

Pannoramic Viewer 1.15.4 User's Guide

> July 25, 2014 Rev. 1

PV1.15.4-UG-EN Rev1

# Contents

Disclaimer	4
Character Formats and Symbols	6
Terms and Abbreviations	7
Keyboard Quick Reference	8
1 About Pannoramic Viewer 1.15.4	10
1.1 3DHISTECH Product Family	10
1.2 Marker Counter	11
2 Installing Pannoramic Viewer 1.15.4	12
2.1 Requirements for Installing and Using Pannoramic Viewer	
2.2 Installation	
2.3 Uninstalling Pannoramic Viewer	26
3 Starting Pannoramic Viewer	27
3.1 Pannoramic Viewer Start-up Options	27
3.1.1 Thumbnail	
3.1.2 Slide Window Defaults	
3.1.3 Resolution	
3.1.4 Viewer	
3.1.5 External Application Call for Image Capture 3.1.6 External Application Call for Annotation Export	
3.1.6 External Application Call for Annotation Export	
3.2 Configuring the Slide Display Scale	
4 Working with Pannoramic Viewer	
4.1 Menu Bar	
4.1 file Menu	
4.1.2 Settings Menu	
4.1.3 Window Menu	
4.1.4 Help Menu	
4.2 Managing Folders in the Navigation Tree	
4.3 Opening Digital Slides	
4.3.1 Opening slides locally	
4.3.2 Opening slides from a 3DHISTECH CaseCenter server	39
4.4 Upload and Download Slides to and from CaseCenter	40
4.4.1 Upload Slides to CaseCenter	
4.4.2 Download Slides from CaseCenter	43
4.5 Viewing Slides	44
4.5.1 Image creation method	44

4.5.2 Displaying information on the slide	
4.5.3 Using the full-screen mode	
4.5.4 Dragging and moving the slide up/down and left/right 4.5.5 Setting the magnification level	
4.5.6 Previewing a smaller area with a higher magnification	
4.5.7 Zooming	
4.5.8 Rotating slides	47
4.6 Working with Z-stack Slides	48
4.7 Working with Fluorescent Slides	49
4.8 Changing Image Properties	50
4.8.1 Setting up colors on a slide	
4.8.2 Setting up colors on a Fluorescent slide	
4.9 Working with Annotations	
4.9.1 Creating Annotations in Pannoramic Viewer	
4.9.2 Managing annotations	
4.9.4 Exporting annotations	
4.10 Measuring in a Digital Slide	
4.10.1 Length measurement	
4.10.2 Determination of perimeter and surface area	58
4.11 Displaying Information Relating to a Digital Slide	
4.11.1 Explanations to digital slide-related information	60
4.12 Batch Processing	62
4.13 Exporting Slides from Pannoramic Viewer	65
4.13.1 Image capture	
4.13.2 Saving a slide 4.13.3 Export to TIFF Image, Pannoramic Slide or to MetaXML	
4.13.3 Export to TIFF Image, Partnoramic Side or to MetaXML	
4.14 Synchronizing Slides in a Set	
4.15 Recording Tracking History	
4.15.1 Retrieving the tracking history	
5 Working with TMA Master Marker	73
5.1 Creating TMA Master Marker	
6 Working with Marker Counter (licensed feature)	
6.1 Managing Marker Categories	
6.1.1 Using existing marker categories	
6.1.2 Exporting Marker Counter results	
7 SlideDriver	
Appendix – Toolbar Buttons	
References	

# Disclaimer

Copyright © 2001-2014 3DHISTECH Ltd. All rights reserved.

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.

3DHISTECH Ltd. is not liable for damage of whatever nature (including, but not limited to, general or specific damage, indirect damage, consequential damage or incidental damage, including the results of the analysis of the digitized slides, for example, change of health status related to erroneous diagnosis from the digitized slide(s)) that stems from or is associated with use of Product, digitized slides, quality of staining, quality of stained slides, quality of used method of staining. 3DHISTECH Ltd. assumes no responsibility for the functionality and fault-free condition of your "application programs" (Workflows, VBA macros, Commander scripts).

#### 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, nor any other regulatory authority. The data and images obtained or viewed using this product are not intended for clinical or diagnostic use.

For performance evaluation only, the performance characteristics of this product have not been established.

#### Third-Party Disclaimer (LibTIFF – TIFF Library and Utilities)

3DHISTECH Ltd. is not liable for damage of whatever nature (including, but not limited to, general or specific damage, indirect damage, consequential damage or incidental damage, including damage resulting from lost profits, interruption of business, loss of business information, pecuniary loss or similar damage) that stems from or is associated with the incorporated part of this Product that is made by Silicon Graphics Inc.

THE SOFTWARE IS PROVIDED "AS IS" AND WITHOUT WARRANTY OF ANY KIND, EXPRESSED, IMPLIED OR OTHERWISE, INCLUDING WITHOUT LIMITATION, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

IN NO EVENT SHALL SAM LEFFLER OR SILICON GRAPHICS BE LIABLE FOR ANY SPECIAL, INCIDENTAL, INDIRECT OR CONSEQUENTIAL DAMAGES OF ANY KIND, OR FOR ANY DAMAGES WHATSOEVER RESULTING FROM LOSS OF USE, DATA OR PROFITS, WHETHER OR NOT ADVISED OF THE POSSIBILITY OF DAMAGE, AND ON ANY THEORY OF LIABILITY, ARISING OUT OF OR IN CONNECTION WITH THE USE OR PERFORMANCE OF THIS SOFTWARE.

#### **Further Information**

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

# **Character Formats and Symbols**

The following character formats and symbols are used in this User's Guide:

Example	Abbreviation or term that is explained in section <u>Terms and Abbreviations</u> (page 7).
Example	Words or characters that appear on the screen. These include field names, screen and window titles, pushbuttons and menu names, paths or options.
	Keys on the keyboard. For example, function keys (such as <b>F11</b> ) or the <b>Ctrl+M</b> key combination.
Example	Cross-references to other documents or sections within this document.
Warning!	Indicates that you need an additional module to use the feature or that there are prerequisites for the task.
Important!	Contains an important piece of information or a recommendation. The application will work if you choose not to follow the recommendation but its performance might be less than optimal.
(!) Tip!	Contains a suggestion about using the application in some other way or to some interesting purpose.

# **Terms and Abbreviations**

Annotation	A particular detail of a digitized slide on which measurements can be performed.
CSV format	Comma Separated Value
Digital slide	Slide digitized with a scanner microscope, for example, Pannoramic Scanner. Consists of an MRXS file and a folder with the same name as the MRXS file
Z-stack slide	A special type of digital slide containing multiple layers (focal planes). Its advantage is that the specimen can be viewed by stepping up and down the planes along the Z-axis.
Field	Annotation in an image analysis module.
Layer	2D image of one focal plane of the scanned slide.
MISP	A segmentation and measuring algorithm is stored in a MISP file.
MRXS	File extension of a digitized Pannoramic slide.
DPI	Dot Per Inch
Lossy compression	Type of data compression by which the original file size can be reduced but some of the actual information is lost.

# **Keyboard Quick Reference**

The following is a list containing keyboard shortcuts and key combinations. This list can be displayed in a pop-up window in Pannoramic Viewer by selecting the **Help / Pannoramic Viewer Help** option from the main menu, or by pressing **F1**.

Keyboard shortcut	Description
Up	Move slide upwards
Down	Move slide downwards
Left	Move slide left
Right	Move slide right
+	Increase magnification
-	Decrease magnification
ESC	Close Properties window
F1	Show help window
F2	Fit scanned area to window
F3	1x magnification
F4	2x magnification
F5	5x magnification
F6	10x magnification
F7	20x magnification
F8	40x magnification
F9	Display one pixel at the highest resolution of the image on one pixel of the monitor
F10	Menu
F11	Toggle full screen mode
F12	Switch to pan mode
Ctrl	During pressing, Pannoramic Viewer switches to pan mode
Ctrl+F4	Close active slide window
Ctrl+Shift+F4	Close all slide windows
Ctrl+M	Show / hide magnifier

Ctrl+O	Open slide or projects
Ctrl+P	Show / hide slide preview panel
Ctrl+S	Saves slide as an MRXS file (image compression method is the same as the original file's)
Ctrl+T	Show / hide slide tree
Ctrl+Z	Change bottom preview mode: (Preview / Slide tracking history / Preview + Slide tracking history

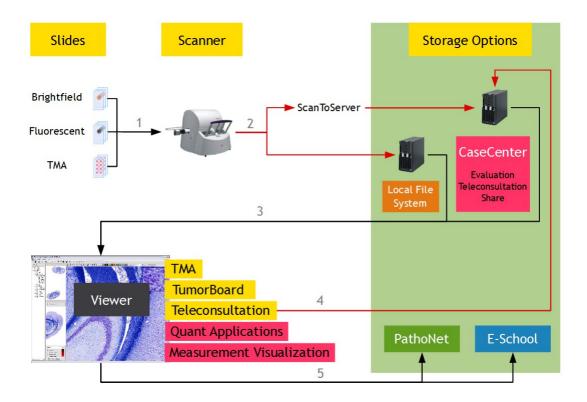
# 1 About Pannoramic Viewer 1.15.4

With Pannoramic Viewer, you can inspect, serialize, and annotate slides digitized with the Pannoramic microscope scanners.

# **1.1 3DHISTECH Product Family**

Pannoramic Viewer is the core 3DHISTECH product. You can purchase additional modules for it to cover various virtual microscopy tasks.

The Pannoramic products support various tasks in the routine and research pathologist's workflow.



- You digitize slides (which can also be tissue microarrays or slides stained with fluorescent probes) with a 3DHISTECH scanner microscope, for example, with Pannoramic SCAN 150. For information on the above mentioned microscope, see *Pannoramic SCAN 150 1.18 User's Guide*.
- 2. The Pannoramic scanner microscope creates digitized slides that can be saved on a local computer connected to the scanner, or if the ScanToServer application is installed, the slides can be uploaded through the scanner software directly to the CaseCenter server where slides can be evaluated, shared, and you can also create Teleconsultation session to discuss specific cases with pathologists.

3. Slides can be opened from the local computer or the CaseCenter via Pannoramic Viewer, you can inspect the local slides and you can create *annotations* on them to mark interesting areas.

Pannoramic Viewer is a platform for the following applications, called modules:

With the **TMA** module, you can view and analyze slide sets in which each slide is a cut from a tissue microarray. Combined with the **TumorBoard** module, the TMA module provides you with a powerful tool to perform research-scale analyses on digital slides.

With the **TumorBoard** module, you can perform an immunohistochemical analysis on a slide set. If you purchase the TMA module as well, you can run your **TumorBoard** analysis on a tissue microarray and score the TMA project in **TumorBoard**.

4. With the **Teleconsultation** module, you can upload slides to a dedicated CaseCenter server and share them with other Pannoramic Viewer users to request their analysis. CaseCenter allows you to view remote slides (slides stored on a 3DHISTECH CaseCenter server). With this application, you can also host a *Teleconsultation* session or participate in a session initiated by another user.

With the Quant applications such as **HistoQuant**, **MembraneQuant**, **NuclearQuant**, **FISHQuant**, **CISHQuant**, **CISH-RNSQuant**, and **DensitoQuant** modules, you can define algorithms to measure various parameters on a slide. Combined with **TumorBoard**, you can run the Quant algorithm on one slide or on a set of slides (grouped into an **TumorBoard** project).

**NOTE: FISHQuant**, **CISHQuant**, and **CISH-RNSQuant** modules can be launched only in the QuantCenter application only.

 You can upload slides to Pathonet or share slides for E-School. For more information on E-School, see *E-School 1.11 User's Guide*. One example of a server-based teleconsultation solution is <u>www.pathonet.org</u>.

## **1.2 Marker Counter**

Pannoramic Viewer includes **Marker Counter as a licensed feature** to count cells and objects manually. You can define counter categories that Marker Counter uses, and you can move and reset Marker Counter on-screen.

After creating a Marker Counter annotation and placing markers within the region, the application automatically generates the values and shows them in a pop-up window. You can use this tool, for example, to keep track of the following data:

- number of marked objects or labeled areas
- percentage of positive cells within the examined area
- average intensity of marked objects within their category

All measured parameters can be stored in the digital slide or can be exported to a database.

