

# Plate-Viewer

## User manual, Original

Published by: ACQUIFER Imaging GmbH  
 Hans-Bunte-Str. 8  
 69123 Heidelberg  
 Germany

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Feb-2022

This manual will guide you through the usage of the ACQUIFER Plate-Viewer. It will provide you with all relevant information required to browse, visualize, and export image data acquired with the ACQUIFER Imaging Machine, configure supervised feedback microscopy experiments and the usage of Plate-Viewer plugins. Please keep this manual in a readily accessible place for future reference.

The Plate-Viewer is shipped with the ACQUIFER Imaging Machine and is pre-installed on the accompanying HIVE-CORE or HIVE-IM system. The Plate-Viewer is intended to be used with image data acquired with the ACQUIFER Imaging Machine only. It is incompatible with any other image data from third party image acquisition devices.

The Plate-Viewer is designed and tested to guarantee optimal performance on a HIVE-CORE or HIVE-IM. Usage on other devices, such as laptops may impair performance and may be affected by software incompatibilities. Any modifications, such as modifying or moving the installation, are discouraged and should not be carried out without prior consultation of ACQUIFER.

The instructions contained in this document are based on state-of-the art technology and standards. This manual is intended to provide correct and accurate information at the date of writing. However, this is subjected to future changes due to upgrades and further developments of the Plate-Viewer and Imaging Machine system. Therefore, ACQUIFER undertakes no liability for any possible errors in this document. ACQUIFER appreciates if you would report any errors, faults, suggestions or comments on the content of this manual or on any of our other documentation ([support@acquifer.de](mailto:support@acquifer.de)).

The information in this manual is subjected to modifications and changes at any time and without prior notification. Please discuss with your ACQUIFER specialist any questions related to the Plate-Viewer that may not be covered by this manual.

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Heidelberg (Germany), February 02, 2022

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## 1 Guidance for the documentation

### 1.1 Purpose

Purpose of this User Manual is to provide persons using the product with adequate and sufficient information for the optimal operation of the Plate-Viewer software. The User Manual is part of the Scope of Supply of ACQUIFER Imaging Machine.

Information and instructions in the User Manual do not exclude the personal responsibility of the operator or user of the Plate-Viewer.

### 1.2 Intended Target Group

This User Manual is intended for the following target group of persons

- Users and staff introduced and trained by ACQUIFER specialists
- Experts having experience in similar software as the Plate-Viewer
- Official Persons operating and using the system

### 1.3 Handling and Safekeeping the User Manual

It is strongly recommended that official operating personal has access to this manual. Please prevent unauthorized persons using or copying partly or wholly this document. Please keep this manual in a readily accessible save place, so all official users and operators, can use it for reference.

### 1.4 Validity

This user manual is exclusively valid only for the Plate-Viewer software version at the time of delivery.

### 1.5 Applicable Documents

Component	Document	Name of Manufacturer
Imaging Machine	manual	ACQUIFER Imaging GmbH, Hans-Bunte-Str. 8, 69123 Heidelberg

## 2 Functional description

### 2.1 Intended Use

The ACQUIFER Plate-Viewer software is designed for browsing, visualization and exporting of image data acquired with the ACQUIFER Imaging Machine, for the configuration of supervised feedback microscopy workflows and execution of image analysis workflows using pre-defined plugins. Image data generated with other digital imaging devices is not supported.

The ACQUIFER Plate-Viewer is not intended to be used on personal laptops or computers other than the HIVE-CORE or HIVE-IM.

### 2.2 Overview

The Plate-Viewer software is compatible with Windows operating systems (Windows 8 and Windows 10 based operating systems). It is tested and approved to work as pre-installed software on a HIVE-Core or HIVE-IM as supplied by ACQUIFER upon delivery. Usage on other devices, such as personal laptops or computers may impair performance or may be affected by incompatibility issues.

### 2.3 Technical Support

If you require further information or additional support, we are more than happy to help you. Please contact your ACQUIFER specialist directly or send an email to [support@acquifer.de](mailto:support@acquifer.de).

## 3 Installation

### 3.1 Software Installation

The software is part of the ACQUIFER Imaging Machine control software and is pre-installed on any HIVE-CORE or HIVE-IM accompanying the Imaging Machine.

The stand-alone software can be installed by extracting the Plate-Viewer.zip archive.

## 4 Operation

### 4.1 Starting the Plate-Viewer software

The Plate-Viewer can be started from the configuration panel of the Imaging Machine control software by clicking the 'Plate-Viewer Launch App' sub-panel. The Plate-Viewer will automatically open the image dataset that was acquired last with the Imaging Machine within the currently active project directory.



Figure 1 | 'Plate-Viewer Launch App' icon in the ACQUIFER Imaging control software to launch the Plate-Viewer

Alternatively, the Plate-Viewer software can be started by double-clicking the icon on the desktop or in the windows explorer.



Figure 2 | Plate-Viewer software icon

#### 4.1.1 Closing the software

To exit the program, close the software window using the X symbol in the right upper corner of the software window.

#### 4.1.2 Main elements of the GUI

Once the program is started the main window will appear on the screen (Figure 3).

The User Interface of the Plate-Viewer software is split into 4 areas: **(1) Main Menu Area:** providing control elements and menus for configuration and software functionalities; **(2) Thumbnail View:** displaying a thumbnail overview of the experiment according to the utilized microplate layout; **(3) Single-Well View:** displaying the current well images as selected in the Thumbnail View at higher resolution; **(4) Expert Panel:** providing controls and menus to modify the visualization and navigate in XYZCT.

