here you can setup parameters of the mail server, add or remove users, and also set the message body in the **Message subject** field by entering a text for example, to inform recipients on the location of the scanner, or the slide set you are working with.

Click ⁝ to test settings (by sending an e-mail to the address defined in the **E-mail To** field) after you have finished to check their relevancy before applying them.

First click **SMTP setup** to display the E-mail settings window, in which you can dedicate an e-mail server through which the software sends e-mails to the selected recipients.
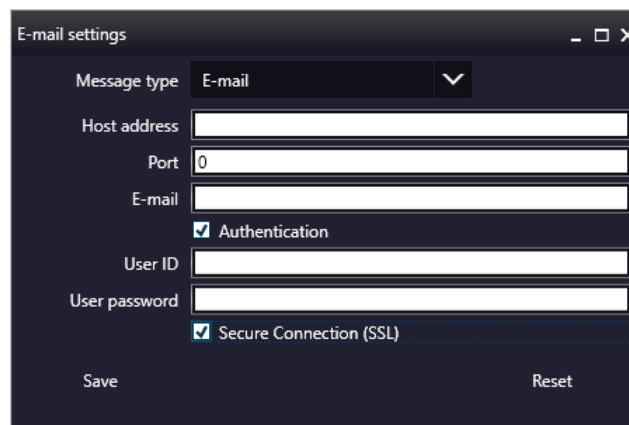
Figure 86 – Messenger Setup window

In the Messenger Setup window the following parameters can be set:

- **Message Type** – E-mail

- **Host Address**

- **Port**

- **E-mail**

- **Authentication** – **User ID** and **User password** are required.

- **Secure Connection (SSL)** – If activated, the messages will be sent through secured connection.

For research and education uses only, not for use in diagnostic procedures.

- • If you have properly set messenger settings, click **Save**.

- • Click **Add new user** in the **Message Call** section of the External application call settings window then enter **Display name** and **Address** of the recipient. Select message functions under the **Send Message if:** field for the selected recipients (from the drop-down list at the top of the section).

- • Click **Delete user** if you want to remove a user from the list after selection.

- • – **Image compensation**

    You can use a saved compensation image whenever compensation-related issues occur (deriving from the slide, illumination, staining or embedding medium irregularities).
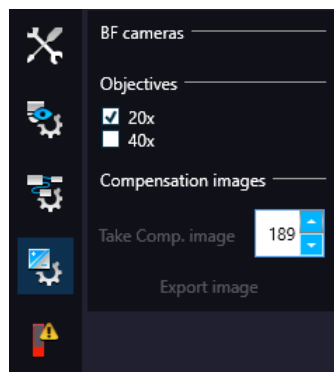
    - ○ **BF cameras** field displays the active camera. Currently, there is only one active camera for Brightfield mode.

    - ○ **Objectives** field displays the objective used for scanning.

    There might be specks or minor etches on the surface of the compensation slide that require modifying the threshold settings. Therefore, the scanner will not use those areas for the creation of the compensation images.

    To modify the threshold settings, set the desired threshold value in the box.

    To create the compensation image(s), click **Take Comp. image**. Click the **Stop** button to interrupt the process. The progress bar at the bottom of the window displays the progress of the compensation image creation.

    - ○ **Export image –** Save compensation image.

- **– Emergency slide operations**

    This panel contains control and maintenance functions.

    ○ **Emerg. position**

        The slide holder will be moved to a position where the slide can be removed manually after opening the cover (see section ***7.3.1 Opening the protective covers***).

    ○ **Slide removed**

        Click this button to notify the software that you have removed the slide manually.
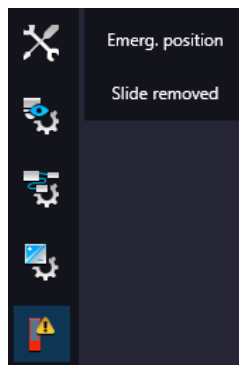
5.7   Hosting

Hosting a live teleconsultation session gives specialists the opportunity to examine the same Live microscopic image during a real-time counseling even if being in laboratories far away from each other. This means multiple users can connect to the device at the same time. The user of the scanner will be the host with direct access to the scanner.

> (!) **Note:**
>
> Both the client's and the host's scanner software version must be identical.

Once client users have connected, they will be able to reach the following functions of scanner:

- Live image

- Preview window

- Profile content

- Stage control

Each participant see the same live image within the same preview window. The active user can navigate on the slide and set up a scanning profile. The **Start scan** function is also available for the active user. Each participant can make comments in a chat window while examining the slide image.