# 2 Installing Pannoramic Viewer 1.15.4

## 2.1 Requirements for Installing and Using Pannoramic Viewer

Operating system	Microsoft Windows 7 SP1 64 bit or Windows 8 EN
Hardware	Minimum: Pentium P4 1.8 GHz, 2GB RAM (4GB RAM if running under Server OS) <b>Recommended</b> : Pentium C2D (DualCore) 2.4 GHz, 4GB RAM
Monitor	Recommended: 96 DPI
Screen resolution	Minimum: CRT – 1280×960 TFT – 1280×1024 Recommended: CRT – 1600×1200 or better TFT – 1680×1050, 1920×1080 or better
LAN/WAN	LAN: 10 Mbit/s or better networking interface Minimum bandwidth to remote servers: 1,000 kbit/s (DSL)
Disk space	Minimum: 300MB

## 2.2 Installation

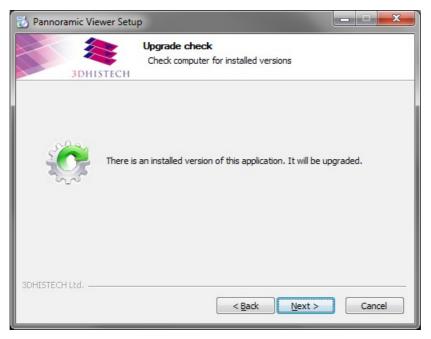
You receive the Pannoramic Viewer installation files on an installation disk, or downloaded in an executable file.

- 1. Start the installation:
  - From the CD/DVD-ROM:
    - I. Insert the Pannoramic Viewer installation disk into the CD or DVD-ROM.
    - II. If the start-up screen does not appear automatically, launch the Pannoramic\_Viewer\_1\_15\_4\_xxxxx\_RTM.exe file in the root directory of the disk.
  - From the compressed file, run the **Pannoramic\_Viewer\_1\_15\_4\_xxxxx\_RTM.exe** file.

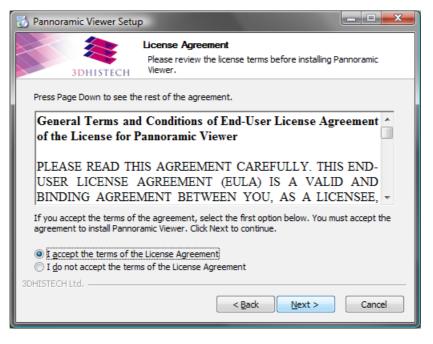
2. Follow the instructions in the installation wizard, and click **Next** to start installation.



3. If you have a different version of the Pannoramic Viewer, it must be upgraded.



4. Accept the End-User License Agreement, and click Next.



5. After the installation of prerequisite files has finished, click Next.

📸 Pannoramic Viewer Setup			_ <b>_</b> X
	<b>quisites</b> w the prerequisites of	the installation.	
Status			
Microsoft Visual C++ 2008 SP1   Microsoft Visual C++ 2010 SP1		Installed Installed	
All of required components are in To continue click Next button.	nstalled.		
3DHISTECH Ltd. ————————————————————————————————————	< <u>B</u> ack	<u>N</u> ext >	Cancel

6. Click **Next** to install SlideDriver Redistributable Package.

🔂 Pannoramic Viewer Setup	
3DHISTECH	Choose Components Choose which features of Pannoramic Viewer you want to install.
Check the components you install. Click Next to continue	want to install and uncheck the components you don't want to e.
Select components to install	SlideDriver Redist x86
Space required: 131.9MB	
3DHISTECH Ltd. —————	< <u>B</u> ack Next > Cancel

7. Choose the installation folder for Pannoramic Viewer. Click **Browse** for locating a different folder than the default one, then click **Next** to continue.

**NOTE:** As during upgrade, the location of the previously installed software cannot be modified, this step is missing from the procedure.

🔂 Pannoramic Viewer Setup
3DHISTECH Choose Install Location Choose the folder in which to install Pannoramic Viewer.
Setup will install Pannoramic Viewer in the following folder. To install in a different folder, click Browse and select another folder. Click Next to continue.
Destination Folder           C:\Program Files\3DHISTECH\Viewer         Browse
Space required: 131.9MB Space available: 11.2GB
3DHISTECH Ltd

8. Select the Start menu folder for creating shortcuts of the program.

📆 Pannoramic Viewer Set	up 📃 🗆 💻
3DHISTECH	Choose Start Menu Folder Choose a Start Menu folder for the Pannoramic Viewer shortcuts.
can also enter a name to o	
3DHISTECH\Pannoramic 3DHISTECH Accessories	Viewer
Administrative Tools Adobe Extras and Upgrades	
Do not create shortcut	
3DHISTECH Ltd,	
	< Back Install Cancel

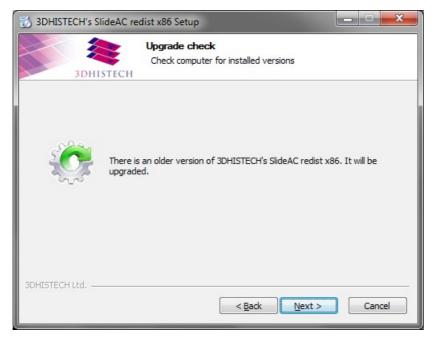
A progress bar and an information box is displayed, showing the status of the installation process.

📸 Pannoramic Viewer Setur	
3DHISTECH	Installing Please wait while Pannoramic Viewer is being installed.
Execute: "C:\Users\TAMAS	~1.CSO\AppData\Local\Temp\nse6777.tmp\CaseCenterClient_Rec
Extract: SlideAC_Redist_ Execute: "C:\Users\TAMA Output folder: C:\Users\T Extract: CaseCenterClient	AMAS~1.CSO\AppData\Local\Temp\nse6777.tmp (86.exe 100% S~1.CSO\AppData\Local\Temp\nse6777.tmp\SlideAC_redis AMAS~1.CSO\AppData\Local\Temp\nse6777.tmp t_Redist_x86.exe 100% S~1.CSO\AppData\Local\Temp\nse6777.tmp\CaseCenterC
3DHISTECH Ltd, —————	< <u>B</u> ack <u>N</u> ext > Cancel

9. The installation procedure pauses to start to install SlideAC Redistributable package. Click **Next** to continue.



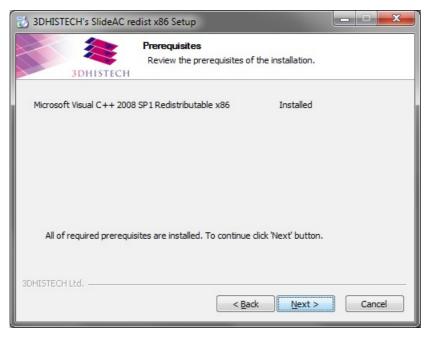
10. If an older version of SlideAC has been found on your computer, it must be upgraded.



11. Click I Agree to accept the EULA, and continue with the installation.



12. The installer checks for the prerequisite of SlideAC, and if a relevant version has been found, click **Next** to continue.



13. The location of SlideAC cannot be modified, so click **Install** to continue.

🔂 3DHISTECH's SlideAC redist x86 Setup						
3DHISTECH	Choose Install Location Choose the folder in which to install 3DHISTECH's SlideAC redist x86.					
Setup will install 3DHISTECH's SlideAC redist x86 in the following folder. To install in a different folder, click Browse and select another folder. Click Install to start the installation.						
Destination Folder C:\Program Files (x86)	\3DHISTECH\SlideAC-redist Browse					
Space required: 280.7MB Space available: 63.4GB						
3DHISTECH Ltd, —————	< <u>B</u> ack Install Cancel					

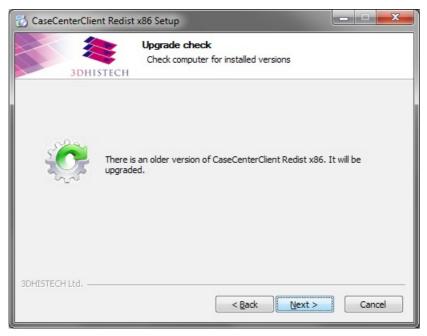
14. If the installation procedure of SlideAC has been finished, click **Finish** to close the window.



15. The installation procedure pauses to start to install CaseCenterClient Redistributable package. Click **Next** to continue.



16. If an older version of CaseCenterClient has been found on your computer, it must be upgraded.



17. Click I Agree to accept the License Agreement, then click Install.



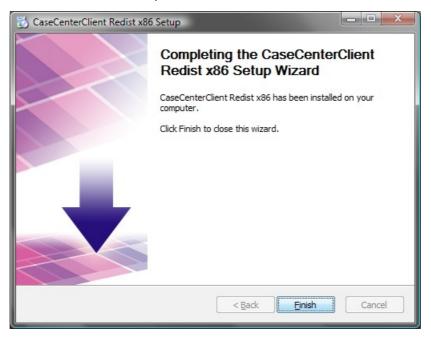
18. The location of CaseCenterClient cannot be modified, so click **Install** to continue.

🔂 CaseCenterClient Redist x86 Setup					
3DHISTECH	Choose Install Location Choose the folder in which to install CaseCenterClient Redist x86.				
	Client Redist x86 in the following folder. To install in a different ct another folder. Click Install to start the installation.				
Destination Folder C:\Program Files (x86)\3	3DHISTECH\CaseCenterClient-redist Browse				
Space required: 5.3MB Space available: 63.4GB 3DHISTECH Ltd,					

A progress bar and an information box is displayed, showing the status of the installation process.

🔂 CaseCenterClient Redist x86 Setup					
Installing           3DHISTECH         Please wait while CaseCenterClient Redist x86 is being installed.					
Registering: C:\Program Files\Common Files\3DHISTECH\TreeManager.dll					
Output folder: C:\Program Files\Common Files\3DHISTECH Extract: C:\Program Files\Common Files\3DHISTECH\CLCHand.dll 100% Registering: C:\Program Files\Common Files\3DHISTECH\CLCHand.dll Extract: C:\Program Files\Common Files\3DHISTECH\SlideIOINet.dll 100% Registering: C:\Program Files\Common Files\3DHISTECH\SlideIOINet.dll Registering: C:\Program Files\Common Files\3DHISTECH\SlideIOINet.dll Extract: C:\Program Files\Common Files\3DHISTECH\SlideIOINet.dll Registering: C:\Program Files\Common Files\3DHISTECH\SlideIOPS.dll Extract: C:\Program Files\Common Files\3DHISTECH\TreeManager.dll 100%					
3DHISTECH Ltd					

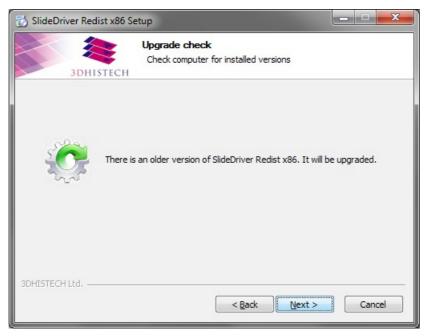
19. Click Finish to finalize and quit the installation of CaseCenterClient Redistributable Package.



20. The installation procedure now is halted to launch the installation of SlideDriver Redistributable package. Click **Next** to continue.



21. If an older version of CaseCenterClient has been found on your computer, it must be upgraded.

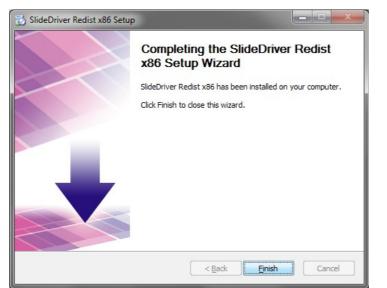


22. The installer checks for the prerequisite of SlideDriver, and if a relevant version has been found, click **Next** to continue.

🔂 SlideDriver Redist x86 Setup					
Prerequisites 3DHISTECH Review the prerequisites of	the installation.				
Microsoft Visual C++ 2010 SP1 Redistributable x86	Installed				
All of required prerequisites are installed. To continue click 'Next' button.					
3DHISTECH Ltd	Cancel				

23. The location of SlideDriver cannot be modified, so click Install to continue.

SlideDriver Redist x86 Setup						
3DHISTECH	Choose Install Location Choose the folder in which to install SlideDriver	Redist x86.				
	Redist x86 in the following folder. To install in a d her folder. Click Install to start the installation.	fferent folder,				
Destination Folder C: \Program Files (x86)\	3DHISTECH\SlideDriver-redist B	zowse				
Space required: 557.0KB Space available: 63.4GB 3DHISTECH Ltd,	< <u>B</u> ack	Cancel				

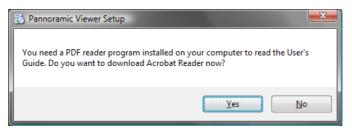


24. Click **Finish** to finalize and quit the installation of SlideDriver Redistributable Package.

25. Leave the **Show User's Guide** option checked to open the folder containing this User's Guide. Click **Finish** to close installation wizard.

