

# JuLI™ Stage

Real-time live cell imaging system

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## User Manual



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## **JuLI™ Stage User Manual**

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The information in this user manual is described as accurately as possible.

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# **1 Safety and compliance**

## **1.1 General safety precautions**

- If water or other material enters the instrument, adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to Product Specifications.
- Do not touch the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltage available at your location.
- This instrument is air-cooled and its surfaces may become hot during operation. During installing, leave a space of more than 10 cm (4 inches) around the instrument and do not place any objects between the instrument and the walls.
- Do not install the instrument on a slant or a place prone to vibrations, which induces the risk of instrument malfunction or damage of the instrument.
- Never insert any objects into the air vents of the instrument as this could result in electrical shock, personal injury, and equipment damage.
- Plug the power cord firmly into the wall outlet and AC adapter.
- To avoid potential shock hazard, make sure that the power cord is properly grounded.
- Be sure to position the equipment such that it is easy to disconnect.
- Turn off the instrument before unplugging the power cord and/or moving the instrument.
- If the instrument is dropped or broken, disconnect the power cord and contact a service person. Disassembling the case will void the warranty.
- Use only authorized accessories (adaptor, power cord, and USB drive).

## 1.2 Safety symbols

Symbol	Meaning
	Caution and Warning
	Protective earth (Ground)
	This instrument and consumables conforms to the EC Declaration of Conformity.
	<p>This equipment has been tested and found to comply with the limits for a Class A digital instrument, pursuant to Part 15 of the FCC Rules.</p> <p>These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.</p> <p>This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.</p>

## 1.3 **Intended use**

 **Warning! Use only as intended.**

The JuLI™ Stage system, including the original accessories may only be used in conjunction with the methods described in this manual. NanoEntek does not assume any liability for any other applications or procedures, including use of individual subassemblies or components for other purposes. The manufacturer does not assume any liability for any other kind of application, including individual subassemblies and the addition of individual components. This exclusion of liability also applies to all service or repair work which was not carried out by authorized NanoEntek service personnel.

**For research use only – not for use in diagnostic procedures**

The JuLI™ Stage is a real-time live cell imaging system suitable for use in research applications such as academic, pre-clinical, and for forensic applications only. Due to its technical design the JuLI™ Stage is particularly suitable for cellular applications.

**Exclusion of Liability for Measurement Results**

NanoEntek does not assume any liability for the correctness of measurement results obtained with the NanoEntek JuLI™ Stage system nor for conclusions based on these measurement results.

## **2 Product overview**

The JuLI™ Stage is a real-time live cell imaging system that can acquire extended image series directly from various cell culture plates (6 to 384 well), flasks, slides and dishes in an incubator. The JuLI™ Stage supports a brightfield and three fluorescence channels, three objective lenses, and sensitive filter-based optics to optimize for various live cell assays. It combines ease-of-use with versatility by offering simple workflows and robust analysis of different readout parameters.

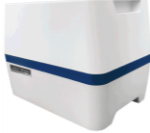










The JuLI™ Stage is suitable for a wide variety of applications:

- Live cell imaging (time lapse)
- Cell culture quality control
- Cell culture optimization
- Cell-based assay optimization
- Angiogenesis
- Scratch-wound assay
- Neurite growth
- Protein expression
- Kinetic imaging and cell differentiation
- Cell growth monitoring
- Stem cell development
- Cell proliferation
- Cytotoxicity
- Cell migration

## 2.1 Scope of delivery

The JuLI™ Stage real-time live cell imaging system is shipped with the following components.

Upon receiving the instrument, please check that all items listed below are included in the shipment. If any part is missing or damaged, contact your local distributor or [sales@nanoentek.com](mailto:sales@nanoentek.com).

<b>JuLI™ Stage</b> 1x	<b>Control Box</b> 2x	<b>Objective lens (4x, 10x, 20x)</b> 3x	<b>Protective covers</b> 2x
			
<b>Connection Cable</b> 2x	<b>Repeater, 5m</b> 2x	<b>Ferrite core</b> 2x	<b>Regional power cord &amp; Adaptor</b> 1 SET
			
<b>JuLI™ Stage Installaion Guide</b> 1x	<b>JuLI™ Stage User manual</b> 1x	<b>PC incl. Mouse &amp; Keyboard</b> 1x	
			

## 3 *Description*

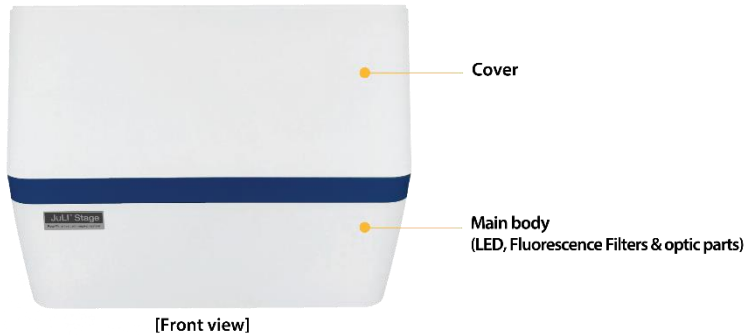
### 3.1 *Front view*

#### **Cover**

Used to protect test samples from external light. Simply put on the cover if you use the JuLI™ Stage on the bench and/or look at light sensitive samples.

#### **Main body**

The main body contains all of the mechanical, electrical, and optical parts.



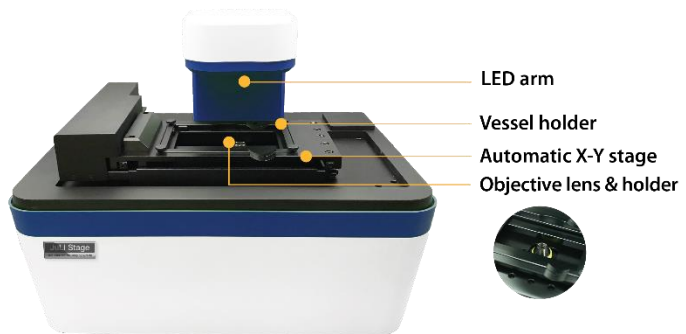
## 3.2 Side view

### LED arm

The arm above the sample holds the LED for the brightfield illumination.

### Automatic XY-stage

The automatic XY-stage precisely navigates to the intended position. The holder is manufactured after SBS standard so that you can insert all standard microplates (6 to 384 well plates). Using one of the JuLI™ Stage vessel holders you can image petri dishes, culture flasks, or glass slides.