For research and education uses only, not for use in diagnostic procedures.

Hosting a teleconsultation session

1. Select **Hosting** from the microscope mode menu

2. Type in your nickname used during the session

3. Click **Start hosting**

4. The software displays the hosting address and the port number
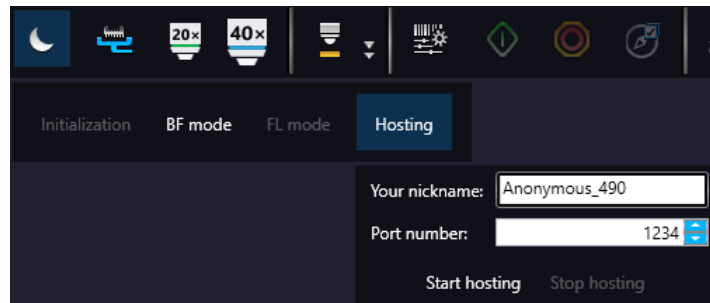
Join teleconsultation as a client

1. Start the *Lite* version of the scanner software

2. Type in your nickname used during the session

3. Enter **Host address** and **Port number**

4. Click **Connect**

On Client side the following functions are unavailable/inactive:

*   Selection box

*   Scan properties

*   Separate scanning

> (!) **Note:**
>
> During a fluorescence Live consultation the shutter of light path will be closed automatically during the host time in order to save the sample from the burnout effect.

5.8   Using Profiles

> ⊙ **Note:**
>
> Saved profile settings cannot be applied if using a different type of Pannoramic scanner.

If you want to scan a group of slides, it is advised to save your settings previously in a profile and continue scanning in Automatic mode. You can save the following settings in a profile:

Scan Mode

- Brightfield

- Fluorescent

Scan Options

- Focus frequency (in Fluorescent mode)

- Focus frequency (in Brightfield mode)

- Compression procedure

- Use of a fixed compensation image (in Brightfield mode – ON/OFF)

- Use of Save focus quality (ON/OFF)

- Use of stitching (ON/OFF)

Determination of the area to be scanned

- Use of automatic threshold specimen

- Use of specimen threshold value

- Use of Brightfield/Darkfield preview image

- Use of marker pen threshold and specimen threshold values

- Use of scan everything within marked area (ON/OFF)

- Remove coverslip function (ON/OFF)

- Filling of holes (ON/OFF)

- Use of removing specks according to the set value (ON/OFF)

- Use saved scan area

- Use selection box (ON/OFF)

Fluorescent settings

- Filter position(s)

- Selected focus channel

- Selected stitching channel

- Flat field correction (ON/OFF)

    - Intensity statistics

    - Intensity reducing

    - Intensity equalization

- Sharpening (ON/OFF)

- Use of skip empty field-of-views (ON/OFF)

- Do not use extended focus on empty field-of-views (ON/OFF)

    - Custom extended focus

Other settings

- Limitation of focus range (ON/OFF)

- Use of objective position

- Enable/disable Multilayer options

- Use of extended focus or Z-stack step size and focus level

- Output resolution

6 Troubleshooting and Maintenance

6.1 Safety Information

Check cables, plugs and connections before operation, and if defective or cannot be replaced, contact Thermo Fisher Scientific Service for support.

Check whether the device is compatible with your local line voltage. It can operate at line voltages 100V to 240V and 50/60 Hz.

Only plug the device in a socket with protective grounding (earth) contact.

Do not operate the device in explosive environment.

Disconnect the device from the power outlet before opening it or replacing a part.

The product is not equipped with protection against hazardous materials (infectious, toxic, radioactive, corrosive substances or other effects damaging to health). Decontaminate infectious materials after use.

Dust, specks and humidity may decrease or harm the operating capability of the device. Protect it with dust cover when not in use. Switch the power off before covering it.

There is crush hazard during operation in the area of input or output. Inserts/magazines should be moved through software. The protective shield of the magazine feed unit can be unmounted when slide scanning has stopped. The device can be opened for service reasons only when switched off, and in cases stated in this User's Guide (for example, changing filters).

Defective devices do not classify as domestic waste. Dispose of the device in compliance with the currently valid legal requirements.

Lumencor SPECTRA 6 illumination unit

- **NEVER** switch on the Lumencor SPECTRA 6 light source when it is not mounted to the microscope.

- Do not cover the unit, as it would cause abnormal temperature rising.

- Ensure that the light source is mounted well and the fixing lever is closed.

6.1.1   Cleaning the device

- Before cleaning, switch off the device and pull the plug out.