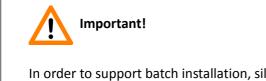
🔂 Pannoramic Viewer Setup			
	Completing the Pannoramic Viewer Setup Wizard		
	Pannoramic Viewer has been installed on your computer.		
	Click Finish to close this wizard.		
	Check to place shortcuts only for current user. Show User's Guide.		
	< Back Finish Cancel		

26. If the installer cannot find Adobe Reader on your computer, the below dialog box appears. Click **Yes**, and the official Adobe website will be opened in your browser where you can download the Adobe Reader installer file from.





Keep the installation disk, or back up the compressed installation file – it is needed for removing the application and also for upgrading the product.



In order to support batch installation, silent mode is available: Pannoramic Viewer can be installed without any user interaction needed, and with no display of the above windows.

Launch **Pannoramic\_Viewer\_1\_15\_4\_xxxxx\_RTM.exe** adding a **/S** switch (upper case is important) to the command line to activate silent mode.

# 2.3 Uninstalling Pannoramic Viewer



The digital slides that you open in Pannoramic Viewer, or the files created by the application are not removed when you uninstall Pannoramic Viewer. You can remove them manually if you do not need them any more.

Start the removal process from the program folder under the Windows Start menu:

- Press the **Start** menu button in Windows.
- Open All Programs/3DHISTECH/Pannoramic Viewer folder.
- Select Uninstall.

or, remove Pannoramic Viewer through the Control Panel:

- Press the **Start** menu button in Windows.
- Open Control Panel/Programs-Uninstall a Program option if using Windows 7.
- Select **Pannoramic Viewer** from the list.
- Click **Remove/Uninstall**.

# 3 Starting Pannoramic Viewer

Start Pannoramic Viewer in any of the following methods:

- From the shortcut on the Desktop
- From the Windows menu: Start/Programs/3DHISTECH/Pannoramic Viewer
- By opening an annotation link
- By opening a Pannoramic slide (.mrxs file)

If running Pannoramic Viewer for the first time, make sure to follow the below procedures:

- Open start-up options of Pannoramic Viewer in Settings / Options (see section <u>3.1</u> Pannoramic Viewer Start-up Options).
- Calibrate the slide display scale-bar (refer to section **3.2 Configuring the Slide Display Scale**).

## 3.1 Pannoramic Viewer Start-up Options

Various parameters are available for a desired user-defined graphical appearance of the Pannoramic Viewer, once the program has started. These properties can be modified by selecting **Options** in the **Settings** menu.

## 3.1.1 Thumbnail

• Size

This option allows you to determine the size of the various preview images (**Small, Normal** or **Large**) displayed when the preview function is on. The larger the thumbnail images are, the more space is available for the name of the digital slide.

• Type

This option defines the representation mode in the thumbnail view.

- **Barcode area** Displays only the barcode area as a thumbnail image in the gallery if the barcode area was recorded as an image by the digitizer. Otherwise, only the digital slide's thumbnail view is displayed.
- **Preview camera image** Displays the barcode area and the digital slide as a thumbnail image in the gallery (as taken by the preview camera) if the barcode area was recorded by the digitizer. Otherwise, only the digital slide's thumbnail view is displayed.
- Low resolution scanned image Displays the digital slide as a thumbnail image (taken by the scan camera) in the gallery.

#### Caching

Options	
Thumbnail (changes will take effect immediately) Size C Small C Normal C Large Caching Cache Folder: C:\ProgramData\3DHISTECH\\Viewer\LocalCache\ Maximum cache size: 50 MB Clear Cache	Resolution (changes will take effect at next Pannoramic Viewer start)         Screen resolution:         96         ÷         DPI         External Application Call for Image Capture (changes will take effect immediately)         Application:       C:\Program Files\IrfanView\iuterview32.exe         Start in:       C:\Program Files\IrfanView         Parameters:       " <image/> "
Slide Window Defaults (changes will take effect at next slide open)         Image: Show slide preview         Image: Show preview in full screen mode         Image: Top preview 'Fit scanned area'         Image: Top scale area station warning         Image: Step size:         Image: Top scale area Network and Internet         Image: Top Image: Top Scale area Network and Internet         Image: Top Image: Top Scale area Network and Internet         Image: Top Imag	Run:       Normal window         Image format:       PNG         Quality factor:       0         External Application Call for Annotation Export (changes will take effect immediately)         Application:          Start in:          Parameters:       " <slide>" <x1> <y1> <x2> <y2>         Run:       Normal window</y2></x2></y1></x1></slide>
Image: Prefetch (recommended)         Image: Show scale bar         Brightfield colors         Fluorescent colors         Viewer (changes will take effect immediately)         Image: Show thumbnails         Image: Show slide tree	Image: Comparison of the comparison

You can locate a caching folder, and determine the disk cache size.

## 3.1.2 Slide Window Defaults

#### Show slide preview panel

This option displays the preview panel in the window of the digital slide if active. Preview panel can be turned off again with the **Ctrl+P** hotkey.

#### Show preview panel in full screen mode

When selecting full screen mode, the slide preview panel is displayed.

#### • Top preview 'Fit scanned area'

If this option is active, the preview image shows only that slide area which is actually covered by a specimen for display.

#### Show magnifier

Allows a magnifier to be superimposed in the top right corner of a digital slide's window. The magnifier can always be switched on and off with the Ctrl+M hotkey.

#### Use BestView for all slides

Optimizes the view according to the "*Best Fit*" method (see section <u>4.8 Changing Image</u> <u>Properties</u>) for the whole slide. If activated, relevant color settings are applied when opening a slide for the first time.

#### Show colorization warning

With this option set, you will be warned every time you open a slide, which colorization differs from the default value.

A dialog box appears at the bottom of the slide window. If you want to reset color settings to default, click **Yes**, otherwise click **No**.

#### Step size

Set how much the keyboard arrows shift the image. For example, if you set the **Step value** to 50%, Pannoramic Viewer moves the focus area with half of the image.

#### Progressive Rendering

This option defines the mode of how the screen is built up and updated. With Progressive Rendering the digital slide is first loaded with a low resolution and afterwards updated with a higher resolution. If Progressive Rendering is disabled, the complete viewing area of the digital slide is shown with the selected resolution, without any intermediate views:

- Always Progressive Rendering is always carried out.
- Local Area Network and Internet Progressive Rendering is carried out only if the digital slide is displayed via LAN or the Internet.
- Internet Progressive Rendering is carried out only if the digital slide is displayed via the Internet (recommended setting).
- **Never** Progressive Rendering is never carried out.

#### Prefetch (recommended)

If this option is active, neighboring areas outside the visible zone are loaded. This way, a faster view build-up process is reached.

#### Show Scale Bar

By enabling this option, a scale bar with a scale value appears in the slide's window. You can also set the colors of the scale bar, the text, and their background both for brightfield and fluorescent slides.

#### Bottom Preview Mode

This is to define if the variable preview of a related specimen (Preview), the tracing mask (Slide tracking history) or both features (Preview and Slide tracking history) are displayed by default when a new digital slide is opened. Toggle between modes with the **Ctrl+Z** hotkey.

#### Bottom Preview Zoom

Allows you to define a default zoom value for displaying the variable preview image. This value, however, can be specifically matched to each digital slide later on.

#### Slide Tracking History Color

This property defines the color of the tracing mask (Slide tracking history). Change color by clicking the vertical bar of this function in the main program window.

### 3.1.3 Resolution

Screen resolution is for setting the currently connected monitor's display screen resolution in units of DPI (refer to section *2.1 Requirements for Installing and Using Pannoramic Viewer*).

### 3.1.4 Viewer

#### Show thumbnails

This option defines if the thumbnail view screen of digital slides is to be in 'ON' or 'OFF' state when a Pannoramic Viewer session starts. If the preview image takes much time to build up (for example, when accessing a remote server), this option can be turned off. It can be turned on again at any time during the program session.

#### Show slide tree

This option defines if the tree view of the digital slides is to be displayed or not when starting the Pannoramic Viewer session.

## 3.1.5 External Application Call for Image Capture

The snapshot function allows you to export the displayed image to raster image viewers or editors (refer to section **<u>4.13.1 Image Capture</u>**). The related settings can be made in this area are as follows:

#### Application

This option defines the program to which the image information is exported. Browse for the folder of the application, and select the executable file.

#### Start in

You can define the working path of the exporting process. It is recommended to define the path in which the program to be called is located.

#### Parameters

 "<IMAGE>": When the image data are exported, the file name of the digital slide can also be transferred.

#### Run

Select the size of the window in which the export procedure is presented:

- **Normal window** The program is launched in a normal window.
- **Minimized** The program is displayed in a minimized window.
- **Maximized** The program is displayed in a maximized window.

#### Image format

The image format to be exported is defined here. The following formats can be selected: JPEG, TIFF, BMP, and PNG.

#### Quality factor

Only if the JPEG image format is selected, you can set the quality factor (ranges from 1 to 100%). This setting is not possible for other image formats.

External Application Call for Image Capture (changes will take effect immediately)				
Application:	C:\Program Files\IrfanView\i_view32.exe			
Start in:	C:\Program Files\IrfanView			
Parameters:	" <image/> "			
Run:	Normal window 💌			
Image format :	PNG			
Quality factor				

## 3.1.6 External Application Call for Annotation Export

When administering annotations, the currently displayed digital slide and a selected annotation can be opened in another program (refer to section <u>4.9.2 Managing Annotations</u>). You can make the corresponding settings in this field.

#### Application

This option defines the program, to which the image information is exported. Browse for the folder of the application, and select the executable file.

#### Start in

You can define the working path of the exporting process. It is recommended to define the path in which the program to be called is located.

#### Parameters

When the image data are exported, other parameters can also be transferred:

- "<SLIDE>" The file name and the path of the digital slide currently displayed are transferred.
- **<X1>** The x value for the top left corner of the annotation is transferred.
- **<Y1>** The y value for the top left corner of the annotation is transferred.
- **<X2>** The x value for the bottom right corner of the annotation is transferred.
- **<Y2>** The y value for the bottom right corner of the annotation is transferred.
- Run

Select the size of the window in which the export procedure is presented:

- Normal window The program is launched in a normal window.
- **Minimized** The program is displayed in a minimized window.
- **Maximized** The program is displayed in a maximized window.

**NOTE:** Annotation coordinates can be exported if necessary. For further information, see section **4.13.4 Export Annotation Coordinates**.

## 3.1.7 Enable the Use of SlideDriver

After the SlideDriver has been connected to one of communication ports (COM port), activate the function in this window, then click the **Auto detect hardware settings** button.

To enable zooming level to have effect on the slide motion speed when rotating X/Y/Z controls of the SlideDriver, first check the **Motion speed depends on actual zoom level** option, then drag the Speed slider towards the desired direction, either Fast or Slow.

For further information on how to use SlideDriver, see section 7. SlideDriver.

# 3.2 Configuring the Slide Display Scale

In Pannoramic Viewer a calibrated scale can be displayed on the digital slide window. Because the scale integrates the current values for display screen resolution and pixel geometry, the display scale must be properly calibrated before using the application. This input is required only once, unless your monitor is replaced with a new one or you change your monitor settings.

1. Open the Slide Display Scale Setup window by selecting the Settings/Slide Display Scale Setup option.

Slide Display Scale Setup			<b>X</b>
Measure the length of the red li	ne in millimeters and e	nter it below.	
•			•
Displayed line length:	Short 🔻		
Measured line length:	179,1	mm	
Extra slide display zoom factor:		2	
Size of the display pixel :	0,12403	mm	
Reset to default			Apply Cancel

- 2. Select the length of the bar (**Short**, **Middle**, or **Long**), measure the displayed line with a ruler, then type in the value in millimeters into the **Measured line length** field.
- 3. Set the Extra slide display zoom factor (enables extra zoom on slides).
- 4. To apply settings and close the window, click **Apply**. To exit the window without applying changes, click **Cancel**. Upon clicking the **Reset to default** button, setup parameters will be reset to their default values.

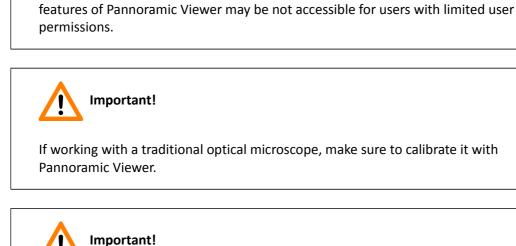
To edit Scale bar settings select **Settings/Options** menu and click either **Brightfield** or **Fluorescent** buttons to open the Scale Bar Colors editor window. Click the palette buttons, and select predefined colors or create a unique one.

Scale Bar Colors	×
Scale bar color:	
	9
Text color:	
	۰
Background color:	
NONE	۰
Apply Cancel	

Click **Apply** when finished editing the color of the scale bar/text/background.

# 4 Working with Pannoramic Viewer

Important!



Since user privileges or access rights depend on Microsoft Windows settings, some

The area of a specific ROI may differ by less than 0.1% when opening the slide with Pannoramic Viewer 1.14.5 or 1.15.4.