## 3.3 Rear view

### Cooling slits

The JuLI™ Stage ventilates the internal parts using 2 fans behind the slits on the back side. Please make sure that the cooling system can operate freely.

### Connection port (device)

Here you can connect the JuLI™ Stage with the control box via the white connection cable (see chapter 2 "Product overview").

### Power inlet

Connect the instrument to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.



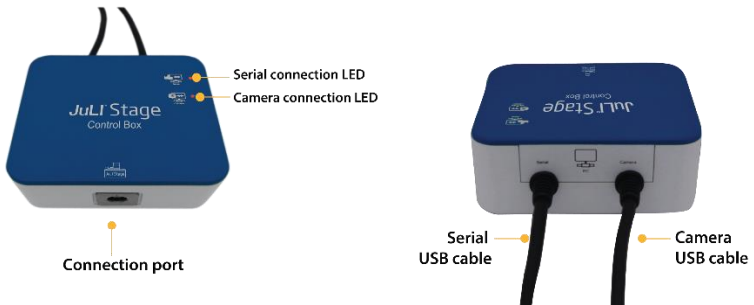
### 3.4 Control box

#### Serial and camera USB cables

The control box can be connected to the PC by connecting the serial and camera USB cables to the USB repeaters. When properly connected the serial and camera LEDs are on.

#### Connection port (control box)

Via the white connection cable the JuLI™ Stage device and the control box can be connected.





## 4 Installation

### 4.1 Unpacking the instrument

1. Open the box and remove the material foam from the box.
2. Carefully lift the instrument out of the box, holding it by the handholds in the bottom of the base.
3. Place the instrument on a flat, levelled surface (table or incubator).



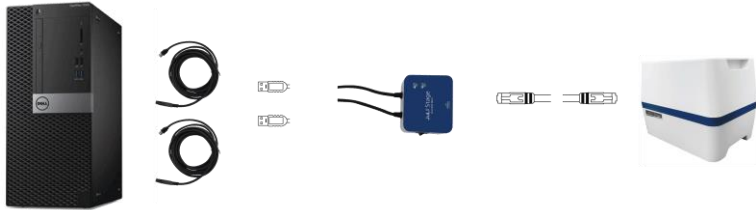
 **IMPORTANT!**

Do not lift the JuLI™ Stage using the LED arm.

## 4.2 Installing the instrument

- !** **Installation:** Please make sure to carefully follow the installation instructions in this manual in order to ensure safe operation. The final installation should look as shown in the following connection overview:

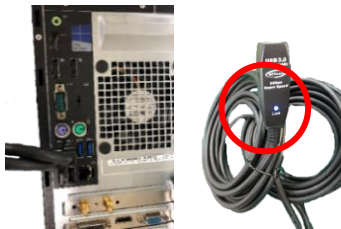
PC ▶ USB-Repeater ▶ USB-Cables ▶ Control Box ▶ Connection Cable ▶ JuLI™ Stage



- !** Make sure no cable is under tension.

1. Connect the PC with the repeater cables:
  - Connect the two repeater cables to the USB3 ports on the back of the PC.
  - Connect the PC to the power outlet using the power cord and turn on the PC.

The blue light on the repeater cables should be on.



2. Connect the other end of the repeater cables to the USB cables of the control box.  
The LEDs on the control box should be red. Protect the cable connection with the plastic cover.



3. Plug the white connecting cable into the control box using the end of the cable *without* the ferrite core.



4. Connect the other end of the connection cable *with* ferrite core to the JuLI™ Stage. Make sure that the connection cable is tightly connected to the connection port. Then connect the power cord.



⚠ Note: Make sure that the JuLI™ Stage has enough space for the cables and proper ventilation on the back side (~ 10cm).

5. Turn on the JuLI™ Stage using the switch on the control box.



6. Start the JuLI™ Stage control software and follow the warm-up guidelines.

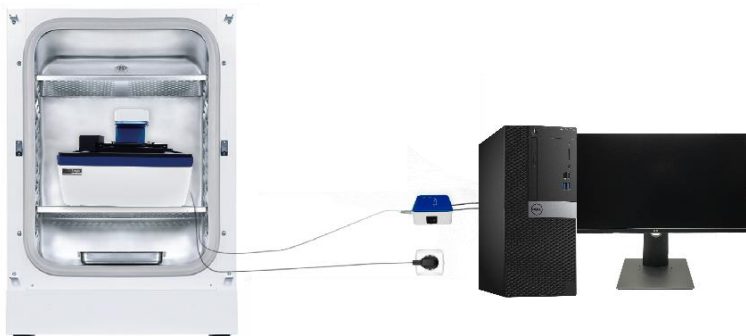


**!** **First use:** Before operating the JuLI™ Stage system for the first time, we strongly recommend allowing the instrument to equilibrate to room temperature for *20 minutes* outside the incubator while the device is turned on.

If the JuLI™ Stage is to be operated inside an incubator, an additional warm-up time of *2 hours* is required to equilibrate it to 37 °C. During this time the JuLI™ Stage must be switched on.

**!** **IMPORTANT!**

When installing the JuLI™ Stage in the incubator, avoid the connection cables to be under any tension. Only the JuLI™ Stage itself may be placed in the incubator, but not the control box or other system components.



 **IMPORTANT!**

Use the cover if the instrument operates on the table to protect test samples from external light.



### 4.3 Operating environment

- Allow at least 10 cm (4 inches) free space at the back of the instrument to allow for proper ventilation and prevent overheating of electronic components.
- Set up the JuLI™ Stage away from direct light sources, such as windows. Ambient room lighting can enter the imaging path and affect the image quality.
- Place the JuLI™ Stage on a level surface away from vibrations from other pieces of equipment.
- Operating temperature range: 5 - 40°C (41 - 104°F)
- Relative humidity range: 20 - 95 %.

### 4.4 Cautions

 **IMPORTANT!**

**First use:** Before operating the JuLI™ Stage system for the first time, we strongly recommend allowing the instrument to equilibrate to room temperature for *20 minutes* outside the incubator while the device is turned on.

If the JuLI™ Stage is to be operated inside an incubator, an additional warm-up time of *2 hours* is required to equilibrate it to 37 °C. During this time the JuLI™ Stage must be switched on.