- Do not let liquid penetrate into the system.

- To clean liquid from the device, use a lint-free cotton cloth. You can moisten the cloth with water.

- To clean greasy surfaces, it is recommended to apply **Isopropanol** or **Ethanol** on a piece of lint-free cotton cloth and gently rub the surface.

6.1.2   Cleaning the magazine

Do not clean the magazines with xylene, because it might harm the plastic. We recommend to apply **Isopropanol** on a piece of lint-free cotton cloth and gently rub the surface instead. In case the magazine is damaged, use either order a new one, as inserting a deteriorated magazine could lead into unsafe loading of slides.

6.1.3   Preventive maintenance

To prevent fungal growth

- Do not leave the equipment in an unconditioned environment with a storage temperature above 60°C (140°F) or below 0°C (32°F), which may damage the equipment.

- Maximum operating temperature is 35°C (95°F) with a relative humidity of less than 75%.

- Install it in a room with proper air ventilation.

- Do not let dust or fingerprints remain on optical surfaces.

6.2   Troubleshooting

This section describes the most common troubleshooting solutions for the Pannoramic 250 FLASH III product. If any problem arises that does not have solution in this manual, contact the service personnel.

The product does not start

- **Power supply is interrupted**

    1. Make sure that both the main power supply switch and the side switch are on (see section ***1.1.4 Main parts of the Base Unit***).

    2. Check cable connections.

Control software has stopped

- **Magazine jammed**

    1. Exit control software.

    2. Switch off control computer and the power supply, disconnect power supply cable (see section ***1.1.4 Main parts of the Base Unit***).

    3. Remove protective covers (see section ***5.3.1 Opening the protective cover***).

    4. Remove slide or magazine manually (see sections ***5.3.4 Removing a jammed or broken slide*** or ***5.3.6 Removing a magazine from the transporting system***). If a jammed magazine cannot be removed, contact service personnel.

    5. Reattach protective covers.

    6. Reconnect power supply cable of the base unit and the control computer to line power supply.

    7. Switch on the power supply of the Pannoramic 250 FLASH III (see section ***1.1.4 Main parts of the Base Unit***).

    8. Switch on the control computer.

    9. Launch control software.

Illumination error! Probably there is a problem with the Xenon FLASH lamp. Please, turn off the scanner.

- Contact Thermo Fisher Scientific Service for support.

Image is black

- Contact Thermo Fisher Scientific Service for support.

Virtual slide is improperly focused almost across its entire surface

- **Focus range enabled**

    - Disable **Limiting focus range**.

Poor image quality

- **Imaging error (for example, chromatic aberrations)**

- Contact Thermo Fisher Scientific Service for support.

> ⚠️ **Warning!**
>
> Do not modify the camera driver settings, as the default settings adjusted by Thermo Fisher Scientific, give the most accurate images.
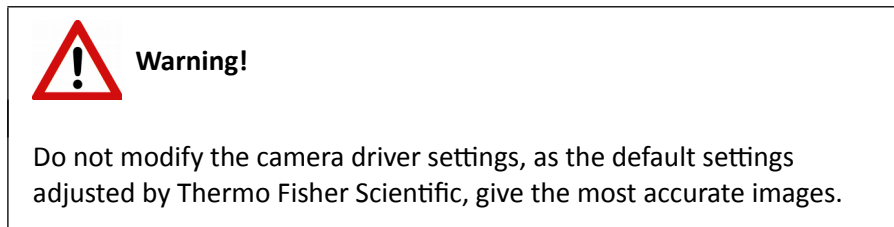
Virtual slides show poor stitching in Pannoramic Viewer

- **Scan camera is not properly adjusted (for example, due to loose fixture)**

- Contact Thermo Fisher Scientific Service for support.

Preview image is too dark / too bright

- **Invalid compensation image for preview camera**

    - Capture a new compensation image for the preview camera (see section ***4.3.6 Service panel***)

Digitization process is extremely slow

- *Extended focusing* **option is activated**

    - Deactivate the **Extended focusing** option if not required.

- *Z-stack* **option is activated**

    - Deactivate the **Z-stack** option if not required.

Image is out of focus (in Manual mode)

- **Focus range limit is activated**

    - Deactivate the **Limiting focus range** option.

- **Focus range setting is incorrect**

  - Change focus range setting.

All individual images show a shading effect

- **Algorithm failed to find empty image fields**

  - Capture a reference image with a blank slide (see section ***4.2.2 Image Compensation***).

6.2.1　Fluorescent Applications (optional)

The switching of the Lumencor SPECTRA 6 cannot be triggered, in spite of trigger connection through the camera.