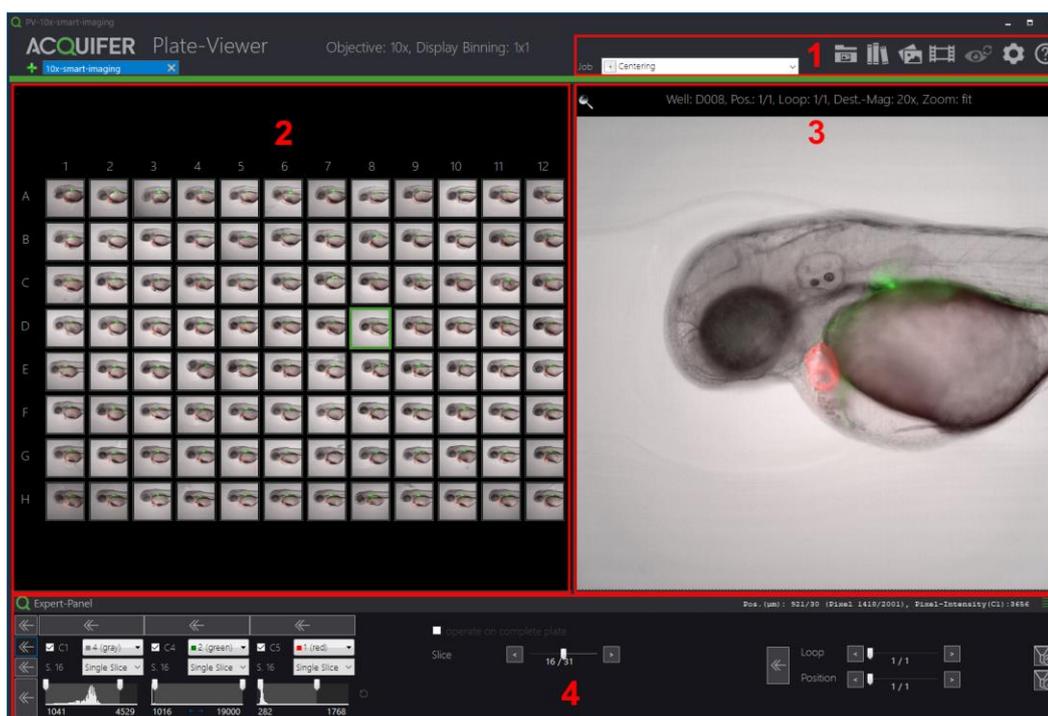


Figure 3 | Main control areas in the Plate-Viewer software. (1) Main Menu Area, (2) Thumbnail View, (3) Single-Well View and (4) Expert Panel

## 4.2 Plate-Viewer Main Menu Area

The Main Menu Area provides access to functionalities to open experiments, configure feedback microscopy workflows, export data or configure the PlateViewer.

- 1) **'Open Image Folder'** (via folder symbol): opens an image folder with image data acquired with the ACQUIFER Imaging Machine and displays thumbnails in the Thumbnail Preview. The plate layout, the objective used, and the number of channels, z-slices, subpositions and loops are automatically detected.
- 2) **'Create Script'** (via Files symbol): Opens a file dialog to select an ACQUIFER Imaging Machine Script file (*\*.imsf*) for subsequent modification by the Plate-Viewer. This function can be used to re-center regions of interest after selecting them in the Single-Well View using the built-in click-tool functionality or certain plugins. Please refer to section 4.6 for more details about the click tool and supervised feedback microscopy experiments.
- 3) **'Store Images'** (via image symbol): Opens a pop-up window that allows to store/save currently displayed image views as *\*.bmp*, *\*.png*, *\*.jpeg* or 16-bit Tiff. By default, a subfolder within the currently opened experiment is used, but it can be freely defined. Images can be saved for individual wells or entire experiments either channel-by-channel or as RGB overlays using the different available options (see Figure 4).

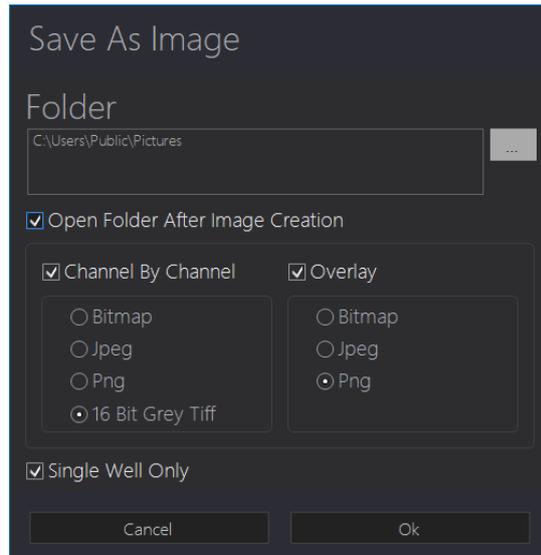


Figure 4 | Sub-menu for saving and exporting individual images

- 4) **'Create Image Sequence'** (via movie symbol): Opens a pop-up window that allows to store/save image sequences either as z-stacks or time-stacks. By default, a subfolder within the currently opened experiment is used, but it can be freely defined. Image sequences can be saved as multipage Tiffs or MP4 movies for individual wells or entire experiments either channel-by-channel or as RGB overlays. The framerate of MP4 files can be freely defined and movies can be optionally compressed using the Codec H264.

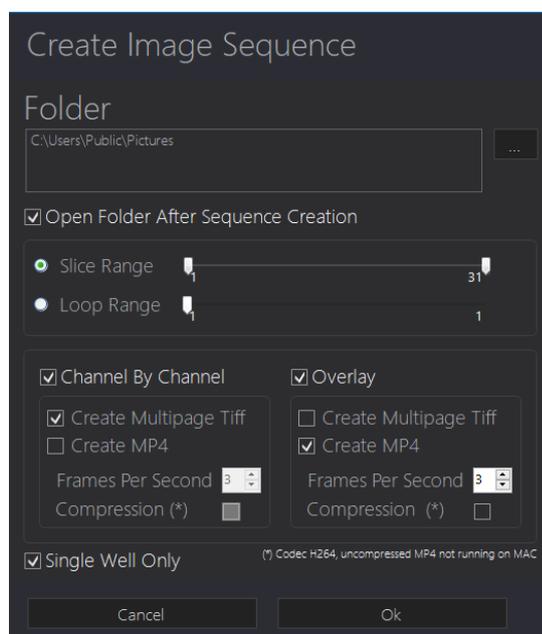


Figure 5 | Sub-menu for saving and exporting image sequences

- 5) **'Reset Changes'** (via eye symbol): reset all changes the user made to the current experiment visualization (e.g. histogram adjustments, LUTs etc.) and restores default settings.
- 6) **'Settings'** (via gear symbol): Opens a pop-up window (Figure 6) that allows to configure default performance and visualization settings, as well as plugin/job configurations. Available settings are the default binning factor of displayed images, the default directory containing scripts to be modified (see section 4.6), options how to handle cached temporary data used to improve browsing performance, maximum number of tabs in the thumbnail view (see section 4.3), default LUTs used for different channels and configuration of jobs and plugins (see section 4.7).
- 7) **'About'** (via question mark symbol): Shows versions number and manufacturer information.