**In an incubator, the JuLI™ Stage and PC must be left turned on at all times.**

**The JuLI™ Stage is optimized for a standard size cell culture incubator.**

It is recommended that the temperature of the JuLI™ Stage instrument can equilibrate to the temperature of the incubator.

**Environmental requirements**

When you use the JuLI™ Stage inside an incubator, make sure that the temperature of the laboratory never exceeds 15°C to 25°C. Temperature outside this range may affect the performance of the JuLI™ Stage.

**The well plate's cover should be wetted using the cell culture media before monitoring.**

This step is recommended to avoid condensation inside the well plate cover for monitoring.

⚠ Note: This procedure can increase the risk for contamination. When properly warmed up some vessels do not show condensation and can be imaged without pre-wetting the lid. This should especially be considered for critical samples.



**Avoid exposing the JuLI™ Stage to UV light.**

UV light may degrade components, including plastics. Damage from UV exposure is not covered under the manufacturer's warranty.

**Always wipe the surfaces with ethanol-soaked paper towels.**

Do not directly spray ethanol anywhere on the JuLI™ Stage.

⚠ Note: The objectives are especially prone to damage by excessive ethanol. Please always clean the objectives with ethanol-wetted objective cleaning paper.

### **Internet Connection and Windows Updates on Control PC**

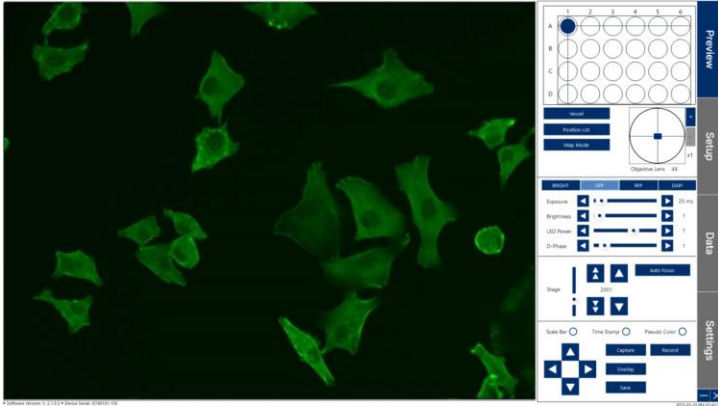
If the control PC is connected to the internet, the Windows® operating system is automatically updated (and rebooted if necessary). In order to avoid errors during operation, we recommend:

- Check for Windows Updates regularly and make sure that all available security updates have been downloaded and installed before starting a measurement.
- Disable internet access temporarily during screening campaigns to avoid unattended installation of updates or reboots during a screening run.

# 5 User interface

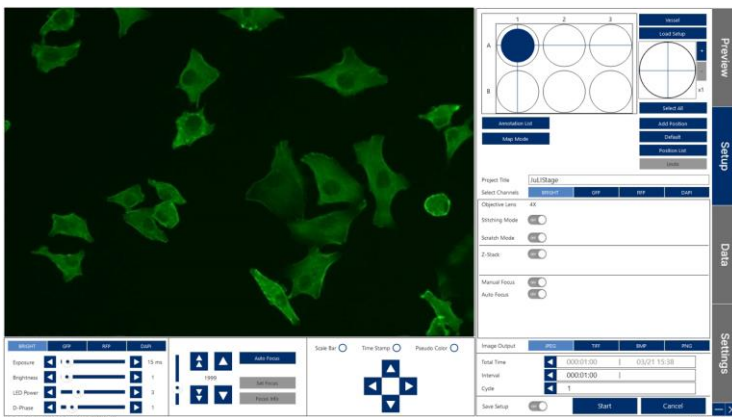
## Preview menu

The **Preview** menu contains all functions to quickly set up the vessel layout, imaging conditions, and acquire snapshots and short movies.



## Setup menu

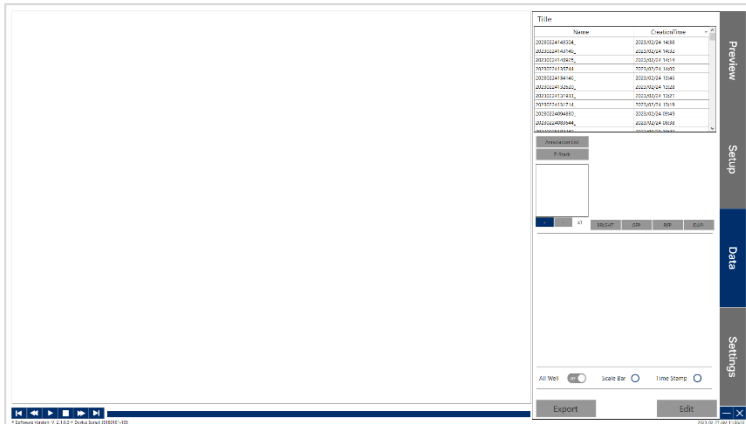
The **Setup** menu includes all functions of the Preview menu (except snapshot and short movie functions) and additionally the experiment settings. For the definition of the experiment you can set an experiment name, layout, manual focus (supported by a focus info map), or autofocus, stitched or scratch mode, image format output, and time lapse preferences. This is where you start your experiments.





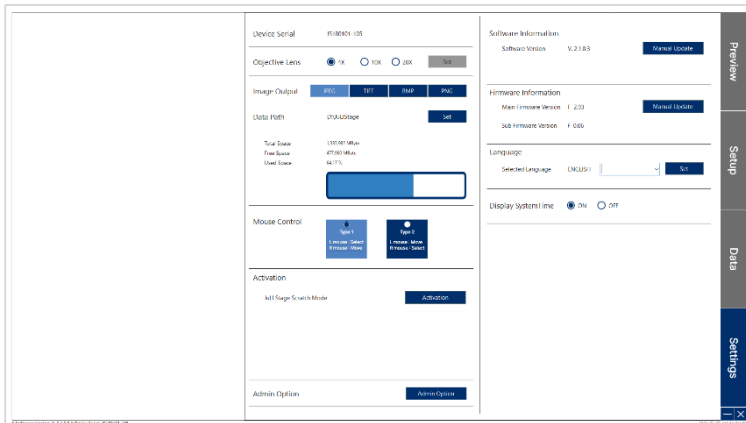
### Data menu

After finishing the experiment you will automatically be directed to the **Data** menu. Here you can review your data.