- Contact Thermo Fisher Scientific Service for support.

No suitable exposure time can be found. Signal is too weak.

- **When using Lumencor SPECTRA 6**

- Contact Thermo Fisher Scientific Service for support.

6.3   Maintenance

This section describes the most common maintenance solutions that can be performed by the user of the product. For maintenance solutions not described in this manual, contact the service personnel.

6.3.1   Opening the protective cover

To perform maintenance work and when slide or magazine jamming occurs, the protective cover(s) of the Pannoramic 250 FLASH III need to be opened in order to eliminate the problem. Before opening the cover, exit the control software, switch off the main power supply and disconnect the power cables (see section *1.1.4 Main parts of the Base Unit*).

> ⚠️ **Warning!**
>
> The opening mechanism is operated by a pre-tensioned hinge. Be sure that before opening the protective cover, open the FL cover first, so it will not lash to the cover during opening. For information on how to open the FL cover, see section *3.3.3. Opening/Closing the FL protective cover*.
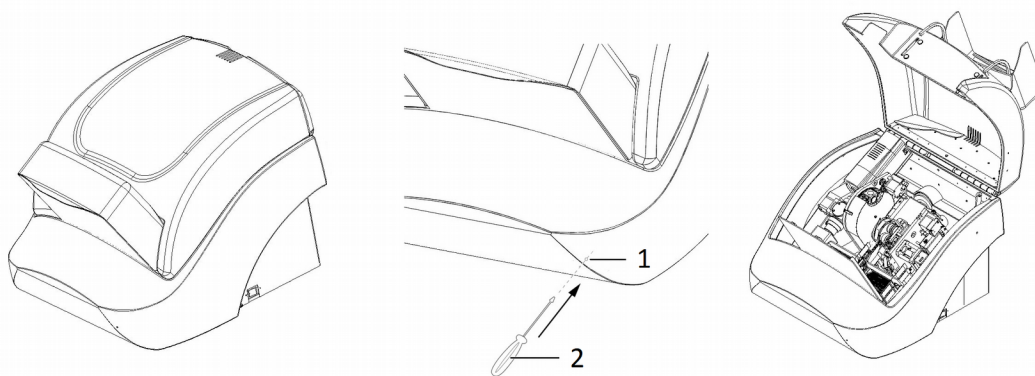
Figure 87 – Opening the cover

To open the cover, you will need a maximum 3mm diameter, 50 mm long screwdriver.

1. Insert screwdriver **(2)** in the cover opener hole **(1)** aligned 30 degrees upwards and parallel to the side of the base unit.

2. Press the cover opener button with caution with the screwdriver.

3. Open the cover.

4. To close the cover, push down the cover on the right side (above the lock) until you hear a clack sound.

6.3.2   Replacing fuses of the main power switch

1. Switch off the main power supply and disconnect the power cable (see section ***1.1.4 Main parts of the Base Unit***).

2. The fuse compartment is located next to the main power supply switch at the back side of the Pannoramic 250 FLASH III.

3. Release lock (**1**) by pushing the lever to the left, so the insert (**2**) can be pulled out.
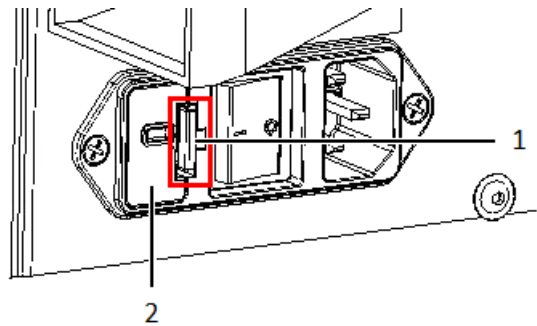
Figure 88 – Fuse compartment