## 4.1 Menu Bar

The main window menu bar located at the upper section of the program window contains menus grouped into the following categories: **File**, **Settings**, **Window**, and **Help**.

### 4.1.1 File Menu

The File menu includes the following options:

 $\sim$ 

trl+N
trl+0
Ctrl+S
Ctrl+E

- Open... Open slide or folder (for further information, see section <u>4.3 Opening Digital</u> <u>Slides</u>)
- Save Slide as... Save slide with a different name, and/or to a different location. Set magnification level, and the location where the slide will be saved.

Save	e As			<b>X</b>
	Image file format : Quality factor : Magnification: : Destination path:	JPEG 90% 1:2 c:\10301-07_HE_I-1		Browse
			Save	Cancel

- Slide Export... Export slide or its annotated areas with a different image compression method (see section <u>4.13.3 Export to TIFF Image, Pannoramic Slide or to MetaXML</u> for more information)
- **Close** Close the active slide window
- Exit Exit Pannoramic Viewer

### 4.1.2 Settings Menu

Settings	
Options	
Slide Display Scale Setup	

- Options Display and SlideDriver settings can be accessed and modified within the Options window. For more information, see section <u>3.1 Pannoramic Viewer Start-up Options</u>.
- Slide Display Scale Setup Set scale attributes, both for Brightfield and Fluorescent slides (see section <u>3.2 Configuring the Slide Display Scale</u> for more information on the settings).

### 4.1.3 Window Menu

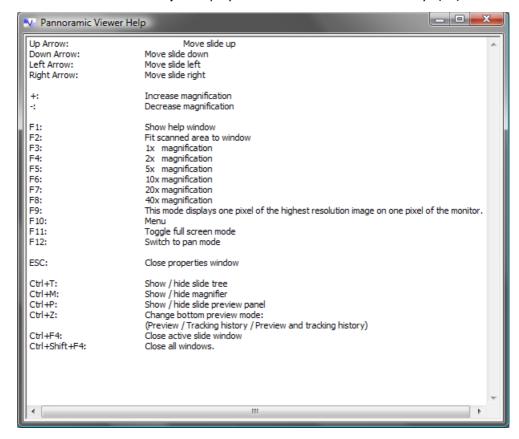
🖶 Cascade
Tile Horizontally
Tile Vertically
Minimize All
Arrange All
Close All Shift+Ctrl+F4
1 Local: 10301-07_HER2_I
2 Local: 10301-07_HE_I-1
✓ 3 Local: 43_BF_Z7_3

- Cascade Slide windows are displayed according to this option by default
- Tile Horizontally Arrange slide windows horizontally
- Tile Vertically Arrange slide windows horizontally
- Minimize All Minimized slide windows are placed to the bottom of the main window
- Arrange All Arranges minimized slide windows to their default position on the main window
- Close All Close all slide windows

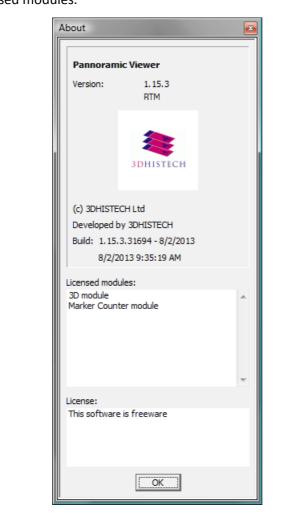
Open slide windows are listed with numbers, and a check mark can be seen on the left side of the active window.

### 4.1.4 Help Menu

F	lelp
	Pannoramic Viewer Help
	About



• Pannoramic Viewer Help – Display a list of control and shortcut keys (F1)



• **About...** – Display developer information about the release version of Pannoramic Viewer and the licensed modules.

# 4.2 Managing Folders in the Navigation Tree

You can open slides one-by-one by browsing them though a file manager software (Windowsembedded or independent).

Slides can be opened faster, however, if you add their folders to the navigation tree on the main program window of Pannoramic Viewer. You can add both local and remote folders to the navigation tree.

### Adding a folder to the navigation tree

- 1. If the navigation frame is not visible, click 📴 to display it.
- 2. Depending on the location of the folder, select the **Local** tab or the **Teleconsultation Server** tab in case you are connected to a CaseCenter server or Pathonet.

Local Teleconsultation Server		
Image: Consultation Server           Image:		
Refresh		
0040	🖻 🛃  😵	
Slide Name	Slide Path	
10301-07_ER_I	C:\SLIDES\WQ\1030	
10301-07_HE_I-1	C:\SLIDES\WQ\1030	
•		

3. In the main window toolbar, click 🛃.

🔨 Open Slide or 3D Project	
Local Network	List Preview
SLIDES SLIDES SLIDES MQ NQ SWShare SWTOOLS SWWork	Name     Size       10301-07_HER_1     771 MByte       TMATMA_Project_01     5 KByte       TMATMA_Project_02     4 KByte
Update Tree Set Home Folder Add Folder	Open Close

- 4. Browse for the directory that you need and then click Add Folder.
- 5. If you want Pannoramic Viewer to start with this folder every time you open the application, click **Set Home Folder**.

**Remove a folder from the navigation tree** by right-clicking the folder, then select **Remove Project from Tree** from the shortcut menu.

# 4.3 Opening Digital Slides

You can open digital slides from a local storage (disk drive or connected drive) or remotely from a CaseCenter server.



### 4.3.1 Opening slides locally

To open a slide you can choose from the following options:

- Select File / Open.
- Press Ctrl+O.
- Click the Button in the toolbar.
- In the navigation tree, select the **Local** tab, browse for the folder and then double-click the slide.
- If the slide is already displayed as a thumbnail in the preview frame, double-click the thumbnail.

### 4.3.2 Opening slides from a 3DHISTECH CaseCenter server

CaseCenter servers are set up by default, so that only authorized users can access them. A server administrator must give you a user name and password before you can open slides from the server in Pannoramic Viewer.

Depending on the type and speed of the connection, some Pannoramic Viewer screens may take longer to load from the server than from a local drive.

- 1. Click the 🛄 icon in the toolbar.
- 2. Enter the **URL** or the **IP address** of the CaseCenter and log in to the server with your user name and password.

Check **Secure connection** option if you want to connect through HTTPS.

3. Click **OK** to continue.

Connect to Teleconsultation Server		
Address :	172.16.252.252	
User Name :	user_01	
Password :	********	
Secure connection :	$\overline{\mathbf{v}}$	
Cancel		

After you logged in to the server, you can see the remote folders and slides in the navigation tree on the **Teleconsultation Server** tab.

Important! If a user is connected to a CaseCenter slide storage server – for example, mapped a network drive or uses a shared printer – slides can be accessed on that CaseCenter server only through HTTP / HTTPS.

# 4.4 Upload and Download Slides to and from CaseCenter

### 4.4.1 Upload Slides to CaseCenter

- 1. Select any of the following options to upload slides to the server:
  - Click in the toolbar, then in the displaying window, enter the **URL** or the **IP address** of the server you want to connect to. Log in with your user name and password.

Select a slide or folder containing a set of slides from the tree in the Local tab. Rightclick the slide or folder, then choose **Upload Slide(s) to Teleconsultation Server**. • Batch upload is available when creating a list of slides from various locations (folders or disk drives).

After selecting a folder or slide in the Local tree, press the  $\bigcirc$  icon below the Navigation Tree panel or the  $\bigcirc$  icon to remove slide(s) from the list. Items added to the list can be uploaded to the server by clicking the  $\bigcirc$  icon.

Local	Teleconsult	ation Server
□····································		
Refresh		
	🕹 🛃 i	• 4 🛛
Slide Nar	ne	Slide Path
10301-0	7_ER_I	C:\SLIDES\WQ\1030
10301-0	7_HE_I-1	C:\SLIDES\WQ\1030
•		4

Type in the **Address** of the server, the **User Name** and **Password** in the Connect to Teleconsultation Server window. Check **Secure connection** option if you want to connect through HTTPS. Click **OK** to continue.

Connect to Teleconsultation Server		
Address :	172.16.252.252	
User Name :	user_01	
Password :	*******	
Secure connection :	$\overline{\mathbf{v}}$	
OK Cancel		

2. Set upload parameters, and click **OK**.

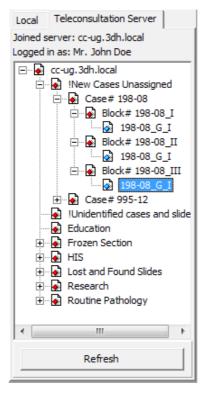
Access Rights Public Remote Path
Allow saving Read only Automatic Delete from Server Never September, 2013 25 26 27 28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2 3 4 5 Control of Pathology Control of Pathology C
Refresh Tree OK Cancel

Access Rights (Pathonet-related option)	If you want to upload the slide to Pathonet, share it by selecting <b>Public</b> , make the slide <b>Read only</b> and/or downloadable by selecting <b>Allow saving</b> .
Automatic Delete from Server	Set the date of deletion from the drop-down list (1/2/3/5 Days, 1/2/3 Weeks, User Defined, or Never). The date can be selected in the calendar as well.
Remote Path	Select the location of the slide on the server.

3. You can either add more slides to the list, change **Slide Parameters**, or remove slides by clicking the **Delete from List** button in the Slide Upload window. When all the slides you want to upload to the CaseCenter server are listed in the window, press the **Start Upload** button.

•	V Slide Upload			
	Slide Name:	Slide Path:	Status:	
	43_BF_Z7_3	C:\SLIDES\43_BF_Z7_3	Prepared for upload	
	Colcc-B-Cat-Erbit-B2	C:\SLIDES\Colcc-B-Cat-Erbit-B2	Prepared for upload	
•			•	
	Slide Parameters Delete from List	Start Upload Stop	Close	

4. To update the tree, click the **Refresh** button.



**NOTE:** When updating the tree, only the content of an expanded folder will be refreshed.

### 4.4.2 Download Slides from CaseCenter

Select any of the following options to download slides from the server:

- Batch download is available when creating a list of slides from various folders:
  - 1. Select a slide or folder containing a set of slides from the tree in the Teleconsultation Server tab.
  - After selecting a folder or slide in the navigation tree on the Teleconsultation Server panel, press the sicon below the Navigation Tree panel or the sicon to remove slide(s) from the list.

002	n 🛃 🛛 🗵
Slide Name	Slide Path
198-08_G_I	!New Cases Unassig
198-08_G_I	!New Cases Unassig
•	•

3. Items added to the list can be downloaded from the server by clicking the icon.

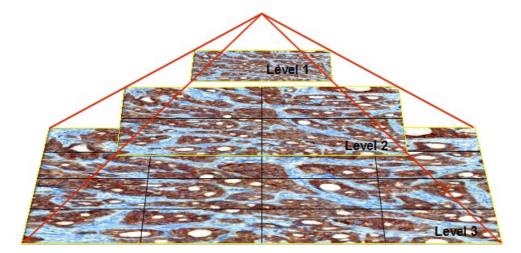
- 4. Browse for the output folder.
- 5. Click Start.
- Slides can be downloaded one by one by using the **Save Slide as...** function described in section **4.13.2 Saving a Slide**.

### 4.5 Viewing Slides

### 4.5.1 Image creation method

Slide images in Pannoramic Viewer are represented by a tiled image pyramid. The image pyramid is a hierarchical structure composed of *n* levels of the same image of different resolutions. This allows the rendering engine to grab only that bit of data that is necessary for a particular view of a digital slide image.

However, if the user is zoomed in to a specific area of a large image, then only those tiles needed to show the specific areas are downloaded. This can lead to very large bandwidth savings because often only some aspects of a large image are interesting to the user.



The below illustration shows what the image pyramid looks like conceptually. At each level of the pyramid, the image is scaled down by 4 (a factor of 2 in each dimension).

At the bottom of the pyramid is the given full size image. Each set of  $2 \times 2=4$  neighboring pixels is replaced by their average as the pixel value of the image at the next level.

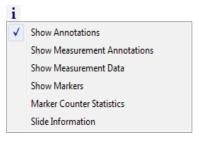
This process, which reduces the image size by half, is repeated n=log2N times until finally an image of only 1 pixel (average of entire image) is generated as the top of the pyramid.

### 4.5.2 Displaying information on the slide

Each digital slide window displays the following two pieces of information:

- The size of the window currently on display in relation to the specimen and the selected magnification.
- The loading progress of a current view: with digital slides available on local sources, the loading
  process is very fast so the progress bar is of secondary signification in this case. However, where
  digital slides on remote servers are accessed, this bar shows the latest progress state of
  downloading.

When clicking the *i* button in the digital slide window, a selection menu with information items in relation with the digitized digital slide (Slide info) appears on the display.



# Important! Select Show Measurement Panel from the Viewer's INFO menu to display detailed measurement information according to annotations only when the analysis process is finished, otherwise the slide must be reopened.