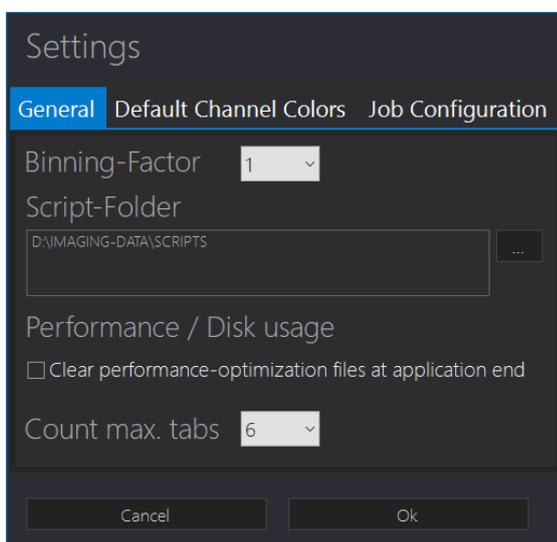


Figure 6 | Plate-Viewer settings menu

### 4.3 Thumbnail View

The Thumbnail View provides an intuitive overview of the acquired image datasets. The layout is automatically adjusted to match the plate layout of the experiment and by default an overlay visualization of all color channels is shown. However, the thumbnail view will adapt to any changes made to the visualization in the **Expert Panel** or **Single-Well View** (see section 4.4).

Multiple experiments can be opened and will be displayed in separate tabs. Each tab can be configured separately. A new tab (experiment) can be opened using the green plus button. Tabs can be un-/docked using the green triangle button. Tip: right-clicking on the tab name will open the experimental folder in Windows Explorer.

Individual wells can be selected by mouse-click. Selected wells will be shown at higher resolution in the **Single-Well View**. Once a single well is selected, browsing through the wells can be done using the arrow keys.

The Thumbnail View has further context dependent functionalities that can be reached by right-clicking individual wells or the general area. These functions will be discussed in the sections 4.6 and 4.7.

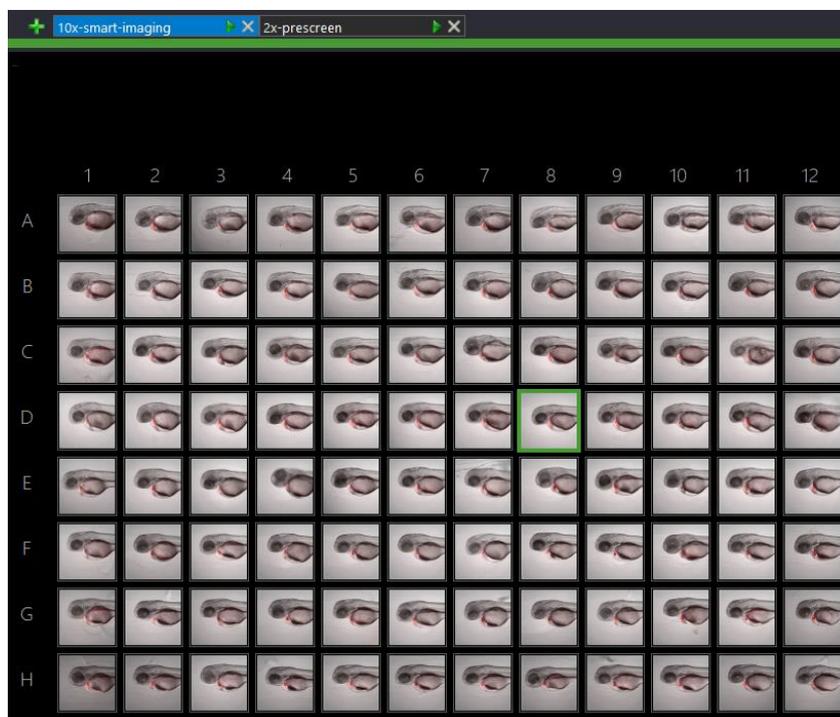


Figure 7 | Thumbnail-View. Shown is the visualization of a 96 well plate.

#### 4.4 Single-Well View

The **Single-Well View** display the currently selected well at higher resolution. It will show an RGB overlay image of the active channels of currently selected z-slices (or z-projection), as well as the currently selected loop and subpositions as selected in the **Expert Panel**. The mouse wheel can be used to zoom-in and zoom-out.

The **Single-Well View** can be undocked from the main interface by clicking the magnifying glass symbol. In the undocked window, navigation through wells can be achieved through the arrow buttons and zooming-in and zooming-out through the mouse wheel or respective buttons.