4. Remove insert from the compartment.
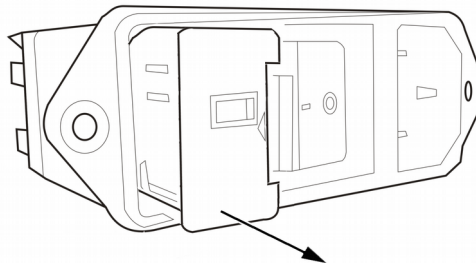
Figure 89 – Removing the fuse insert

5. Replace fuses, put the insert back into its mounting, and push it inwards until you hear a snap, so the lever locks.
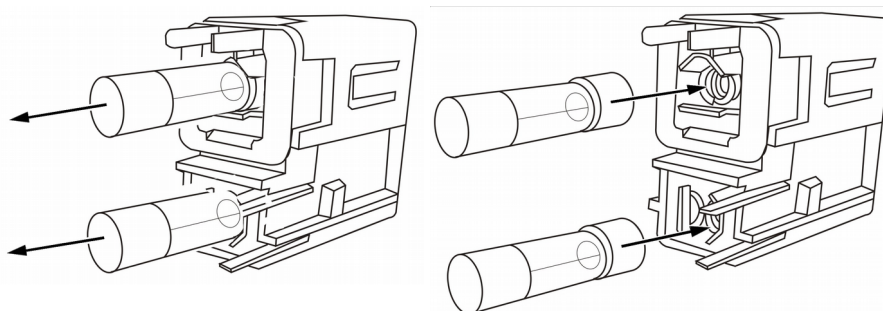
Figure 90 – Replacing the fuses

6.3.3   Removing a jammed or broken slide

If a slide got jammed or has suffered breakage during the process of digitization, stop the process and, if possible, move the slide holder to a position where the slide can be easily removed by clicking the **Emergency Manual Slide Removal Position** button (see section **_4.3.6 Service panel_**).

> ⚠️ **Warning!**
>
> Crush hazard! Before you remove a jammed slide, cancel the running digitization process, terminate the software session and turn the power off.

Any manipulation performed on a mechanical component must be followed by a complete device initialization routine (software restart).
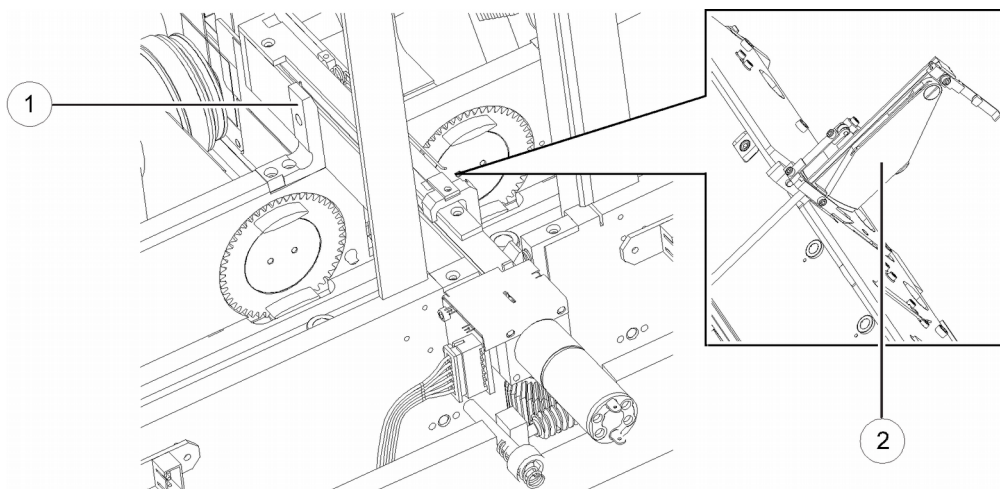
Figure 91 – Removing the slide loader

1. Open the protective cover (see section **_5.3.1 Opening the protective cover_**).

2. Push locking lever **(1)** to the left, and keep the lever in this position.

3. Seize the slide loader **(2)** by the end where the lever is and turn it up by the necessary degree to make the removing of slide possible.
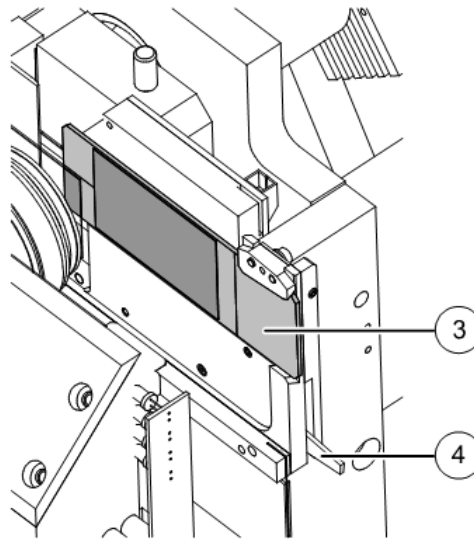
Figure 92 – Removing a slide

4. Press clamping lever **(4)** down. The slide position lock releases.

5. Retrieve slide **(3)** from the holder.

6. Release clamping lever **(4)**.

7. Turn down the slide loader **(2)** back into the original position and pull it inwardly (to the Pannoramic 250 FLASH III), until the guiding pin connects with the motor block groove again.