### 4.5.3 Using the full-screen mode

To switch to full-screen mode, click the  $\square$  button in the main toolbar or press **F11** on the keyboard. To return from the full-screen mode, press **F11** on the keyboard.

### 4.5.4 Dragging and moving the slide up/down and left/right

# (!) Tip!

Use clicks in the preview window rather than holding down the mouse button when you move around on a slide: Pannoramic Viewer renders the image faster if you move the mouse in discrete steps rather than with a continuous movement. When using SlideDriver as the operating device for moving the image, press the button defined for X,Y resolution change to enable faster movement.

To enable moving and dragging, click the <sup>1</sup> button in the slide toolbar. Then use any of the following three options:

- a) With the mouse: Click an anchor point in the slide, hold the left mouse button and drag the slide to the desired direction.
- b) **From the keyboard:** Click the area of the slide that you want to inspect, then use the arrow keys on the keyboard to move the image up/down or left/right.
- c) With the SlideDriver: Turning the upper knob (Y) moves the slide horizontally, and turning the lower knob (X) moves the slide vertically.

### 4.5.5 Setting the magnification level

You can set the magnification level in the toolbar at the top of the slide window:

```
Fit 1X 2X 5X 10X 20X 40X 1:1 Magnification: 15
```

Use one of the preset magnification levels.

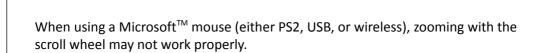
- Fit the entire specimen is visible, fits to the size of the window
- 1:1 one camera pixel corresponds to one pixel on the monitor

You can also type in the value of magnification level manually in the text box. To apply the desired magnification level, hit **Enter**. Finally, click the area that you want to inspect with that magnification. Note, that the highest magnification ratio that can be achieved on a slide is 2:1, meaning that one pixel of the digital slide is presented on two pixels in the slide window.

### 4.5.6 Previewing a smaller area with a higher magnification

- 1. To enable the magnifier (a small preview window in the top-right corner of the slide window), click the <a>button</a> on the slide toolbar, or use the **Ctrl+M** hotkey.
- 2. Move the mouse cursor to the area that you want to see in the magnifier.

### 4.5.7 Zooming



### Zooming around a point

Important!

- 1. Click the N? button on the slide toolbar if not activated.
- 2. Select the point that you want to inspect then zoom with the mouse scroll wheel, with the SlideDriver, or with keyboard keys:
  - Use the mouse wheel. You can modify zoom level by rolling the mouse wheel back and front.
  - Roll the Z wheel of the SlideDriver to zoom in or out.
  - Use the plus (+) and minus (-) keys on the keyboard.

### Zooming to an area

- 1. Click 🖸 on the slide toolbar.
- 2. Select the area that you want to inspect with the left mouse button then release it.

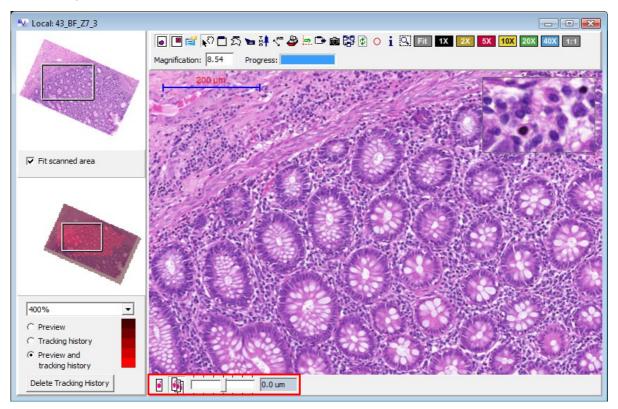
### 4.5.8 Rotating slides

With this feature you can rotate slides by degrees, and flip them vertically or horizontally. The feature-related buttons are displayed in the main toolbar of Pannoramic Viewer after opening a slide.

<b>:</b>	Rotate slide 90° left
<b>.</b>	Rotate slide 90° right
••	Rotate slide 180°
	Flip slide horizontally
	Flip slide vertically
	Slider to rotate slide by from 0 to 360 degrees
<b>*</b> 1	Reset rotation

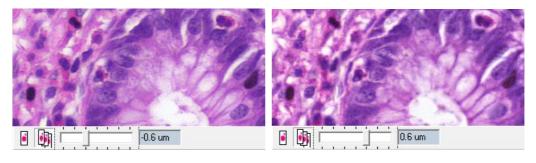
# 4.6 Working with Z-stack Slides

Multilayer scanning at different focal planes of a specimen on a whole slide is available for you with Pannoramic scanners such as Pannoramic 250 FLASH II, SCAN 150, MIDI, and DESK. The resulting slide is a three-dimensional, so-called Z-stack slide. Pannoramic Viewer allows you to view a specimen by stepping up and down in the focal planes along the Z-axis, which is especially useful for viewing "thick" specimens.



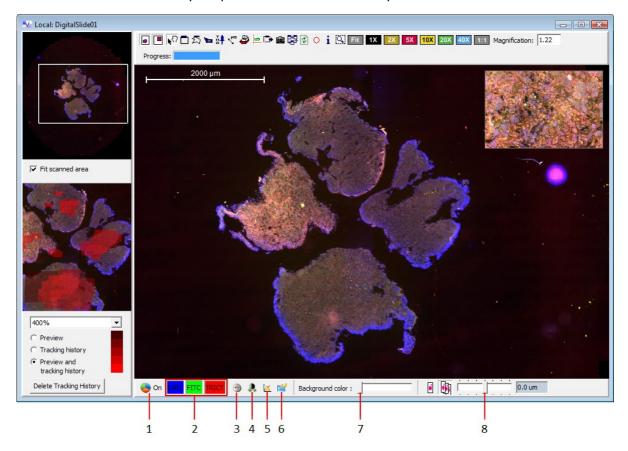
If a Z-stack slide is opened in Viewer, a toolbar is displayed at the bottom section of the slide window. The default view of the slide is presented in the below image (the prime focal plane is displayed).

Upon clicking the 🗐 button, the Z-stack view is turned on. The slide bar includes notches corresponding to the number of focal planes generated during the scanning of the specimen. Drag the slider left (step below the prime focal plane) or right (step above the prime focal plane) or roll the Z wheel of the SlideDriver to move between focal planes. Below are images of two planes, one step below and one above the zero plane.



# 4.7 Working with Fluorescent Slides

Pannoramic Scanner allows you to create slides with maximum 9 channels in fluorescent mode. For more information, see *Pannoramic SCAN 150 1.18 User's Guide*. When you open a fluorescent slide, Pannoramic Viewer offers you options that are available only for these kind of slides.



- 1. Turn on/off colors
- 2. Left click on a label: switch on/off current channel

Right click on a label: change the color of the channel (pseudo-colorization)

- 3. Set optimal brightness/contrast value considering minimum and maximum contained gray or color value (depends on zoom factor, all channels)
- 4. Set optimal brightness/contrast/gamma value (depends on zoom factor, all channels)
- 5. Set default value
- 6. Properties window: adjust brightness, contrast and gamma channel by channel
- 7. Change the background color of the slide in gray-scale
- 8. Z-stack slider

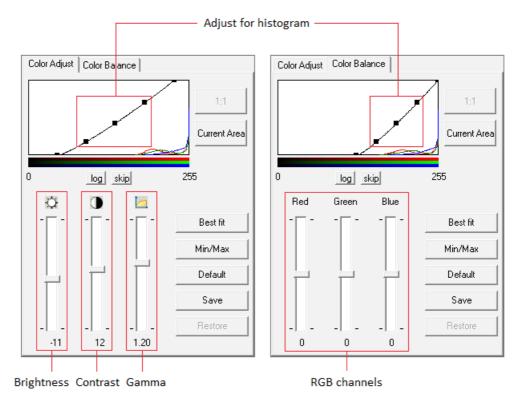
# 4.8 Changing Image Properties

If the <sup>feff</sup> button is activated, the software displays a window where you can make modifications in property settings regarding brightness, contrast, gamma value, and color matching for each single digital slide.

Important!
If you use a high-quality screen, the default settings for color reproduction, gamma, brightness and contrast are adequate. These functions are mainly intended for highlighting specific image contents for training and documentation purposes or to match monitor screen features. User-modified settings are saved specifically for the active digital slide.

**NOTE:** The applied color settings of the slide is used when creating Gallery or Classification Gallery in the Measurement Visualization module, and when activating the **Enhance Color** option in case working with HistoQuant or FISHQuant.

### 4.8.1 Setting up colors on a slide



If a slide is open, click the  $\mathbf{E}$  button on the toolbar to open the **Properties** window.

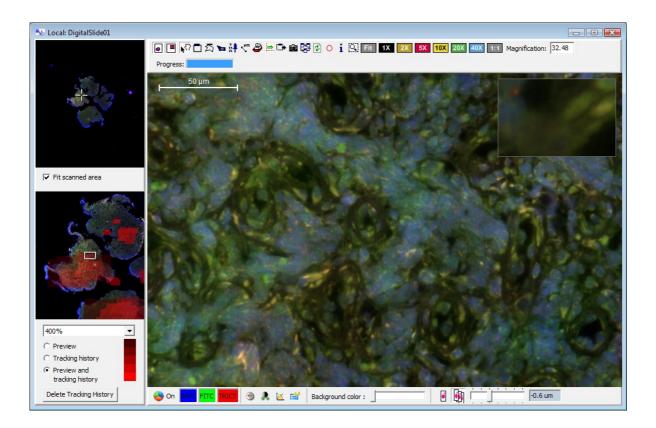
- If the **Current Area** button is activated, the histogram of the currently displayed image segment is shown.
- If **1:1** is active, the histogram for the complete digital slide is shown. The option **1:1** is available only if the specimens were digitized by means of a Pannoramic Scanner control software version 1.14 or higher.
- In the upper part of the window there is a graphic image of the color values of the currently displayed digital slide or image segment. The black line with squares represents the characteristic curve, and it can be shifted to left/right or upward/downward by dragging the squares. If brightness, contrast, gamma, or RGB values are changed, the sliders below are set automatically to the corresponding positions.

Representation between the gray-scale value (ranges from 0 to 255) and the number of gray-scale values is linear. For logarithmic representation the **log** button must be activated.

- Use Best fit button for optimal setting on the basis of optimal values stored during the scanning of the specimen (only for fluorescent slides). This setting is based on a threshold value five per thousand for all brightfield slides and for fluorescent slides scanned with older version of Pannoramic Scanner software. In brightfield mode, all three color channels (RGB) are optimized (Color Balance tab). For fluorescence, this function can be executed for each single channel.
- The Min/Max button is used for optimal setting by extending the histogram between the minimum and maximum values. In brightfield mode, all three color channels (RGB) are optimized (Color Balance tab). For fluorescence, this function can be executed for each single channel.
- The three sliders have a direct influence on brightness, contrast and gamma value on the Color Adjust tab, and on RGB channels on the Color Balance tab. By clicking the **Default** button, the sliders for brightness and contrast are reset to the default value 0, the gamma value is set to 1.00 (brightfield) or 1.00 (fluorescence).
- Save settings by clicking the Save button. Restore is used to recall the settings again. This
  function refers only to the current digital slide. If another digital slide is called, the saved
  values will not be available.

### 4.8.2 Setting up colors on a Fluorescent slide

If specimens were digitized in the fluorescence mode, all channels are displayed in the bottom part of the slide window. The software supports up to nine channels. In the example below, three channels were digitized (DAPI, Alexa 488 and Cy3).

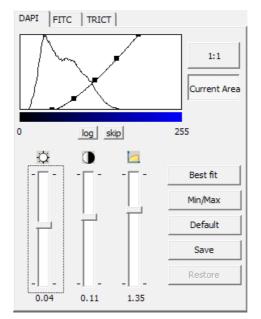


The pseudo-colors are taken over as specified in the Pannoramic Scanner control software before digitization, but can still be changed in Pannoramic Viewer.

There are various options for changing the representation of digital slides:

- Individual channels can be shown or hidden by clicking on the respective channel
   DAPI (Blue) FITC (green) Bhodamine (grange)
- By clicking with the right mouse key on the respective channel, another pseudo-color can be selected for this channel.
- Channels are displayed in their pseudo-colors when the Solution is on. To display the digital slide in gray levels, this button must be in 'OFF' position. For gray-level images, only one channel can be displayed.
- The <sup>(\*)</sup> button serves to optimize the digital slide for all channels through the Best fit function (for optimizing single channels, see section 4.8.1.Setting up colors on a slide).
- The substitution is used to optimize the digital slide for all channels by extending the histogram (Min/Max) (for optimizing single channels, see section 4.8.1.Setting up colors on a slide).
- By clicking the 🖾 button, the default settings are restored.
- The non-digitized region of a digital slide is displayed with a gray-scale value that has been defined by means of the slider **Background color**. A relatively dark background is recommended (slider shifted to the left).