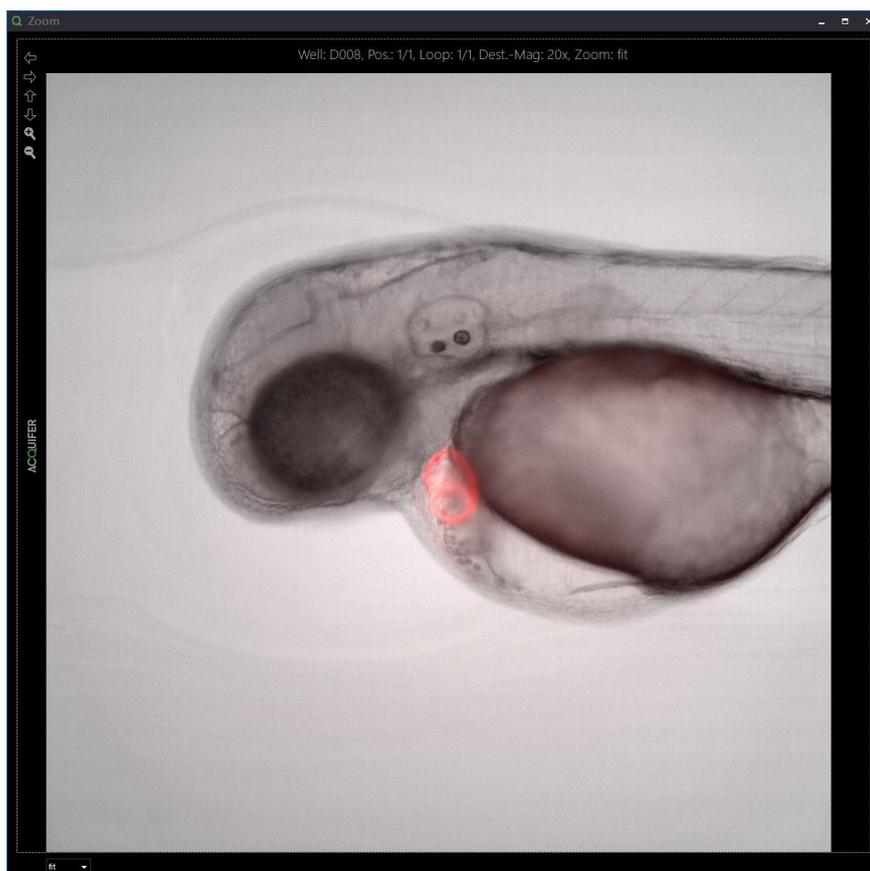


Figure 8 | Undocked Single-Well View. Shown is the visualization of a single well.

## 4.5 Expert Panel

The Expert Panel allows to change the visualization of data and browse through XYZCT.

### 4.5.1 Channel configuration

On the left-hand side, a configuration matrix menu is located to configure each channel individually. By default, any changes are applied to a single well only to reduce latencies and save computation time. However, all settings can be readily transferred to all other wells by clicking the arrow buttons surrounding the configuration matrix menu. Arrows on the top will apply all settings within a column of the menu and arrows on the left-hand side will apply all settings within a row of the menu, respectively. The arrow in the left corner will apply all settings to all wells.

The top row of the menu allows to select if a channel is displayed in the **Thumbnail View** and the **Single-Well View** by clicking the corresponding tick-box. The LUT of a channel can be selected using the drop-down menu.

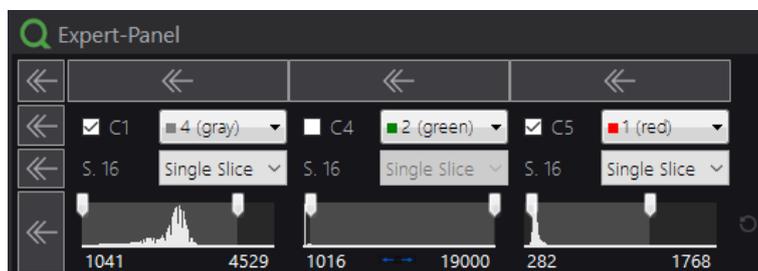


Figure 9 | Matrix menu to configure visualization of different channels

The middle row allows to choose the display mode of image data that consists of more than one z-slice as either single or individual z-slices, or as minimum or maximum intensity projections.

The bottom row allows to adjust the display scaling of the images by moving the sliders on the histogram, i.e. the grey-scale pixel value of the left slider will be shown as black and the grey-scale intensity value of the right slider will be shown as white. The histograms are automatically adjusted such that the borders correspond to the minimum and maximum grey-scale intensity of the currently selected image. Borders can be manually set by right-clicking on the histogram. All histogram changes can be reset by clicking the curved arrow next to the histogram or bottom row, respectively.

#### 4.5.2 Dimensionality browsing

Navigation through XYZT and subpositions can be done using the three sliders for **Slice** navigation (= z-slice), **Loop** navigation (= timepoint) or **Position** navigation (= subpositions). Navigation to other planes can be done by clicking the arrow buttons or moving the slider. The currently viewed position and the maximum number of images for respective dimensions is indicated.

The user can either browse individual wells or all wells simultaneously by clicking the **'operate on complete plate'** tick-box. Please note that operating on all wells simultaneously may result in latencies especially when browsing larger datasets.

Visualization settings can be saved or loaded using the 'Load Filter' and 'Store Filter' button on the very right of the panel. This is useful to quickly apply non-standard configurations to similar datasets.

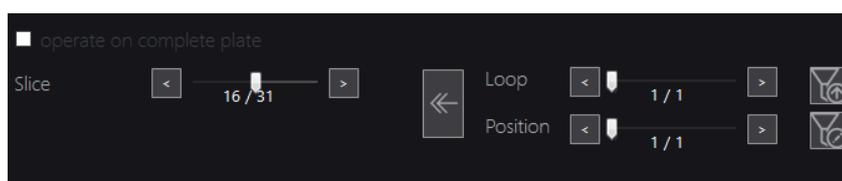


Figure 10 | Sliders for multidimensional navigation

## 4.6 Click-Tool functionality for supervised feedback microscopy

The Plate-Viewer can be used to setup supervised feedback microscopy workflows, in particular re-centering workflows. The user can select XY coordinates and regions of interest that should be centered and imaged in a subsequent experiment. Once selected the Plate-Viewer software can modify an existing Imaging Machine script file (\*.imsf) to replace all *GotoXY* commands. The modified script can then be loaded and executed in the ACQUIFER Imaging Machine control software.

The classical use case for this workflow is to acquire pre-screen data at lower resolution (e.g. 2x) and open it in Plate-Viewer. Then the user selects the ROI and modifies an existing template script (\*.imsf) containing instruction for an higher resolution or time-lapse experiment. Once executed, the modified script leads to acquisition of the automatically centered ROIs.

### 4.6.1 Selecting ROIs for automated centering and imaging

To select a XY coordinate for automated centering and imaging, one can simply click onto the image in the **Single-Well View**. A **centering-cross** will indicate the selected XY position. Additionally, a **centering-box** is displayed that will indicate the field of view with different objectives. The currently selected destination objective is indicated above the preview image. Please note that only a single XY coordinate can be selected per image of a well or subposition due to the structure of \*.imsf files. To select multiple positions it is needed to image the structure multiple times using subpositions.

Right-clicking on the Single-Well view will open a context menu for configuration of the Click-Tool.

- **Clear centering position** will delete the currently selected XY coordinate and ROI.
- **Change centering-box color** allows to change the color of the bounding box to optimize visibility on different LUTs.
- **Show centering-box** tick-box switches the visibility of the centering-box.
- **Show centering-cross** tick-box switches the visibility of the centering-cross.
- **Select objective** submenu allows to select the destination objective and thus the size of the centering box indicating the field of view of the chosen objective.