8. Press the slide loader **(2)** down to mechanical stop position, in which the locking lever must engage **(1)**. Use moderate force to press the loader part opposite the motor in clamp direction, until this part mechanically engages, too.

9. Close the protective cover again (see section ***5.3.1 Opening the protective cover***).

10. Launch the control software.

6.3.4   Installing and Removing the Filter Module (Fluorescent Option)
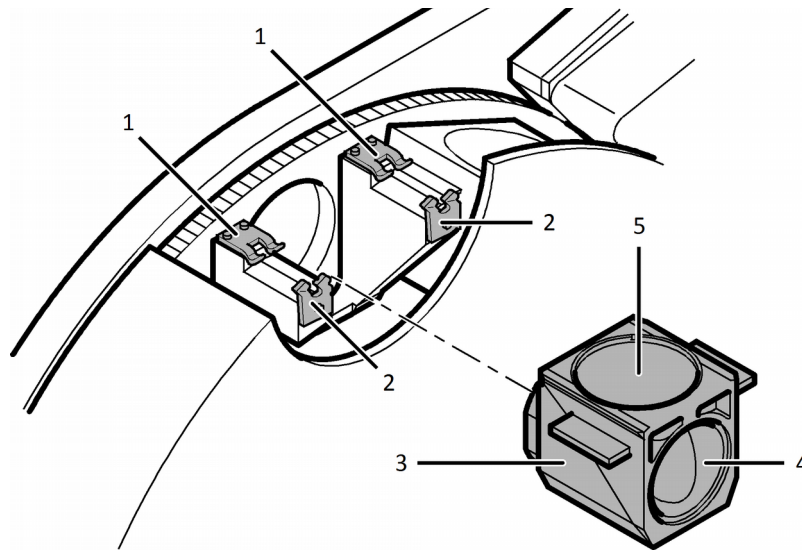
Figure 93 – Installing and removing the filter module

1.   Turn off the illumination unit.

> ⚠️ **Warning!**
>
> Crush hazard! Stop the running digitization process before you proceed with filter module installation or removal.

2.   Open the protective cover (see section ***5.3.1 Opening the protective cover***).

3.   Select the desired filter in the corresponding software window (see section ***4.2.3 Microscope Settings/Filter Settings panel***). The filter will be positioned in such a way that the user can access it without having to turn the filter wheel manually.

4.   Enter the respective parameters in the input fields.

5.   Hold the filter module **(3)** by its handling pads (one on the right, one on the left (lateral) side of the module) and insert it in forward tilted position from above into the upper spring-loaded clamps **(1)** at the filter wheel.

> ⚠️ **Warning!**
>
> Avoid touching the surface of the filters, and store the modules in their own boxes when not used.

6. Press the filter module down, until it safely connects to the bottom spring-loaded clamps **(2)** of the filter wheel. Mounting position of the filters: excitation filter **(5)** and emission filter **(4)**

7. To remove the filter module, first slightly tilt it out of its upper spring-loaded fixtures, then pull it out of the bottom spring-loaded components and take it off.

8. Close the protective cover (see section ***5.3.1 Opening the protective cover***).

9. Turn on the illumination unit again.

6.3.5   Removing a Magazine from the Transporting System
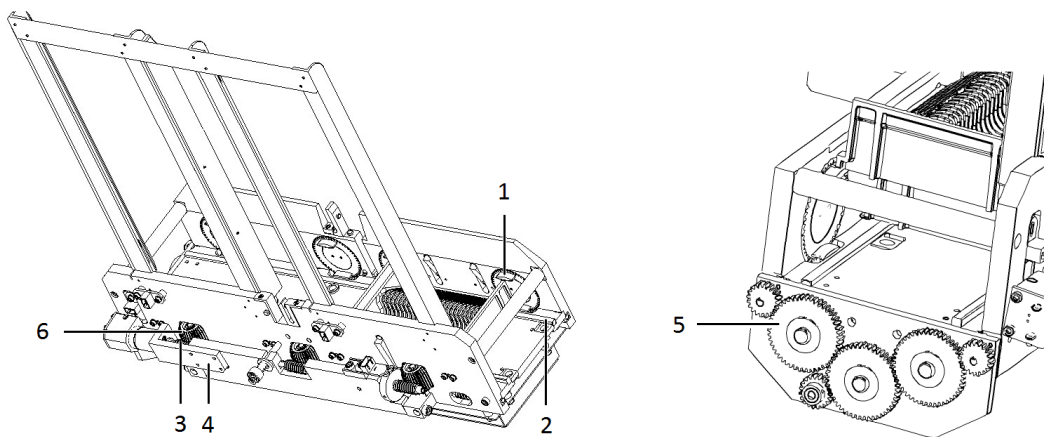
Figure 94 – Removing a magazine

1. Open the protective cover (see section ***5.3.1 Opening the protective cover***).

> ⚠ **Warning!**
>
> Crush hazard! Remember to stop the running digitization process, terminate the software session and turn power supply off before you remove a jammed magazine (see section ***1.1.4 Main parts of the Base Unit***).