### Settings menu

Within the **Settings** menu you can revise the device serial, change the objective lens, choose the data path, review the remaining disc space, choose your mouse control configuration, review activated software, update the software, and firmware, and toggle on or off the system time which is displayed on the bottom right of the window (neither can the user enter the admin option, nor are there any options for alternative languages).



## 6 Basic operation

The JuLI™ Stage is controlled by a PC (monitor resolution 1920x1080) via wireless mouse and keyboard.

**Note:** This manual describes all operations using the default mouse setting (Type 1). If necessary, you can change the mouse setting in the **Settings** menu.



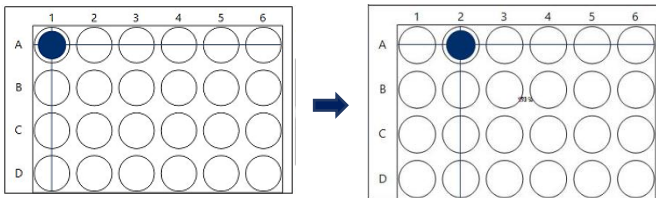
### 6.1 Moving

#### Plate navigation



Right mouse

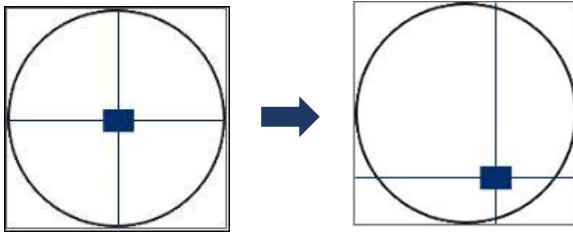
In both the **Preview** and **Setup** menu you can move to the center of a well by clicking it using the right mouse button.



In this example the well A2 was right-clicked which is indicated by a blue circle as shown above.

#### Well navigation

Within a well you can move to any position by right-clicking into the well-representation in the **Preview** and **Setup** menu.

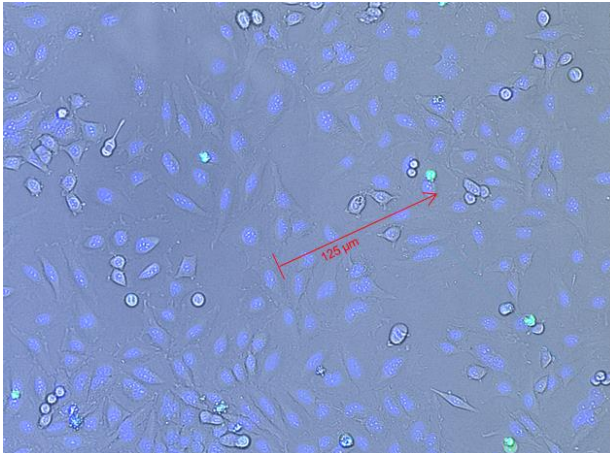


The selected position is marked by a blue square and is equivalent to the field of view.

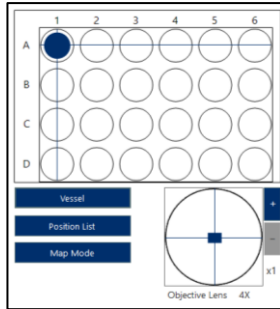
☞ Note: The field of view for the 4x, 10x, and 20x objectives is 3.55 mm<sup>2</sup>, 0.58 mm<sup>2</sup>, and 0.14 mm<sup>2</sup>, respectively.

### Well navigation using the image

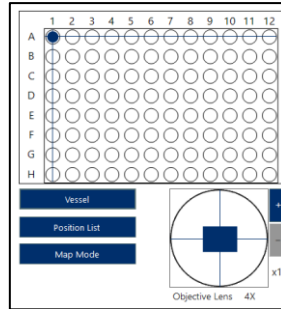
Clicking the right mouse button and dragging over the image will move the current position towards the direction of the arrow in the **Preview** and **Setup** mode. The moving distance is indicated beside a red line as shown below.



☞ Note: The size of the blue square represents the field of view and can change depending on the objective and vessel type.



24 well plate



96 well plate

⚠ Note: When moving between wells, a popup message will appear to wait for the stage to finish moving. If you click **Cancel**, the stage will immediately stop at the current position.



## 6.2 Selection

### Well selection



Left mouse

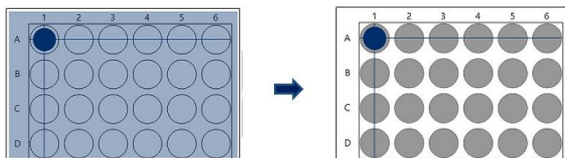
A well can be selected by left click which will highlight the well with a grey color. Clicking the well a second time will de-select it.

⚠ Note: Because it is part of setting up an experiment, wells can only be selected in the **Setup** menu.

Multiple wells can be selected by left-clicking and dragging over all desired wells.

⚠ Note: To de-select multiple wells hold the shift-key, left-click and drag over all wells that are supposed to be de-selected.

When clicking **Add Position** a position in the center of all selected wells can be added to the **Position List** (these positions will be imaged when the experiment is started). These wells will then appear light blue.



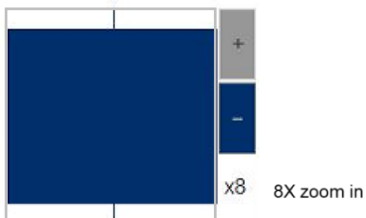
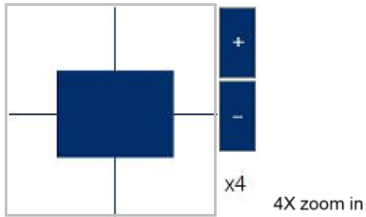
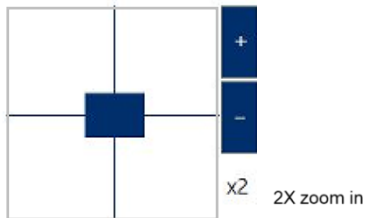
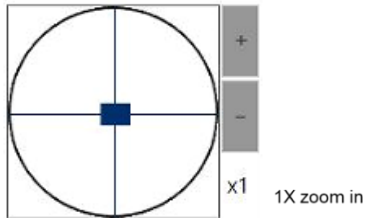
### Selecting additional positions

In order to add multiple positions to a well left-click into the well-representation. Selected positions are highlighted by a light blue square (double-click to delete). Click **Add Position** to add these positions to the **Position List**. Subsequently the squares will appear dark blue.

**Note:** Selecting a well and positions within this well without clicking **Add Position** will result in these positions not being included in the imaging experiment.