Regions of interest and XY coordinates need to be identified for each well individually; however, not all wells need to be annotated. Wells without ROI annotation will be automatically omitted in subsequent screening experiments. Therefore, the click-tool workflow cannot just be used to center ROIs but also to filter out unwanted wells or samples.

Wells can be selected in any order from the Thumbnail preview. For rapid annotation, the space bar on the keyboard can be used to automatically move to the next well in the Single-Well View and place a centering-cross and centering-box at the center of the image. Existing centering-cross can be moved anytime or deleted using the context menus.

To provide an intuitive overview, all selected ROIs are also visualized in the thumbnail preview. The **Centering-visualization** can be adjusted to show the centering-box, a symbol indicating that the well was annotated or both by right-clicking on the Thumbnail Preview and choosing the respective option from the context menu. Additionally, the context menu of the Thumbnail Preview allows to remove annotations.

#### 4.6.2 Modifying and executing an \*.imsf script

Centering coordinates can be used to modify an existing ACQUIFER imaging Machine \*.imsf script file. An \*.imsf script is loaded using the **'Create Script'** button in the main menu area. The Plate-Viewer will then automatically modify the target script, i.e. existing XY coordinates will be overwritten with new values according to the centering-cross annotations, and non-annotated well coordinates are deleted. A new script file will be generated in the directory the template script was loaded from with the file name addition *\_CENTERED* and all capital letter (e.g. Input: *..\script.imsf* → Output: *..\SCRIPT\_CENTERED.imsf*). Upon successful modification of the template script the Plate-Viewer software will report the number of successfully modified XY coordinates and the number of removed wells in a pop-up window as blocks modified or removed.

The modified or re-centered script can be loaded in the ACQUIFER imaging Machine control software's **'Smart Imaging'** tab and executed by clicking the **'Run'** button. Please refer to the ACQUIFER Imaging Machine control software manual.

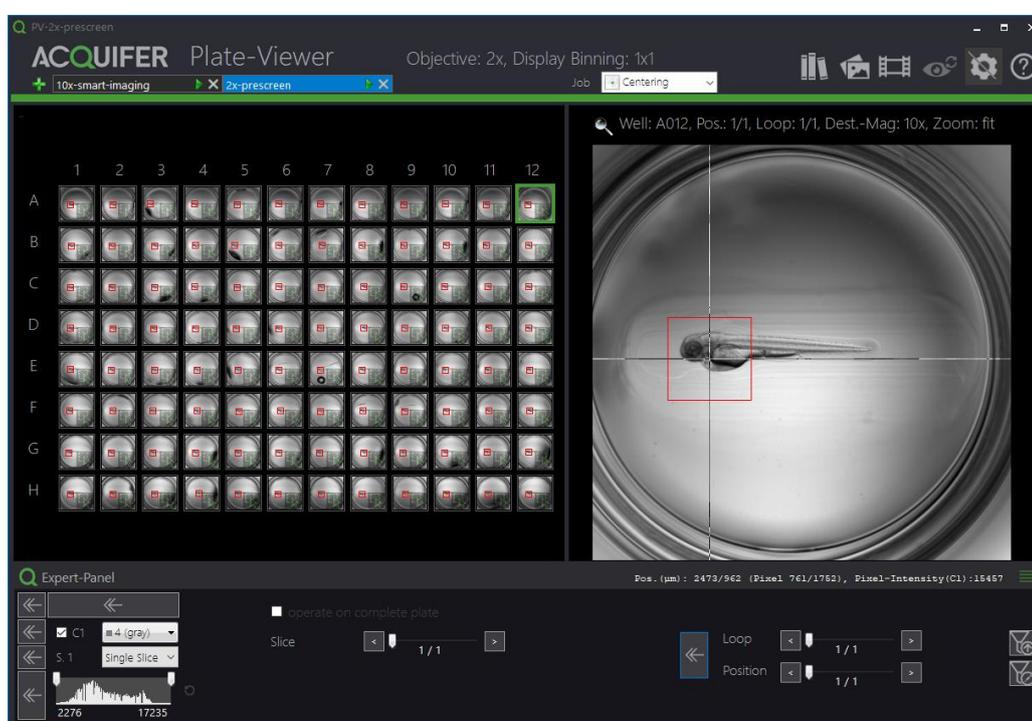


Figure 11 | Click-Tool functionality. View of a full 96 well plate experiment after selection of 96 regions of interest. The Single-Well View shows the centering-cross and centering-box. The Thumbnail View the centering box and a symbol indicating that regions of interest were selected for individual wells

## 4.7 Plugins and Jobs

The Plate-Viewer can execute external programs using the Job or Plugin interface. Plugins can be configured using 'Settings' -> 'Job Configuration'. In brief, a command line call and the content of an input CSV file can be configured. The external program can retrieve information from the input CSV file to configure a processing task and can send back processing results to the Plate-Viewer using an output CSV file. The generation of new plugins is intended for advanced user only. Please contact [support@acquifer.de](mailto:support@acquifer.de) or your ACQUIFER specialist for further information on Plate-Viewer plugins.

### 4.7.1 Executing Plugins and Jobs

The Plate-Viewer is optionally shipped with a set of plugins to automatically detect regions of interest for automated placement of centering-crosses and centering-boxes based on object detection algorithms (e.g. template-matching plugin based on [Thomas, L., Gehrig, J. BMC Bioinformatics \(2020\)](#)). Additionally, demonstrator plugins for image processing and analysis may be installed. The detailed usage of optional plugins is introduced during training upon system installation depending on customer requirements. Please contact [support@acquifer.de](mailto:support@acquifer.de) or your ACQUIFER specialist for further information on available Plate-Viewer plugins. In general, plugins can be accessed and executed using the 'Job' dropdown menu in the main menu area and clicking the green triangular play button next.

### 4.7.2 Laser plugin

If the purchased ACQUIFER Imaging Machine is equipped with a laser module, the **laser plugin** can be used to configure automated photomanipulation experiments. The laser plugin can be accessed from the drop-down menu 'Job' in the main menu area and can be recognized by the flash symbol.

Once the laser plugin is active, the user can annotate images with rectangles, lines and polygons within the **Single-Well View**. The different labelling tools can be selected in the right-upper corner of the Single-Well View. These annotations represent the areas or **laser regions** that will be illuminated by laser light in a subsequent step. Multiple annotations can be placed on a single image. Wells annotated with a laser region will be marked with a flash symbol and yellow frame in the Thumbnail Preview.