> ⚠ **Caution!**
>
> Check that the magazine is not tilted, so that it rests on both horizontal guide rails.

2. If the magazine is in horizontal position, tilt the upper part of the horizontal magazine drive plate **(4)** from the magazine loader unit far enough for the driving gear **(6)** to disengage from the driving worm gear **(3)**.

3. Push the magazine on the guide rails **(2)** to the right to remove it from the transporting system, as to the left the Adimec camera is on the way, blocking the path.

4. If the magazine is tilted because of twisted tappets **(1)**, first restore the tappets to their initial position. They are correctly positioned if vertically aligned exactly one above each other. Turn the gear **(5)** by hand, until the tappets are in their initial position again and the magazine flatly rests on both horizontal guide rails.

> ⚠️ **Warning!**
>
> Danger of pinching! Turn the knurled wheel slowly with caution and take care of your fingers!

> ⓘ **Note:**
>
> Any manipulation performed on a mechanical component (for example, transporting system) by the user must be followed by a complete Pannoramic 250 FLASH III initialization routine (software restart).

6.3.6   Transporting

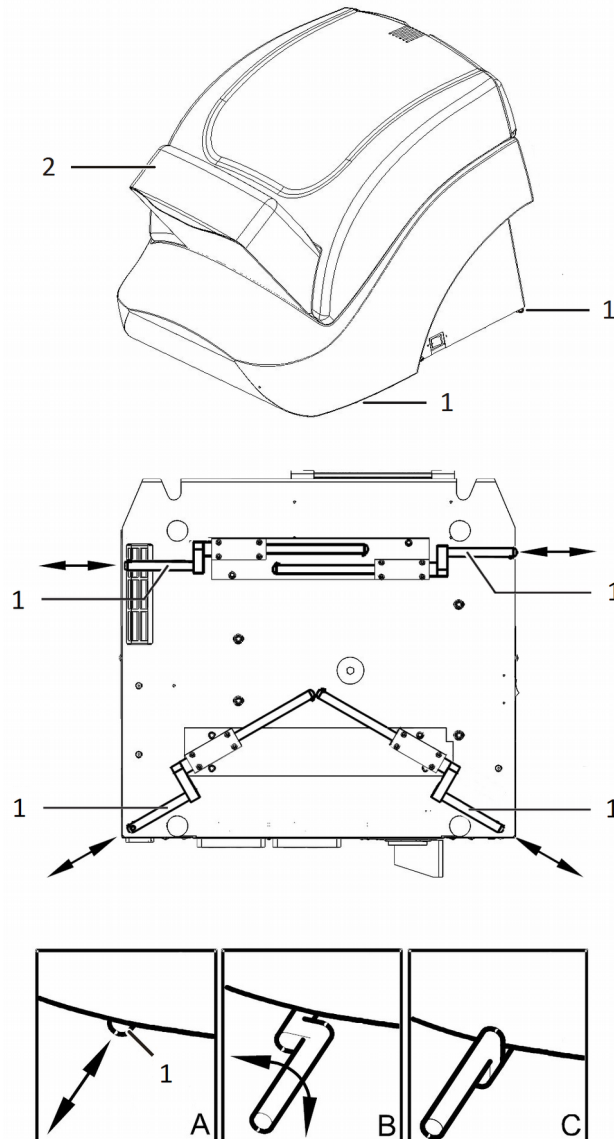The following procedure shows how to transport Pannoramic 250 FLASH III.

Figure 95 – Transporting handles

> ⚠️ **Caution!**
>
> Do not use the FL protective cover **(2)** to raise, shift or transport Pannoramic 250 FLASH III!

> ⊘ **Note:**
>
> The base unit has four carrying handles for transporting. Two or four people can carry the base unit using these carrying handles. To determine the appropriate number of transporter people, observe the laws of your country regarding load carrying capacity limitations. You can find the weight data of the base unit in section *6. Technical Data*.