### 6.3 Position zoom

You can zoom in or out of the position clicking + or – on the right side of the well-representation.



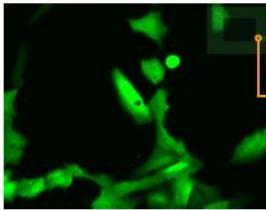
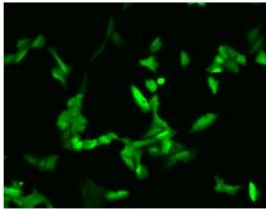
## 6.4 Mouse wheel



Mouse wheel  
(Up and down)

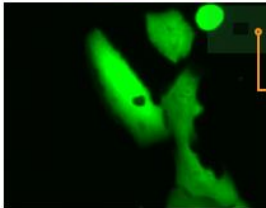
### Zoom-in and out

Scroll up or down in the image view of either the **Preview** or **Option** tab to increase or decrease the size of the image. The zoom function can also be used in all other software modules.



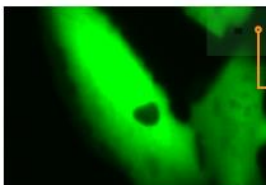
< 1<sup>st</sup> zoom in >

Magnified navigation



< 2<sup>nd</sup> zoom in >

Magnified navigation

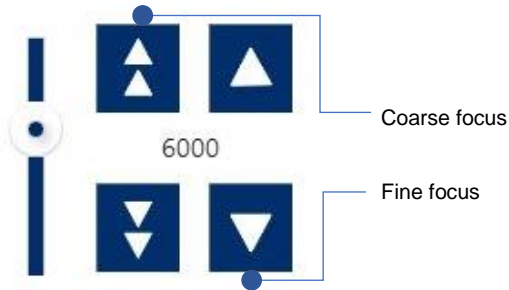


< 3<sup>rd</sup> zoom in >

Magnified navigation

### Focus adjustment

While holding the shift key you can scroll up or down to focus the sample. It is the same function as the fine focus arrow buttons on the screen.

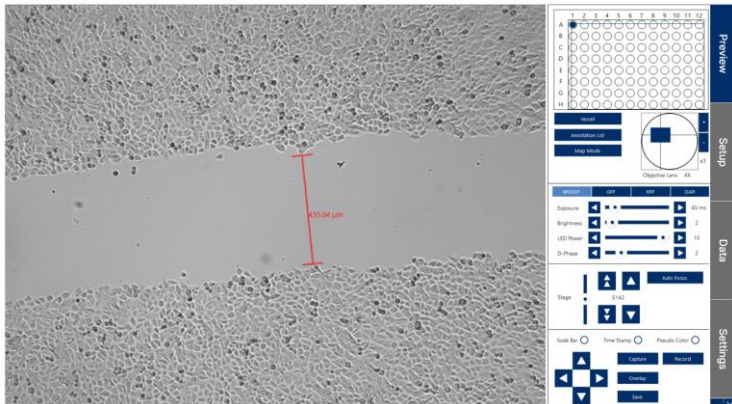


⚠ Note: The coarse focus is not supported by the mouse wheel.

### Measure distance and size

To measure distances or the size of an object click the mouse wheel at one side of an object and hold the mouse wheel down until you reach the end of this object. The length of the bar will be shown in micrometer.

⚠ Note: The size measurement function can be used in both the **Preview** and **Setup** menu.

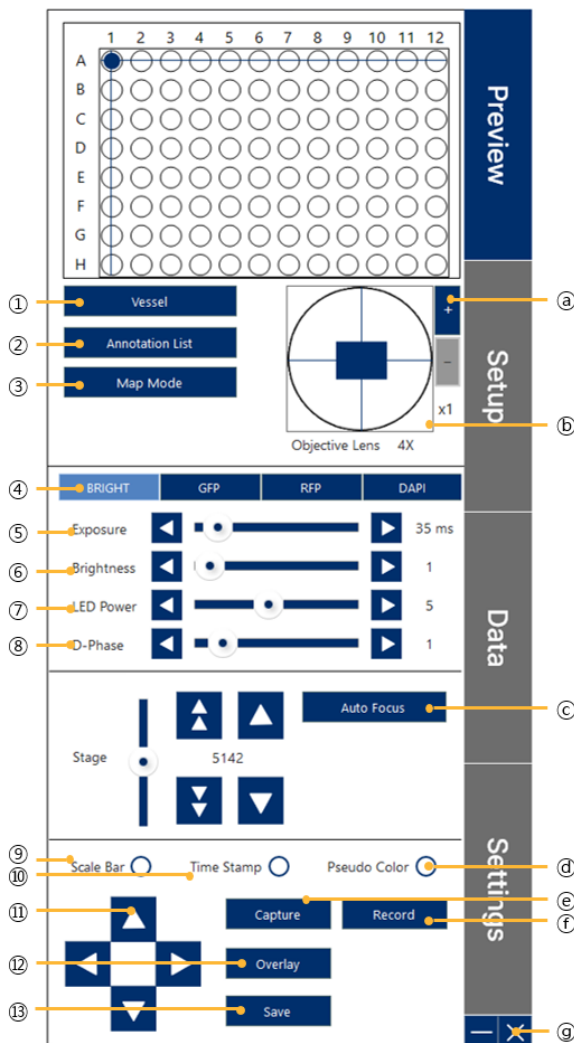




# 7 Operation

## 7.1 Preview menu

The **Preview** menu allows you to select basic system functions and is the first screen after start-up. It is available for checking the sample, setting the optimized focusing, capturing and merging the images.

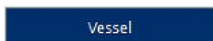


- ① Vessel type / calibration
- ② Annotation list
- ③ Map mode button
- ④ Channel selection
- ⑤ Exposure control
- ⑥ Brightness control
- ⑦ LED power control
- ⑧ D-phase control
- ⑨ Scale bar button
- ⑩ Time stamp button
- ⑪ XY direction control
- ⑫ Image overlay button
- ⑬ Image save button
- a Well zoom in & out
- b Well navigation
- c Auto focus button
- d Pseudo color button
- e Capture image
- f Record short movie
- g Minimize & close buttons

### Vessel type

In this menu you can select your vessel type and calibrate the well positions of this plate.