Each laser region can be configured from a context menu accessible by right-clicking on the annotation:

- **Laser object properties:** opens a sub-menu to specify the relative laser intensity and the number of iterations of laser illumination.
- **Clear current laser object:** deletes the current annotation.
- **Clear all laser objects (current well):** deletes all current annotation from an individual well.
- **Clear all laser objects (complete plate):** deletes all current annotation from the entire experiment.
- **Fill current laser object:** either fills the current annotation for area-illumination or removes filling for outline-illumination.
- **Copy laser object:** copies the current laser object
- **Paste laser object:** pastes the copied laser object to a new position or well

To start a photomanipulation experiment, click the 'RUN' symbol (green triangle) next to the 'Job' dropdown menu. This will open a user dialog to specify some global parameters:

- **Runs:** specification of the number of repeats of the photomanipulation experiment. Please note: This is the number of repeats for all annotated regions. This is in addition to the number of iterations for individual annotations.
- **Step size:** parameter to control the step size of the laser, i.e. galvo mirror system, to adjust energy density at the photomanipulation sites in addition to the laser intensity. It can be set either by entering a number in the control field or by using the slider.
- **Accurate lasering (instead of fast lasering):** Tick-box to enable/disable the accuracy mode. It is recommended to enable accuracy mode. For further information, please refer to the laser hardware manual.
- **Current Well only:** Tick-box to laser only the currently selected well.

**Important:** Please make sure the ACQUIFER imaging Machine control software is in live mode and the correct channel is selected before continuing!

By pressing 'OK' the photomanipulation experiment is started. Depending on the complexity, number, size and fill ratio of annotations the time until it starts varies and may take a few moments. A status log window will inform the user about the state and progress of the experiment.

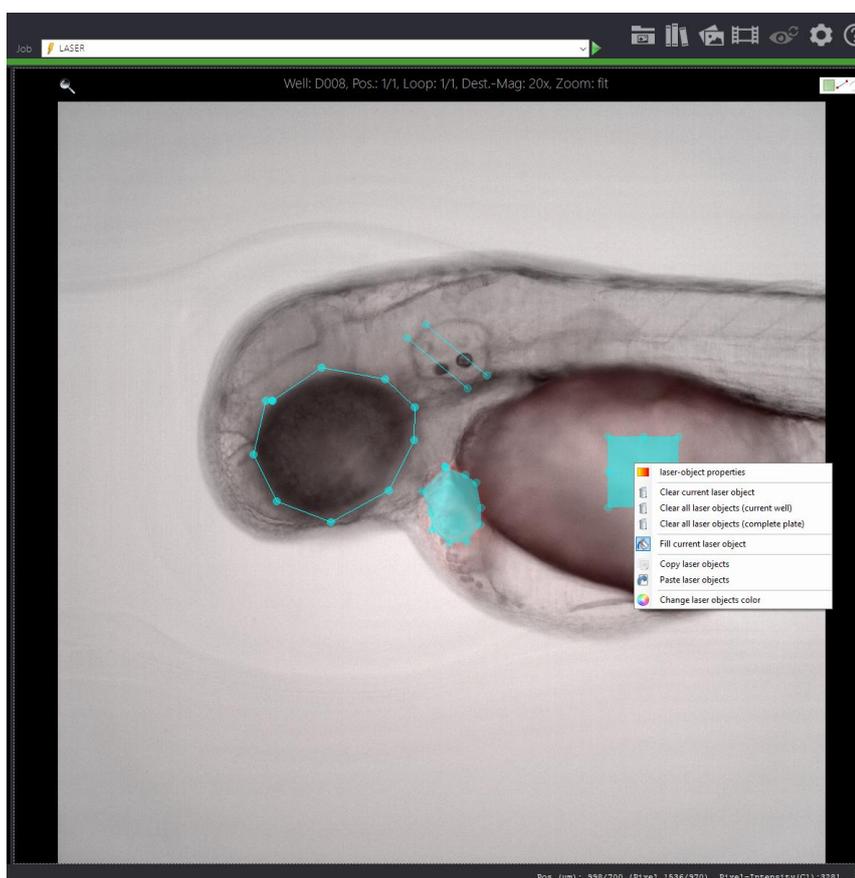


Figure 12 | Single-Well view with annotation for laser photomanipulation. Shown is a single image with different regions of interest and the context menu to configure a single annotation.

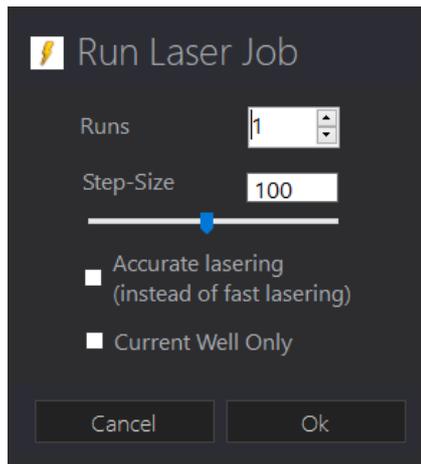


Figure 13 | User dialog to configure global parameters of the photomanipulation experiment. 'OK' will start the experiment.

## 5 Trouble Shooting

There might be rare situations when the Plate-Viewer does not react or acts unexpectedly. In many cases, this can be solved by simple procedures, modifying settings or by using built-in troubleshooting functions of the software.