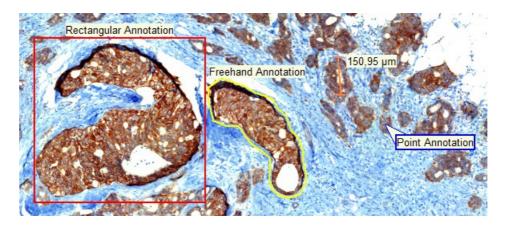
The  $\stackrel{\text{result}}{=}$  button opens a **Properties** window, which allows contrast, brightness, gamma and other values to be set for the individual channels. For details on the settings, see section <u>4.8.1.Setting up</u> <u>colors on a slide</u>.



# 4.9 Working with Annotations

You can add layers (annotations) to digital slides to mark specific areas of the specimen. Annotations can be superimposed on a digital slide. To show previously created annotations on the slide, click the **i** icon, then activate the **Display Annotations** feature. This feature can be activated upon creating a new annotation.

### 4.9.1 Creating Annotations in Pannoramic Viewer



### Rectangular annotation

If the D button is on, a rectangle area can be marked by clicking into the digital slide with the left mouse button and keeping it depressed; or a rectangular annotation can be created of the slide image currently visible in the slide window by pressing the annotation button of the SlideDriver.

As soon as either of the buttons is released, you are prompted to enter the name of the annotation (**Title**) and a comment in the **Create or Edit Annotation** window.

Create or Edit Annotation	×
Annotation parameters	
Title: Rectangular Annotation	
Title background color: Outline color:	
Ok Cancel	

While the title is a compulsory entry item which is indicated in that digital slide, the comment is an optional entry. In addition, a frame line color *(Outline color)* and a background color *(Title background color)* can be defined for the caption area.

#### Freehand annotation

If the  $2^{2}$  button is on, a click into the digital slide with the left mouse button sets the starting point for freehand drawing. Further clicks with the left mouse button allow you to mark/outline any desired image area. For more accurate detection results, leave some gap between the outer cells of the area and the outline while creating an annotation. The end point of a given freehand drawing line must be located near the starting point for the marked area to be actually enclosed. To call the window for specifying *annotation* parameters, click the right mouse button. Parameters can be specified in much the same way as for rectangular-shaped *annotations*.

#### Point annotation

To mark just a specific point rather than a whole area, the  $\ge$  button must be turned on. To define an *annotation*, click a desired spot with the left mouse button. A special input window for definition of properties appears.

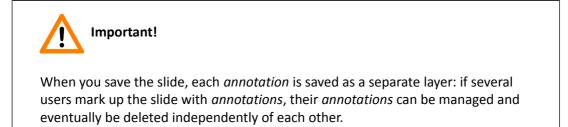
Create or Edit Annotation	×
Annotation parameters	
Title: Point Annotation	
Comment:	_
	~
Direction : Title background color: Outline color:	
Ok Cancel	

An *annotation* name must be typed into the **Title** entry field. This name will also appear in the digital slide view. A **Comment** to this *annotation* is an optional entry item. A desired frame line color for that *annotation* can be selected in the **Outline color** input box. A background color for the *annotation* area can be defined via **Title background color**. **Direction** allows you to determine how the caption box is displayed to the given point.

### Measurement annotations

A measurement annotation is provided for displaying a required distance or spacing.

Click the S<sup>m</sup> button, then select the **Create Distance Measurement Annotation** entry item. By clicking with the left mouse button the marked spot may be chosen as starting point now. By keeping that key depressed, dragging it accordingly and releasing it, the end point of measurement is defined.



### 4.9.2 Managing annotations

Click the  $\clubsuit$  button on the slide toolbar, and then a window appears showing all the *annotations* and measurement annotations on the slide.

Manage Annotations
Annotations:
Rectangular Annotation (2) Perimeter: 351.2 um; Area: 7645.34 um2; Z-Stack laver: 2
Freehand Annotation (2) Perimeter: 381 um; Area: 5246.54 um2; Z-Stack layer: 2 Point Annotation (2) Perimeter: - ; Area: -
Distance Measurement annotations:
44.02 μm (2)
Locate         Edit         Delete         Save to CSV         Send Link to Clipboard         Export         Scanmap         Close

- Locate Change the slide display view locating the selected annotation.
- Edit Open a window containing options for editing the properties of a previously marked *annotation*.

Create or Edit Annotation	X
Annotation parameters	
Title: Rectangular Annotation	
	4
Title background color: Outline color:	
Ok Cancel	

- **Delete** Delete the selected annotation.
- Save to CSV Save the information contained in *annotations* and measurement annotations as a file. Values are separated by a semicolon.
- Send Link to Clipboard Copy a link to the selected annotations to the clipboard.

- Export The currently displayed digital slide can be opened in a predefined program and the marked *annotation* is transferred with its position data. For information on defining an external application, see section <u>3.1.6 External Application Call for Annotation Export</u>.
- **Close** Close this dialog.

### 4.9.3 Creating a link to an annotation

# You can use lin

Tip!

You can use links to *annotations* (and therefore to slides) in E-School training or exam materials. When you copy the link in the address field of a web browser, Pannoramic Viewer opens and the slide to which the *annotation* belongs is opened, not the *annotation* directly.

- 1. Click the 🇳 button on the slide toolbar.
- 2. Select the *annotation* that you want to save as a link.
- 3. Click Send Link to Clipboard.

Send Link of Bookmarks to Clipboard		
Magnification:	<ul> <li>✓ mview: school link</li> <li>✓ exam: encrypted link</li> <li>✓ Direct link</li> </ul>	
Send Link to Clipboard Close		

- Magnification select the desired magnification level
- mview: school link choose this option to generate a normal link to annotation
- **exam: encrypted link** generate link to *annotation*, but only with limited functionality (creating and editing are not possible)
- direct link full path, or relative path is created to the slide
- 4. Open a text editor and copy the link into a text file.

### 4.9.4 Exporting annotations

- 1. Click the 🗳 button on the slide toolbar.
- 2. Select the *annotation* or *annotations* you want to convert to another format.
- 3. Click Export:
  - Measurement annotations can be exported to a CSV file.
  - Non-measurement annotations can be exported to a HTML file, that includes a referenced JPG file for each *annotation*.

**NOTE**: Annotation coordinates can be exported too, for further information see section <u>4.13.4 Export</u>\_<u>Annotation Coordinates</u>.

# 4.10 Measuring in a Digital Slide

### 4.10.1 Length measurement

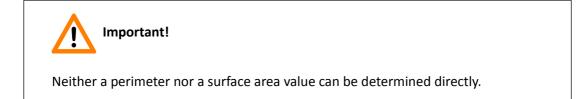
You can perform length measurement jobs via related *annotations*. Although such *annotations* are saved and maintained as part of a digital slide, they can be deleted again. Refer to section <u>4.9</u> <u>Working with Annotations</u> for a detailed description of the procedure to generate measurement annotations.

Apart from this working approach, it is also possible to perform temporary length measurements:

- 1. Press the State button.
- 2. Select the Measure Distance entry item.
- 3. Define a starting point with the left mouse button, and move the mouse to the desired end point keeping it depressed.

The distance reading from the starting point to the current position is indicated in  $\mu$ m. If the mouse button is released, a new starting point can be defined.

### 4.10.2 Determination of perimeter and surface area



To define any of these two parameters, an area must be defined at first, using rectangular or freehand *annotations* (refer to section **4.9 Working with Annotations**).

Having completed this, click the  $\stackrel{\textcircled{}}{\Rightarrow}$  button to turn the **Manage Annotations** window on. This window lists the perimeter and surface area values related to the given *annotation*. To save these data, click **Save to CSV...** (refer to section <u>4.9.2 Managing Annotations</u>).

# 4.11 Displaying Information Relating to a Digital Slide

After clicking the **1** button and selecting **Slide Information**, a window with information items relating to the digitized digital slide is displayed. These information items depend on the hardware used, the hardware control software version, and the mode of digitization.

🖤 Slide Information		×
Optical parameters		
Objective name:	Plan-Apochromat	
Objective magnification:	40x	
Camera adapter magnification:	1x	
Camera type:	PCO.edge	
Micrometer / pixel X:	0.162500	
Micrometer / pixel Y:	0.162500	
Scan information		
Scan start [DD:MM:YYYY] [HH:MM:SS]:	15/02/2013 09:23:58	
Scan duration [HH:MM:SS]:	02:28:07	
File size:	2413.17 MByte	E
Slide dimensions in pixels (width x height):	60416 x 67328	
Scanned field of view count :	4470	
Profile name:	40082-13-2	
Profile name:	40082-13-2	
Fluorescence parameters		
Filter1 (DAPI)		
Exposure time [ms]:	150	
Digital gain:	2	
Number of focus layers:	7	
Focus step size:	3	
Bit depth per channel:	8	
Filter2 (FITC)		
Exposure time [ms]:	250	
Digital gain:	2	
Number of focus layers:	7	
Focus step size:	3	
Bit depth per channel:	8	
Filter3 (TRICT)	050	-
	61 HUH 8114 Port 40082 / 13	
Rotate Barcode / Label Area by 180°	Ok	

### 4.11.1 Explanations to digital slide-related information

### **Optical parameters**

- **Objective name** Shows the name of the objective used for imaging.
- **Objective magnification** The given specimen was digitized with an objective with a physical magnification of 20x.
- **Camera adapter magnification** Shows the magnification of the TV adapter (this information appears only if digitization was carried out with version 1.9 or higher).
- **Optovar magnification** Shows the magnification of the Optovar used (this information appears only if digitization was carried out with version 1.9 or higher). This information is displayed only for digital slides digitized in the brightfield mode.
- **Camera type –** AxioCam Mm Rev.3 was used to digitize the digital slide.
- Micrometer / pixel X Pixel resolution is 0,322500 μm in X direction for this digital slide. This value depends on Pannoramic Scanner control software settings and your hardware configuration.
- Micrometer / pixel Y Pixel resolution is 0,322500 μm in Y direction for this digital slide. This value depends on Pannoramic Scanner control software settings and your hardware configuration.

### Scan information

- Scan start This digital slide was digitized on March 4, 2009 at 11:56:58.
- Scanning duration Digitization time was 1 hour 20 minutes and 33 seconds.
- File size This digital slide occupies about 240.76 MB of hard-drive space.

### Important!

If a digital slide is opened via a teleconsultation server, no respective value is indicated.

• Slide dimensions in pixels (width x height) – The size of this slide is 38,656 x 50,688 pixels (each single image has a size of 1,400 x 1,000 pixels in standard configuration).



The slide size is referred to the total theoretically digitized area of a specimen, not to the actually digitized specimen.

- Scanned field of view count To create the digital slide, a total of 1815 single images were taken.
- **Profile name** The name of the profile under which the slide was digitized.

If a slide was digitized in fluorescent mode, the individual channels are displayed with the respective parameters.

### Fluorescence parameters (only for fluorescent slides)

- Filter 1 (B) The name of the filter is indicated in brackets. It is indicated as defined by the user.
  - Exposure time (ms)

This entry item informs on the exposure time for the individual image fields for this channel.

- Filter <n> The name of the filter is indicated in brackets. It is indicated as defined by the user.
  - Exposure time (ms)

This entry item informs on the exposure time for the individual image fields for this channel.

### Image parameters

- Image compression This entry item shows, whether a compression of the image data was carried out (Yes) or not (No).
- Image file format This parameter indicates the image file format.
- **Quality factor** In the case of a JPEG compression the quality factor is indicated here.

### Preview image

Depending on the equipment of the digitizer, an additional image of the barcode area is displayed or not.

### Rotate Barcode / Label Area by 180°

Press this button to rotate the barcode image by 180°.

# 4.12 Batch Processing

By clicking the 🛃 icon on the bottom of the tree-view panel of the main window, the selected slides and their annotations will be listed in the Batch Processing window in which an specific Image Analysis application can be selected for each annotation.

1. Select a slide or folder in the tree-view panel, then click the 🕥 button to add them to the list. Remove a slide from the list by clicking the 의 button.

O 🕒 🛃	$\overline{\otimes}$
Slide Name	Slide Path
10301-07_ER_I	C:\SLIDES\NQ\103
10301-07_HE_I-1	C:\SLIDES\NQ\10
•	۰.

- 2. If you want to upload slides to the CaseCenter server, click the 🛓 button.
- 3. Click the 🖾 button when finished, select **Process with Legacy Quants** option if you want to run batch processing by using MISP files, otherwise click **Process with QuantCenter Quant** to run batch processing by using scenarios.