1. Click **Vessel**.



2. Select the vessel type used in the current experiment.

Vessel Type List

Well Plate

6 Well Plate    12 Well Plate    24 Well Plate

48 Well Plate    96 Well Plate    384 Well Plate

96 Well Plate (Spheroid/Scratcher)

Culture Dish

35mm Dish    60mm Dish    100mm Dish

Flask

250ml Flask    750ml Flask

Slide

Glass Slide

Calibration

3. Click **Vessel** again to close the window without calibration.

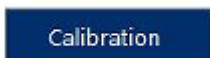
☞ Note: Only vessels belonging to the **Well Plate** type can be calibrated, other vessel will inactivate the **Calibration** button.

☞ Note: In order to see the well borders during a vessel calibration make sure to have the BRIGHT light source turned on.

☞ Note: There are certain combinations of vessel type and objective lens which are not suitable for a vessel calibration: for example, using a 20x lens and a 384 well plate will lead to the 4 edges of the well not being visible at a glance. In such cases, use the next higher magnifying lens, e.g. the 10x objective to calibrate the 384 well plate. The resulting calibration data are also valid for a 20x measurement.

### Vessel calibration

1. Click **Calibration**.

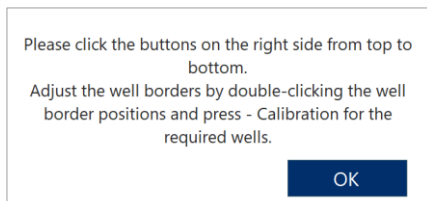


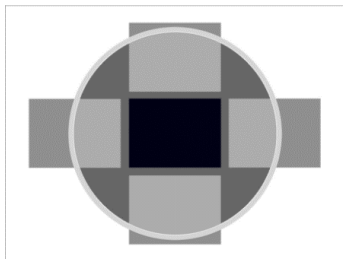
2. Follow the calibration guide on the right from top to bottom.

- a) Click **Move to A01 (or B02)** and **Check A01 (or B02)**. After the well borders were captured set the red calibration lines onto the well borders by double clicking. Then click **Calibration A01 (or B02)** to confirm the new definition.
- b) Click **Move to B02 (or C03)** and **Check B02 (or C03)**. After the well borders were captured move the red calibration lines onto the well borders by double clicking. Then click **Calibration B02 (or C03)** to confirm the new definition.

☞ Note: If the well borders are way off, you can move the well edges into the right position by using the arrow buttons for coarse movements. For smaller corrections, click on a position in the image view, hold the mouse button down, move the image in a direction, and stop the movement by releasing the mouse button again.

☞ Note: 96 well plates will automatically be presented with round wells, while 384 wells are always drawn as squares.





3. Click **Save** to save the calibration information.

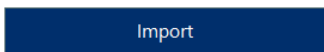


ESP Note: The calibration information is always only saved for the plate type currently in use. It will be applied to the current and all following measurements. However, the vessel calibration needs to be repeated whenever the plate type (number of wells and/or manufacturer) is changed.

4. Click **Set Default Position** to reset the plate dimensions to the default values.



5. Save or load the plate information that has been calibrated with the **Import** and **Export** button.



## Map mode

To browse big samples, look for imaging positions, or calibrate the stitching you can use the Map Mode.

Click **Map Mode** and then **Make Map** to open the menu.

1. Select the channel, map range, and check whether or not to use the autofocus or to separate the channels (if **Separate Channel** is turned off, all selected channels will be overlaid in the map).
2. Click **Make Map** and browse the enhanced view of your sample in the map view.
3. Click **Cancel** or **Apply** to return to the map mode.
4. To toggle the map view click **Load** (in **Preview** tab only). The navigation zoom can be toggled by clicking **N** (in **Preview** tab only).
5. In the **Setup** tab you can also add these positions to the position list

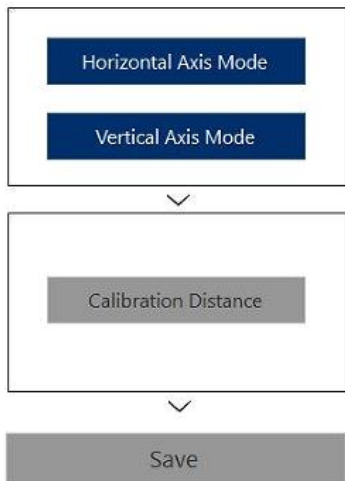


## Calibration for map mode (Stitching calibration)

If there are unwanted overlapping regions in stitched images or during map mode you can correct these.

1. Click **Calibration** in the menu.
2. Select **Horizontal Axis Mode**.
3. Click **Calibration distance** to adjust the Y-axis overlapping location. Drag the images into appropriate positions.
4. Click **Vertical Axis Mode**.
5. Click **Calibration distance** to adjust the X-axis overlapping location. Drag the images into appropriate positions.

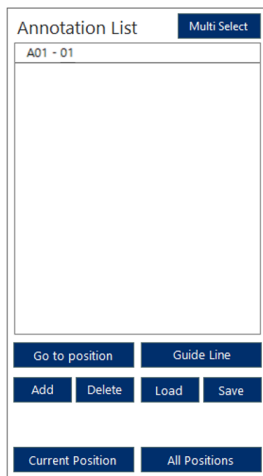
6. Click **Save** and **X** to finish stitching image calibration.



### Annotation List

The **Annotation List** serves to mark and annotate exact locations in the well that are of special interest. These positions are then also available in the **Setup** menu.

⚠ Note: Only in the **Setup** menu these positions can also be added to the **Position List** by clicking **Current Position** or **All Positions**.



1. Click **Guide Line** to get an idea where exactly the center of the image is.
2. Navigate the guide lines onto the position and click **Add** to set a mark and assign a name or description to the position.

### Channel selection

1. By clicking one of the channel buttons the corresponding LED is activated.
2. To turn off the LED please click the same channel button again or simply activate another channel.

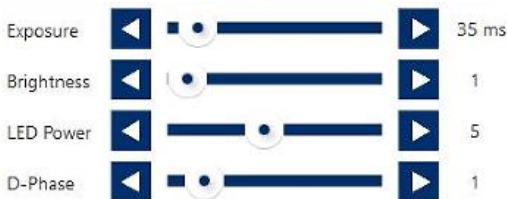


☞ Note: If not deactivated the LED will remain active. To reduce photobleaching/phototoxicity please minimize illumination time by deactivating the LED whenever possible.