Problem	Solution
Error message: 'no images found in folder'	<ul style="list-style-type: none"> <li>• Ensure you selected an experimental folder generated by the ACQUIFER Imaging Machine.</li> <li>• Check if the experiment has been modified, e.g. partial deletion, renamed files etc.</li> </ul>
Error message: 'unable to find the specified file'	<ul style="list-style-type: none"> <li>• Check if the folder <i>log</i> is present and if it contains a valid <i>*.imsf</i> file.</li> <li>• Check that you use the correct version of Plate-Viewer for your version of ACQUIFER Imaging Machine control software.</li> </ul>
Error message: 'Access denied' or 'Experiment is already open'	<ul style="list-style-type: none"> <li>• Ensure that the experiment is not open in a different instance of Plate-Viewer, e.g. another tab or another windows account.</li> <li>• Ensure that you have the windows permission to view and modify the experiment.</li> <li>• Advanced users with permission only: delete the <i>lock.lck</i> file from the directory.</li> </ul>
Plate-Viewer does not start or crashes after start.	<ul style="list-style-type: none"> <li>• Reboot Windows</li> </ul> <p><u>Advanced users and system administrator only!</u></p> <ul style="list-style-type: none"> <li>• Check in the task manager if PlateviewerHost is running while Plate-Viewer software has not started. End the task.</li> <li>• Delete the file C:\ProgramData\PlateViewer\%username%\_PVS.xml. Warning this will delete all Settings made in the 'Settings' area of the PlateViewer. Do NOT delete any other xml files.</li> </ul>
Images load slowly and navigation shows latency	<ul style="list-style-type: none"> <li>• When working with very large datasets change the display binning in 'Settings' to 2, 4 or 8.</li> <li>• Disable the tick-box 'operate on complete plate'.</li> <li>• Check if there is high computational load caused by other processes on the system.</li> </ul>
The data visualization and/or centering-cross positions are wrong for multiple plates and/or subpositions and cannot be easily reverted	<ul style="list-style-type: none"> <li>• Reset all changes by clicking 'Reset Changes' in the main menu.</li> </ul>

## 6 Contact Manufacturer

ACQUIFER Imaging GmbH  
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69123 Heidelberg  
Germany

If you need further assistance, please contact: [support@acquifer.de](mailto:support@acquifer.de)

**Authorized person for technical documentation**  
**ACQUIFER Imaging GmbH**

Hans-Bunte Str.8  
69123 Heidelberg  
Germany

Place and Date of issue

Heidelberg, Germany, February 16, 2022

Ralf Mulflur, General Manager

## 7 Examples

### 7.1 Illustration of workflow for supervised feedback microscopy

The click-tool workflow is intended to readily configure supervised feedback microscopy experiments within the Plate-Viewer software. The workflow is designed to select regions of interests for subsequent automated centering and imaging at higher resolution. Thus, it enables the targeted acquisition of highly consistent datasets of regions-of-interest or rare events.

In the [example](#), the workflow is illustrated for the acquisition of head regions of zebrafish embryos. However, this workflow can be applied without any modification to any other samples (e.g. cell clusters, spheroids/organoids, tissue regions etc).

#### 7.1.1 Acquisition of a pre-screen dataset

In a first step, a dataset needs to be generated. Therefore, the microplate is imaged at a magnification that provides sufficient detail to identify the region of interest. The magnification should be chosen in a way so that the field of view is large enough to see all relevant areas of the specimen. Additionally, this pre-screen experiment should be configured to contain all relevant color channels that allow the region selection based on fluorescent features. After acquisition the data is opened in Plate-Viewer.

**Example:** A zebrafish containing 96-well plate was imaged at low resolution with a 2x objective in the bright field channel. The field of view of the 2x objective allows to capture the entire well of the 96 well plate and thus also the entire embryonic body including head, trunk and tail.

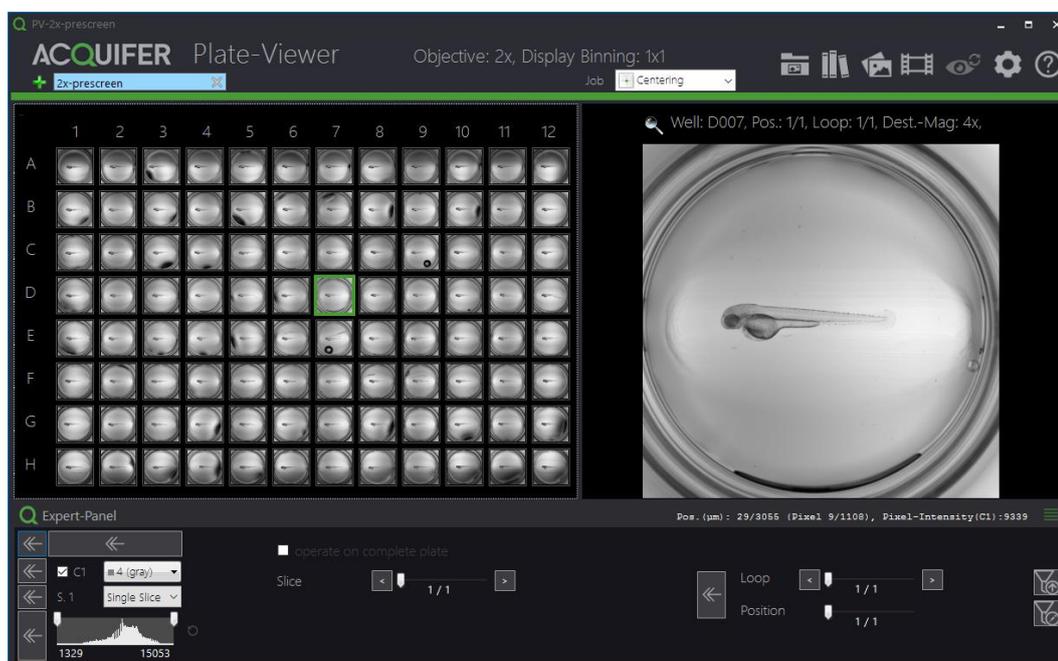


Figure 14 Pre-screen data. Shown are entire wells containing zebrafish embryos imaged with a 2x objective.

### 7.1.2 Selection of centering coordinates

The regions of interest are selected in the **Single-Well** view by simply clicking on the image. It is recommended to select the right target magnification from the context menu. The bounding box will adjust its size according to the magnifications. This helps to position the bounding box and ensure that all relevant structures will be present in the high-resolution image. Wells or subpositions that should be omitted in the main-screen are simply not selected.

Plugins for automated identification of regions of interest are available. Please discuss with your ACQUIFER specialist or contact [support@acquifer.de](mailto:support@acquifer.de) for further details.

**Example:** The centering-cross and centering-box were positioned such that the head, heart region, yolk sac and parts of the trunk are within the field-of-view of the 10x objective.