Select Batch Processing type	
Legacy Quants	Quants in QuantCenter
Legacy NuclearQuant Legacy MembraneQuant Legacy HistoQuant DensitoQuant	NuclearQuant CellQuant MembraneQuant PattemQuant HistoQuant FISHQuant CISHQuant CISH-RNSQuant
Process with Legacy Quants	Process with QuantCenter Quants

4. If you have selected the **Process with Legacy Quants** option the upper, and if **Process with QuantCenter Quant** is selected the bottom window appears.

🚺 Image Analysis Batch Processin	g		
Slides           Select all slides           10301-07_HER2_I           43_BF_Z7_3	Annotations Whole slide Every annotation Rectangular Annotation (2) Freehand Annotation (2)	MISPs Add / Remove + -	Add
Batch Processing List			
Slide	Annotation	MISP	Remove
10301-07_HER2_I	Rectangle Annotation	01	
10301-07_HER2_I	Freehand Annotation	01	
10301-07_HER2_I	Freehand Annotation	DensitoQuant_MyProfile	
10301-07_HER2_I	Freehand Annotation	Her2	
43_BF_Z7_3	Rectangular Annotation (2)	DensitoQuant_Default	
Export Settings			
Save Region measurements			
Save Cumulative measurements	Save	to single CSV	
		Browse Batch Processing	Close

Image Analysis Batch Processing	9		- <b>X</b>
Slides Select all slides -	Annotations Whole slide Every annotation	Scenarios Add / Remove + -	
Colcc-B-Cat-Erbit-B2 DigitalSlide01 10301-07_HER2_I Batch Processing List	555 Det tumor area 1 Annot_01 (-1)	Estrogen Her2 Ki67 P53 Progesterone	Add
Slide	Annotation	Scenario	Remove
DigitalSlide01	555	Her2	
DigitalSlide01	Det. tumor area 1	Her2	
DigitalSlide01	Annot_01 (-1)	Her2	
Ĺ		Batch Processi	ng Close

- 5. Select the slide(s) you want to process (or activate the **Select all slides** option to select every slide listed), then select the annotation(s) from the list (or activate **Every annotation** option, and to process the entire area of the selected slide(s) click **Whole slide** option).
- 6. Select the MISPs or scenarios, depending on the specified processing type you want to run.
- 7. Click the Add button to move the process to the Batch Processing List.
- 8. Activate **Export Settings** options if necessary, either removal of a process is available by selecting it and clicking the **Remove** button.
- 9. Finally, click the **Batch Processing** button when finished.
- 10. After measurements are performed (and saved to a file), close the window.

# 4.13 Exporting Slides from Pannoramic Viewer

Pannoramic Viewer allows you various possibilities to export slides or *annotations* to different file formats.

### 4.13.1 Image capture

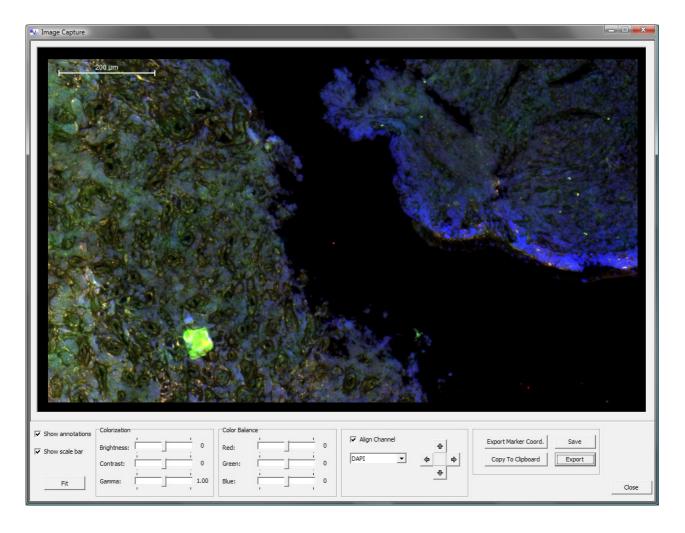
1. Set the magnification factor and shift the image in the slide window as needed.

💘 Image Capture				
50 µm		Rectangular Annotation (2)		
✓     Show annotations     Colorization       ✓     Show scale bar     Brightness:        Contrast:        Fit     Gamma:	Color Balance Color Balance Red: Green: 1.00 Blue:	0     Export Marker Coord.       0     Copy To Clipboard       0     0	Save Export	Gose

2. Click the 📫 button in the slide toolbar.

3. Modify the image properties with the editing options, for example, change its brightness, contrast, and/or gamma correction values as needed.

If a slide was digitized in fluorescence mode, by checking the **Align Channel** function, color channel layers can be individually moved to the desired position with the arrow buttons.



- 4. Export the image or annotation coordinates:
  - Click **Copy To Clipboard** to put the image to the Windows clipboard so that you can use it in another application.
  - By selecting Export, the displayed image will be opened with the raster image viewer application set previously in the Options window (section <u>3.1.5 External Application</u> <u>Call for Image Capture</u>).
  - Click Export Marker Coord. button to save annotation coordinates (for details, see section <u>4.13.4 Export Annotation Coordinates</u>).
  - Click **Save** to save the image to file into a specific folder.

### 4.13.2 Saving a slide

- a) Manually, outside of Pannoramic Viewer:
  - save the Pannoramic Slide and the folder with the same name as the MRXS file
- b) From Pannoramic Viewer:
  - press **Ctrl+S** on the keyboard

### 4.13.3 Export to TIFF Image, Pannoramic Slide or to MetaXML

With the **Slide Export** function you can export a whole slide or just the annotated areas to TIFF Image, Pannoramic Slide or to MetaXML file format. Choose **Slide Export** from the **File** menu or press **Ctrl+E**.

V Slide Export	
Original Settings Slide type: Brightfield Image file format : JPEG Quality	iactor : 100%
Output Settings         File format :       TIFF Image         Magnification:       1:1         Tile size :       256 x 256         Overlap       %         Image codec:       Uncompressed         Quality:       -         Image codec:       Uncompressed	Output Image Information         Selected image information::         Image type:       TIFF Image         Image size:       556 x 331 Pixels         Tile size:       256 x 256 Pixels         Number of tiles:       6         Estimated image file size:       1.1 Megabytes         Aggregated Export Information       Number of exported images: 1
Burn in slide markers     ✓ Z to Slides       R0I Selection     Available regions:       Whole Slide     Caption01 (1)       ->     <-	2 Esitmated total disk size: 1.1 Megabytes Selected Region of Interest
=> <= Destination Path: c:\43_BF_Z7_3	Browse 3 Save Cancel

# Important!

When you save the slide as a TIFF Image, the format of the generated image is a **tiled TIFF**. Therefore, applications such as *Windows Picture and Fax Viewer* **cannot** open the created file, because of its type. To save a standard TIFF file, use the image capture function (refer to section <u>4.13.1 Image capture</u>).

### 1. Output settings

You can set up the output file parameters in this window region.

- a. Output file formats TIFF Image, Pannoramic Slide, and MetaXML
- b. Magnification the level of magnification
- c. Tile size size of tiles from 256x256 up to 2048x2048
- d. **Overlap** (only for Pannoramic Slides and MetaXML) the percentage of overlap between two tiles
- e. Image codec for TIFF Image: uncompressed or JPEG; for Pannoramic Slide: JPEG, PNG, BMP; for MetaXML: JPEG, PNG, BMP, TIFF
- f. **Quality** Set the JPEG compression level.



With lossy compression, the image quality gets worse depending on the compression level. The lower the compression value the poorer the image quality.

- g. **Burn in slide** *annotation* if checked in, the *annotation* is burned in the slide as the part of the image (except for Pannoramic/MRXS slides)
- h. **Burn in slide markers** can be checked only if you choose TIFF Image or MetaXML as an output format.
- i. **Z to Slides** in case the original slide is a Z-stack slide (containing multiple layers focal planes), the exported slide will include the Z-planes.
- j. **Output channels** modify settings mainly if a fluorescent slide is to be exported. It has three options:
  - I. Multi channel: a selected channel can be replaced by another one, written into the new slide (for example, you can set a GGR channel instead of BGR).
  - II. Single channel: one selected channel is written in the new slide.
  - III. Current View: the current color settings is written in the slide.

### 2. ROI (Region of Interest) Selection

You can select the whole slide, or just some annotated areas for export. You can select the annotated areas for export using the  $\xrightarrow{}$  button, or remove them from the selection using the  $\xrightarrow{}$  button. Also, you can select all the *annotations* with the  $\xrightarrow{}$  button or deselect them with the  $\xrightarrow{}$  button.

### 3. Destination path

The path where your slides will be stored. Slides are named after the *annotation* names (if any), otherwise you can give an alternative file name.

# Warning!

Depending on your settings, the size of the exported area, and the number of exported areas, the size of the exported file can be quite large in some cases, and the process can take unexpectedly long time.

### 4.13.4 Export Annotation Coordinates

Coordinates of annotation breakpoints (corner points) can be exported into a CSV file. Coordinate values given in micrometers are based on the scanner motor coordinates.

**NOTE:** Marker annotation coordinates are also exported this way. Refer to section <u>6. Working with</u> <u>Marker Counter (Licensed Feature)</u>.

- 1. Create annotation(s) and/or markers (see section <u>4.9.1 Creating Annotations in Pannoramic</u> <u>Viewer</u>).
- 2. Click the 🔄 button in the slide toolbar.
- 3. Enter the name of the file and specify where the CSV file should be saved, then click **Save**.

Batch export is available when creating a list of slides from various locations (folders or disk drives).

After selecting a folder or slide in the Local tree, press the silon below the Navigation Tree panel or the silon to remove slide(s) from the list.

Export starts on items added to the list by clicking the icon, then browsing for a folder to store CSV file(s).

Local Teleconsultation Server		
□       MQ         □       0301-07_HER2_I         □       misps         □       TMA_Project_01         □       TMA_Project_02         □       0301-07_ER_I         □       10301-07_ER_I         □       TS_Project_01         □       TB_Project_01         □       TB_Project_01         □       TB_Project_01         □       SLIDES		
Refresh		
0 0 4 2 5 8		
Slide Name Slide Path		
10301-07_ER_I	C:\SLIDES\WQ\1030	
10301-07_HE_I-1 C:\SLIDES\WQ\1030		
•		

# 4.14 Synchronizing Slides in a Set

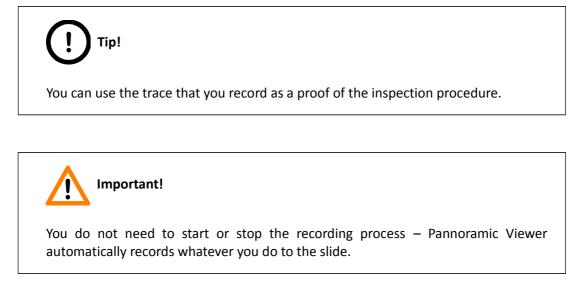
One of the advantages of virtual microscopy over the traditional microscopes is that once you digitized the slide, you can inspect a specimen with different stains.

In Pannoramic Viewer, you can synchronize slides that belong together, so when you move one of the slide image(s) horizontally or vertically, Pannoramic Viewer shows the same area in the other(s) as well. Besides, magnification levels of the slides are synchronized, and when modifying zoom on one slide, the same step is applied on the other slide(s).

- 1. Open the slides that you want to synchronize. It does not matter whether you arrange them in cascading or in any other way.
- 2. Select the same magnification level for all slides.
- 3. Select an easy-to-distinguish detail that is visible on all slides and move it to the center of the window for each slide.
- 4. Click the 🛱 button in the toolbar to synchronize the slides.

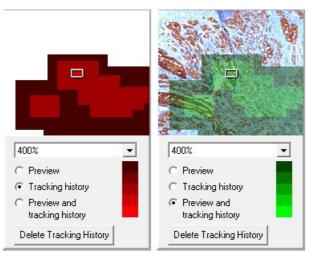
# 4.15 Recording Tracking History

Pannoramic Viewer is able to record the track of the mouse movement on the slide and what magnification levels you select at various spots. This tracing mask is saved as a superimposed layer rather than and integral part of the image. A position mask is even preserved on closing and reopening of the present digital slide.



### 4.15.1 Retrieving the tracking history

If **Preview** is 'ON', only a preview of the digital slide is displayed without a position mask. If the **Tracking history** option is selected, only the position mask is displayed, while the variable preview screen is 'OFF'.



To display both the variable preview screen and the position mask, click the **Preview and tracking history** radio button in the panel or, to select it as default, choose the **Preview and Slide tracking history** option in the Options window (refer to section <u>3.1.2 Slide window defaults</u>).

The position mask appears as a color mask. Click the color bar to switch between the colors (red/green/blue) of the tracing mask. To remove the tracing mask, click the **Delete Tracking History** button.