### Acquisition settings

The acquisition settings allow the user to adjust the exposure time, brightness (digital intensity gain), and the power of the LED. D-Phase increases the stereoscopic features of an image, giving it a stronger depth and thus slightly enhances edges. However, it can also create slight background noise.

- Exposure range : 5 ms - 500 ms
- Brightness range : 0 - 40
- LED power range : 1 - 10
- D-Phase range: 0 - 10



☞ Note: When setting long exposure times, high LED power and high brightness for the GFP channel, a brightfield-like background image may appear. Try to avoid this by using bright GFP-samples or low to medium GFP exposure, brightness, and LED-power settings.

### Auto focus

To use the image based auto focus click **Auto Focus**.



### Scale Bar

Click **Scale Bar** to display a predefined scale bar on the image in the bottom left corner.



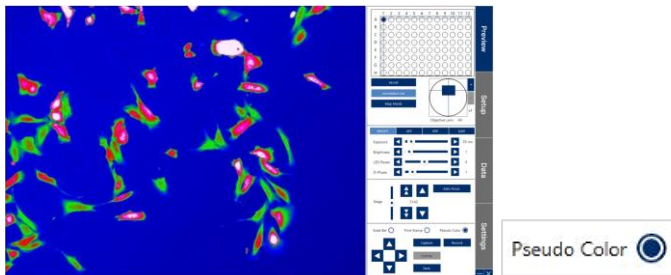
### Time Stamp

The **Time Stamp** shows the number of images and the time point of the time series measurement in the bottom right corner of the image.



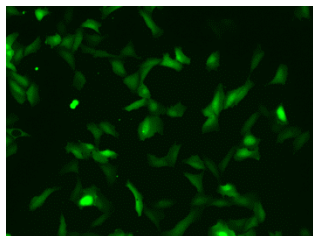
### Pseudo color

During the adjustment of acquisition conditions, the option **Pseudo Color** can help to visualize image illumination. Dark pixels are blue which change to green, red, and finally white for saturated pixels.



### Image snapshots

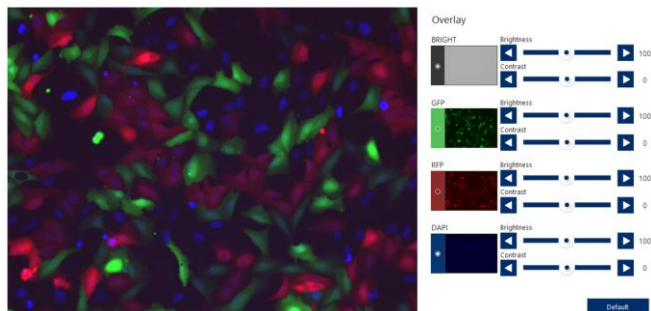
1. Adjust the image settings and click **Capture** to capture the image.



2. Repeat for all desired channels.



- Click **Overlay** and edit your images if necessary



- Activate any combination of channels by clicking the blue dot on the left side of the small images.
- Click **Save** to save the snapshot as jpeg, tiff, bmp, or png.

### Record a movie

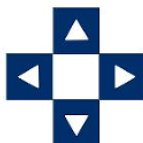
Clicking **Record** opens the live-movie menu. Here you can record a movie of the image view at 10 FPS (frames per second) over a maximum time of 55 minutes.



<sup>EP</sup> Note: Do not minimize the software or put other windows in front of the image viewer during recording because this will be visible on the movie as well.

### XY direction control

Clicking any of the direction arrow buttons will move the stage slightly in that direction.



<sup>EP</sup> Note: You can also move in any direction using right-click and drag or left-clicking any position of the well navigation.

**Minimum & Close button**

Minimize or close the control software.

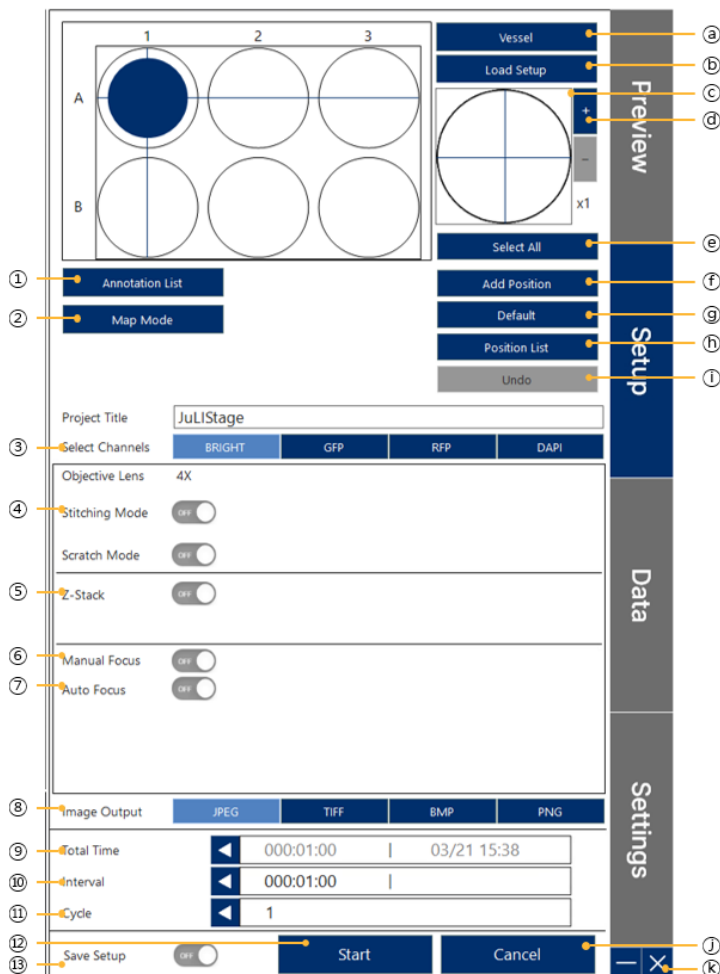


## 7.2 Setup menu

The **Position** menu contains all functions to easily set up an experiment.

☞ Note: All sections covered under **Preview** also apply to the **Setup** tab if not stated otherwise. Sections previously covered are marked with (P).

☞ Note: The acquisition settings and all corresponding functions can be set up below the image view similar to the acquisitions settings in the **Preview** tab.