### 7.1.3 Generating, modifying and executing a script

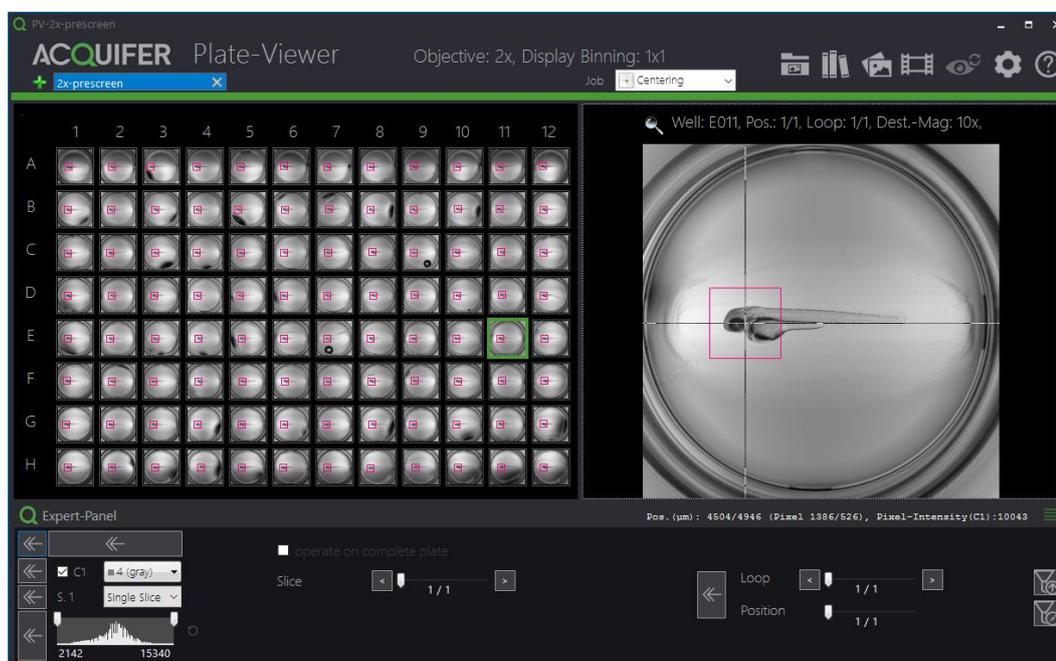


Figure 15 | Selection of region of interest. Shown is pre-screen data with annotated centering-crosses and centering boxes

The selected regions of interest can be used to modify an existing Imaging Machine script (**template script**) such that all well or subposition XY center coordinates are replaced with new XY coordinates corresponding to the centering-cross coordinates. In addition, all non-selected wells or subpositions are removed.

The existing script is generated with the ACQUIFER Imaging Machine control software. It must include all imaging parameters such as channels, objectives, loops, autofocus. Therefore, a target region is manually centered and focused, and imaging parameters are adjusted to produce the desired image data. This configuration is then saved as IMSF script file in the Smart Imaging tab of the Imaging Machine control software (for details please see Imaging Machine manual). The so-called **template script** must contain all well and subposition coordinates that are selected in the pre-screen as new positions cannot be added by the Plate-Viewer. It is recommended to

select all wells before generating the script as excess coordinates and associated commands are automatically removed by the Plate-Viewer. Also the number of subpositions should match or exceed the number of subpositions in the pre-screen.

The **template script** can then be automatically utilized in the PlateViewer. This will produce a new script in the target directory with the file name addition `_CENTERED` and all capital letter (e.g. Input: `..lscript.imsf` → Output: `..SCRIPT_CENTERED.imsf`) (see section 4.6.2). As the template script will not be deleted or modified, it can be re-used for other experiments; thus, users can build up a library of commonly used template scripts.

To run a modified script, open it in the Smart Imaging tab of the Imaging Machine control software and click 'Run'. After execution it can be opened and explored with the Plate-Viewer like any other regular dataset.

**Example:** A template script was generated with instructions to image regions of interest in 3 channels (BF, FITC, TRITC) and 31 z-slices with a 10x objective after execution of a 2-step software autofocus procedure.

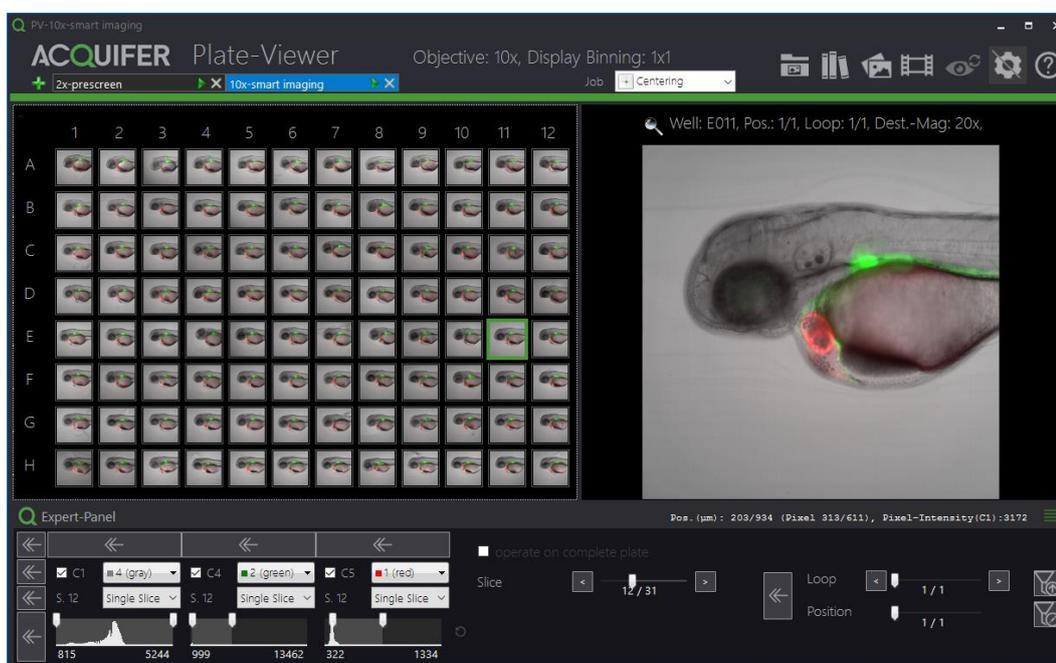


Figure 16 | Visualization of main screen data. Shown are regions of interest as RGB-overlay images after automated re-centering and imaging based on the modified template script.