The intensity of a color indicates the magnification at which a particular sub-area was examined. Brighter color shadings suggest greater magnification factors. A position is not saved, unless a certain magnification level was exceeded. Otherwise, no diagnosis is possible.

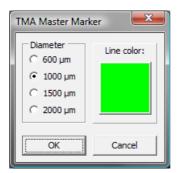
# 5 Working with TMA Master Marker

TMA Master Marker feature allows you to place markers on TMA Master slides, that the TMA Master control software analyzes and uses the markers as selected areas of the tissue to be removed from the donor block and transported to the recipient block.

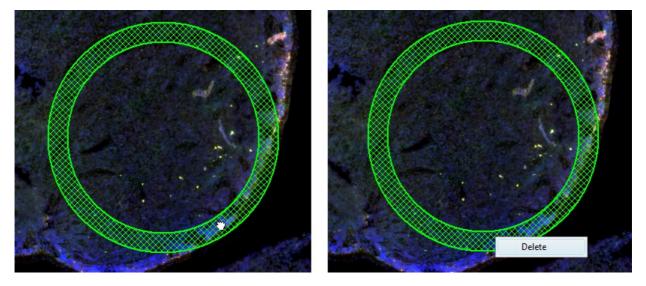
## 5.1 Creating TMA Master Marker

- 1. Click the 📕 button on the slide window toolbar, and select the **Create** menu.
- 2. Set the diameter of the marker (that corresponds to the size of the puncher in the TMA Master unit). You can also choose a distinctive color for the marker(s) either of different or same sizes.

Each time you want to change the marker diameter and/or color, click the  $\frac{1}{2}$  button.



- 3. Click OK or Cancel.
- 4. When placing markers with the left mouse button, mind that the cross-hair cursor in the slide window is presented as the center point of the marker.
- 5. You can drag a marker on the slide by the ring area of the marker (a hand cursor appears).
- 6. To remove a marker, right-click on the marker and select **Delete**.



If you press the <sup>H</sup> button then select **Manage** option you will be available only to move and delete markers.

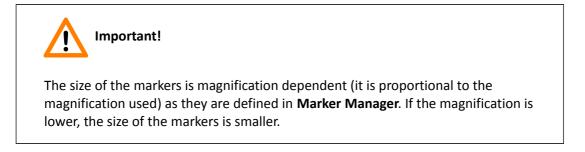
The slide containing TMA Master markers can be used later with the TMA Master software application to determine which areas of the donor block should be transferred to the recipient block.

For more information, see TMA Grand Master 1.2 User's Guide or TMA Master 1.16 User's Guide.

# 6 Working with Marker Counter (licensed feature)

With **Marker Counter** you can create marker categories, and place markers to point to specific areas of an *annotation*.

## 6.1 Managing Marker Categories



- 1. To set up a Marker Annotation, press the  $\bigcirc$  button on the slide window toolbar.
- 2. Select the Manage Marker Category menu item.

Marker categories			
Add Modify Settings			
Name: positiove			
, , , , , , , , , , , , , , , , , , ,			
Fill color: Line color:			
Filled circles			
© Transparent circles			
Diameter: 10 µm			
Line width: 1 pixel			
Add to saved categories			
OK Apply Cancel			

- 3. On the Add tab in the **Marker categories** window, give the name of the marker category, select unique Fill and Line colors if necessary.
- 4. Choose Filled circles or Transparent circles to determine the visualization type of markers.
- 5. Type in the **Diameter** and **Line width** values of markers.
- 6. Check Add to saved categories to add the marker category to Saved categories.

#### **Modify Marker categories**

On the Modify tab of the Marker categories window, select a category and modify its attributes if necessary.

Marker categories
Add Modify Settings
Select: positive
Name: positive
Fill color: Line color:
Filled circles
C Transparent circles
Diameter: 10 µm
Line width: 1 pixel
OK Apply Cancel

#### Marker category settings

You can manage marker categories on the Settings tab. There is a possibility of deleting, and also adding them from the Used categories to the Saved categories column, and vice versa.

Marker categories			
Add Modify Set	ttings		
Used categories	Saved categories		
positive     negative	negative		
Delete =>	<= Delete		
ОК	Apply Cancel		

- 7. Click **Apply** to save changes and leave the window open, or **OK** to save and close the window.
- 8. Click **Cancel** to abandon changes and exit window.

### 6.1.1 Using existing marker categories

- 1. Create an annotation on the target area.
- 2. Select the marker category and place markers on the objects to be measured in the target area.

○ 🗠 i 🔍 Fit 1X 2X 5X 10	<b>X</b> 20	0X 40X 1:1
Manage Marker Category	1	
Select Marker Category	✓	positive
		negative

- 3. If you want to delete markers, right-click on them, and select **Delete**.
- 4. Click the 1 button, and select the Marker Counter Statistics menu item.
- 5. The Marker Counter Statistics window appears on the screen showing values for each category regarding the overall area, number, relative number, and frequency of markers within the given annotation.

<	Show Annotations	Annotation	Name:	mc_test	•
	Show Measurement Annotations	Overall Ar	ea:	0,68	mm2 👻
	Show Measurement Data	Number of	Markers:	5	
< ]	Show Markers	Frequency of Markers: 7,319E+00			
✓]	Marker Counter Statistics	Category	Number	Rel. Number	Frequency
	Slide Information	negative	2	40 %	2,927E+00
		positive	3	60 %	4,391E+00

- 6. If you need an additional marker category which does not exist yet, create it.
- 7. Save or export (Copy to clipboard) the measured parameters and images by clicking theor the button.

If the markers are inside of an annotation (also free-hand annotation), they are summed for that region. In this way it is very easy to produce cell number/area ratios for an actual studying.

### 6.1.2 Exporting Marker Counter results

You can display the most important values for each marker category in the Marker Counter window and you can export them as a CSV file:

- Number of marked objects / labeled area (or objects / standard area).
- Percentage of positive cells in the cell population, for example, in a tumor.

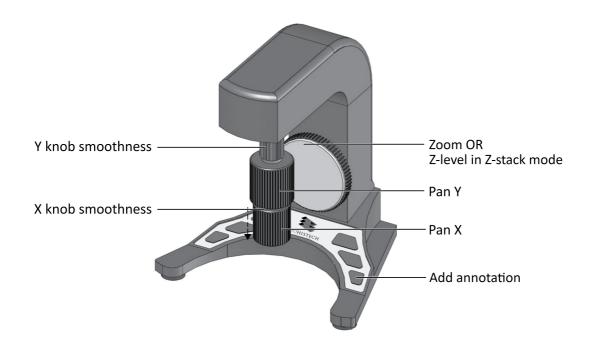
In representative areas the number of positive cells must be divided by the sum of positive and negative cells and multiplied by 100.

Marker coordinates can also be exported. For further information, see section <u>4.13.4 Export</u> <u>Annotation Coordinates</u>.

# 7 SlideDriver

SlideDriver is a 3-axes operating device, that is able to carry out rapid and accurate slide positioning across the complete travel range within microns.

The adjustable drive knobs enable an ergonomic adaptation to friction and height. The friction knob with which you can set the resisting force of the X knob can be accessed when the X knob is pulled down.



Main features of the device:

- alternative velocities for XYZ
- high-resolution, proportional positioning
- fast and sensitive slide positioning across the entire travel range (in sub- $\!\mu$  area at the same time)
- ergonomic adjustment for friction and height (telescopable mechanism)

# **Appendix – Toolbar Buttons**

Main window toolb	ar buttons	
鼲	Show slide tree	Turns slide tree for digital slides on or off
	Show thumbnails	Turns thumbnail view screen (gallery) on or off
<b>2</b>	Open slide	Opens a dialog window for adding a folder or opening a single slide
30	New 3D project	Launches a 3D project under the 3D Reconstruction Module
τ <u>α</u>	Connect to teleconsultation server	Logging in to a teleconsultation server to view digital slides
INT	Host teleconsultation	If this icon is selected, the host will create a group (online teleconsultation)
ţ.	Join teleconsultation	If this icon is selected, a client may join a group (online teleconsultation)
	Disconnect from teleconsultation server	Separates the user from teleconsultation server
	Tile vertically	Arranges several windows currently open horizontally one beside the other
	Tile horizontally	Arranges several windows currently open one below the other
	Cascade	Arranges several windows currently open in a cascade
	Synchronize all slide windows / Slide windows synchronized	Links open windows for simultaneous navigation
×	Full screen	Maximizes currently selected window to cover the entire screen area
<b>.</b>	Rotate 90° left	Rotates Slide by 90 degrees left
	Rotate 90° right	Rotates Slide by 90 degrees right
	Rotate 180°	Rotates Slide by 180 degrees
	Flip horizontal	Flips Slide horizontally
H	Flip vertical	Flips Slide vertically
	Rotate slider	Rotates Slide by dragging the slider
	Image Analysis Modules	Launches a window for the Image Analysis Modules (DensitoQuant, HistoQuant, MembraneQuant, NuclearQuant)
	QuantCenter application	Launches the QuantCenter application modules (PatternQuant, HistoQuant, MembraneQuant, NuclearQuant, CellQuant, CISHQuant, CISH-RNSQuant, FISHQuant)

Digital Slide window	toolbar buttons	
•	Show / hide preview panel	Turns preview image on or off
	Show / hide magnifier	Turns magnifier on or off
<b>⊫</b> °	Properties window	Shows or hides an operator panel for color matching
<b>₽</b> 07	Pan mode	Allows you to shift the window contents using the mouse or keyboard commands if active
	Create rectangular annotation	This function is used to create a rectangular-shaped annotation
ភ	Create polygonal or freehand annotation	This function is used to create a freehand-drawn annotation
1	Create point annotation	This function creates an annotation that points to a specific spot
ă <b>₽</b>	Create TMA Master Marker	By clicking this button you can place markers on TMA Master slides
ζ <sup>μm</sup>	Measurement options	Allows you to measure a distance in a virtual slide and/or create a measurement annotation
2	Manage / locate annotations	Opens a window for organizing of the various annotations
let.	Export annotation coordinates	Exports coordinates of annotation breakpoints (corner points) to CSV file in micrometers.
<b></b>	Create slide report	Selects annotations for consolidation in a report
<b>T</b> ÎT	Capture image	Creates a bitmap snapshot of the virtual slide
¢	Show slide on tree	By clicking onto this button, you can mark the entry that relates to a currently opened virtual slide, if the slide tree is shown
i	Information options	Displays a menu for turning view screen for annotations / measurement annotations / measurement data / markers on and off, and opening virtual slide information window
<u>C</u>	Zoom to selected area mode	You can select sample areas to be enlarged via mouse selection
Fit 1X 2X 5	X 10X 20X 40X 1:1	You can select from predefined magnification levels
4	Measurement Visualization	Measurement visualization as histogram, scatter plot, or an image gallery (available only for Image Analysis Modules)
0	Marker Counter	Activates Marker Counter feature (licensed – optional)
	Z-Stack Slide – Default View	The zero/prime focal plane is displayed of a Z-stack slide
	Z-Stack Slide – Z-Stack View	Turns on Z-stack view, enables the movement below or above the default focal plane
1.2 um	Z-Stack Slide – Slider	Move between focal planes by dragging the slider
Digital Slide window	toolbar buttons for fluorescence s	lides only
٩	Turn color channels on/off	Changes between gray-level and color view

DAPI (Blue) FITC (green) Rhodamine (orange)		Shows all available channels; they can be turned on or off
•	Optimum displaying	Optimizes the view according to the Best Fit method for all channels
	Optimum channels	Optimizes the view according to the Min/Max method for all channels
<u>ند</u>	Default colors	Resets the settings to the default values
Background color :		This slider is used for setting background color of the slide window

# References

Pannoramic 250 FLASH II 1.18 User's Guide

Pannoramic SCAN 150 1.18 User's Guide

TMA Grand Master 2.2 User's Guide

TMA Master 1.16 SP1 User's Guide

# Index

A	
Annotation7, 11, 27, 31, 32, 45, 53, 54, 55, 56,	57, 58, 59, 62, 64, 65, 68, 69, 75, 77, 81
В	
Batch Process	62, 64
Brightfield	
C	
CaseCenter	10, 11, 38, 39, 40, 42, 43, 62
Classification Gallery	
CSV	7, 56, 58, 59, 77
F	
Field	
Fluorescent	
G	
Gallery	
H	
Histogram	51, 52, 81
1	
Image Analysis	
L	- 40 -0 6- 60 -4
Layer	
M	
Marker	
Measurement	
MISP	
MRXS	
	11
Pannoramic Viewer	11
<b>R</b> RGB	E1
S	
S Scatter plot	01
Segmentation	
Segmentation	
Share	
SlideDriver	
T	
Teleconsultation	10 11 38 /0 /1 60 80
U	
Upload	11
opioud	