To transport Pannoramic 250 FLASH III do the following:

1. Switch power supply off (see section *1.1.4 Main parts of the Base Unit*) and detach all other cables.

2. Pull carrying handles out **(1)** until mechanical stop position **(A)**.

3. Turn carrying handles by 90° **(B)** towards the base unit to secure them.

4. Now you can transport Pannoramic 250 FLASH III with the help of another person.

5. After finishing transportation, push carrying handles back into normal position **(C)**.

7   Technical Data

Dimensions (width × depth × height)

Base unit	approx. 68×69×55 cm
Base unit with fluorescence option	approx. 68×95×55 cm

Weight

Base unit	approx. 46 kg
Base unit with fluorescent option	Approx. 50 kg

Ambient conditions

Transportation / shipment (packed)

Permissible ambient temperature	-20°C to +70°C

Storage

Permissible ambient temperature	+0°C to +60°C
Permissible relative air humidity (non-condensing)	75%

Operation

Permissible ambient temperature	+10°C to +35°C
Permissible relative air humidity	75%
Altitude at installation site	max. 2000 m
Atmospheric pressure	500 hPa to 1060 hPa
Degree of pollution	2

Operating data

Intended site	closed room facilities
Electrical protection class	I
Internal protection degree	IP 20
Electrical safety	in compliance with DIN EN 61010-1:2001, DIN EN 61010-2-101:2002 (IEC 61010-1:2001, IEC 61010-2-101:2002)
Over-voltage category	II
Radio interference suppression	in accordance with EN 55011 class B
Noise immunity	in accordance with EN 61326-2-6:2005
Base unit input voltage	100-240V AC
Power consumption of the complete device	max 350W
Fuses	2x T3.15A, 250V

Light sources

Halogen lamp (transmitted light operation)	Precision-Aligned Pulsed Xenon Light Source
Average service life of halogen lamp	2×10^9 flashes
Lumencor SPECTRA 6 light source	SPECTRA Light Engine 6
Estimated service life of the Lumencor's light source	15 000 h

This User's Guide describes Lumencor SPECTRA 6 as light source.

Slide identification

Slides are identified by a VRmagic VRmC-8+ PRO preview camera. Label area is saved as image and barcode is interpreted as a character string.

Scan cameras

pco.edge 4.2 13.3 mm × 13.3 mm sCMOS (CIS2020)	
Pixel size	6.5 µm x 6.5 µm
Camera resolution	2048 x2048 active pixel
Camera max. speed	100 fps at full resolution (2048 × 2048 pixel)
Bit-depth	16 bit
Pixel resolution with 20x objective and 1x C-mount adapter	0.33µm
Pixel resolution with 20x objective and 1.6x C-mount adapter	0.20µm
Pixel resolution with 40x objective and 1x C-mount adapter	0.16µm
Pixel resolution with 40x objective and 1.6x C-mount adapter	0.10µm
Cooling	Peltier with forced air (fan)
Connection type	Dual Camera Link Full (10 taps, 85MHz)
Adimec QUARTZ Q-12A180 camera Global Shutter CMOS	
Pixel size	5.5µm × 5.5µm
Camera resolution	4096 (H) × 3072 (W) pixel
Max frame rate (with 40x objective)	180 fps
Bit-depth	8 bit
Pixel resolution with 20x objective and 1.6x C-mount adapter	0.24µm
Pixel resolution with 40x objective and 1.6x C-mount adapter	0.12µm
Connection type	CoaXPress – 4 lane
Point Grey Grasshopper3 (GS3-U3-51S5M-C) Global Shutter Type CMOS (Sony IMX250)	
Pixel size	3.45µm × 3.45µm
Camera resolution	2448 (H) × 2048 (W) pixel
Frames rates	75 fps
Bit-depth	10-bit / 12-bit

Pixel resolution with 20x objective and 0.63x C-mount adapter	0.27 μm
Pixel resolution with 20x objective and 1x C-mount adapter	0.17 μm
Pixel resolution with 40x objective and 0.63x C-mount adapter	0.14μm
Pixel resolution with 40x objective and 1x C-mount adapter	0.09 μm
Connection type	USB 3.0

Control computer, minimum system requirements

Operating System	Microsoft Windows 7 Professional SP1 64-bit EN
CPU	2x Intel Xeon E5-2670v3 2.30GHz 30MB cache
RAM	8x 4GB DDR4-2133 rg ECC
Hard disk	3 TB
Optical drive	DVD-RW
Display	96 dpi
Graphics	SXGA (1280×1024, True color)
Input devices	Keyboard, mouse
Ports	1x CoaXPress Quad; 1x Double CameraLink port; 1x FireWire (1394 A); 4 × USB 2.0; GB Ethernet

Index