- ① Annotation list
- ② Map mode button (P)
- ③ Channel selection (P)
- ④ Stitching mode toggle button
- ⑤ Z-Stack toggle button
- ⑥ Manual focus toggle button
- ⑦ Auto focus toggle button
- ⑧ Image output button
- ⑨ Total time (time lapse)
- ⑩ Interval time (time lapse)
- ⑪ Cycle number (time lapse)
- ⑫ Start experiment
- ⑬ Save setup toggle button
- a Vessel calibration (P)
- b Load experimental setup
- c Well navigation (P)
- d Well zoom in & out (P)
- e Select all wells button
- f Add position button
- g Default button
- h Position button
- i Undo button
- j Cancel button
- k Minimize & close button (P)

### Navigation position set

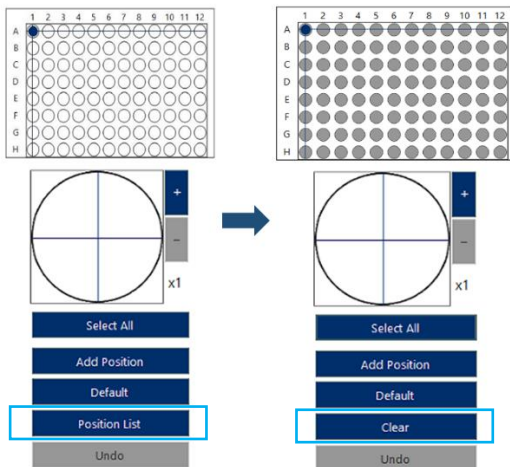
- To select all wells click **Select All**.
- After selecting one or multiple wells, clicking **Add Position** will add the center position of each selected well to the **Position list**.
- There are two ways to proceed if more than one position inside a well is supposed to be measured in only selected or in all wells of the measurement:
  - Select wells first, then select e.g. 5 positions inside the well via mouse click and then click **Add Position**.
  - Select wells, then select one more position inside the well, click **Add Position**, select wells, then select a third position, click **Add Position** and so on.

Both ways allow the user to create measurements, where different wells can but must not have identical measurement positions inside the wells.

- **Default** selects the center position for all selected wells
- **Undo** deletes the last assigned position.
- The **Position List** contains all positions for imaging including the X, Y, and Z coordinates.

Well	Position Index	X Axis	Y Axis	Z Axis
A01	00001	20883	136056	0
A02	00001	38972	136056	0
A03	00001	57261	136056	0
A04	00001	75350	136056	0
A05	00001	93339	136056	0
A06	00001	112128	136056	0
A07	00001	130417	136056	0
A08	00001	148706	136056	0
A09	00001	166995	136056	0
A10	00001	185284	136056	0
A11	00001	203573	136056	0
A12	00001	221862	136056	0
B01	00001	20883	117931	0
B02	00001	38972	117931	0

- **Clear** will delete all positions from all selected wells.

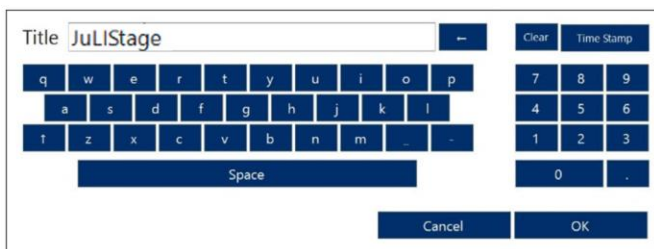


### Project Title

Project Title

Before monitoring, assign a specific title for the project using the pop-up keyboard.

☞ Note: Time Stamp will add the date and time to the name, which improves chronological sorting of the projects.



### Load Setup

Click **Load Setup** to import the experimental setup and layout from previous projects.

- **Vessel Type:** Filter for vessel type.
- **Time Range:** Filter by time.
- **Delete:** Deletes the selected setup and layout.

Load Setup

**Vessel Type List**

**Well Plate**

6 Well Plate  
  12 Well Plate  
  24 Well Plate  
 48 Well Plate  
  96 Well Plate  
  384 Well Plate  
 96 Well Plate (Spheroid/Scratcher)

**Culture Dish**

35mm Dish  
  60mm Dish  
  100mm Dish

**Flask**

250fl Flask  
  750fl Flask

**Slide**

Glass Slide

**Time Range**

All Data  
 Days ago (Last Used)

180720 A549 mln ABT
190813_j1 pj1 statin dose resp _ 2nd_SW
190909 H9 TPX2 WT D43 Dox 4 8 16
200831 H9-Derived CM QC LUT 50uM test
20180719175529_
20200805_sw_etc inh LOS J1
20200805_sw_etc inh LOS J11
20200805_sw_etc inh LOS J12

Delete

Cancel
OK

### Select Channels

Make sure to activate all relevant channels for the experiment.



☞ Note: Light blue means active, dark blue means inactive.

### Stitching Mode

To acquire bigger areas, stitching of multiple field of views can be performed (1x1 - 99x99, 1 - 9801 images). All images are separately saved together with the stitched image. However, the stitched image will be shown as single dataset.

Stitching Mode  ON Whole Well  OFF 0 x 3 x 0 x 3

☞ Note: The stitching settings will be applied to all positions.

☞ Note: Stitching and Z-stack acquisition are mutually exclusive.

### Z-Stack

The Z-stack option enables imaging of several layers of an object. The number of planes ranges from 3 to 35 with a thickness from 0.5  $\mu\text{m}$  – 10  $\mu\text{m}$ .

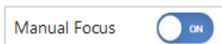


The stack can be acquired downwards, upwards, or up- and downwards from the current position.

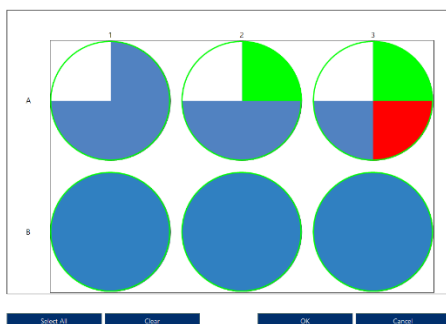
⚠ Note: Stitching and Z-stack acquisition are mutually exclusive.

### Manual Focus

Using **Manual Focus** images will be acquired in a Z height defined by the user.



1. Activate the manual focus.
2. Focus on your object manually or using the auto focus and click **Set Focus** for each channel in the focus adjustment area below the image view.
3. Repeat this for all relevant positions.
4. Review all positions clicking **Focus Info** (see below)
  - A1: Only for brightfield a manual focus has been set.
  - A2: Brightfield and GFP have a set focus.
  - A3: Brightfield, GFP and RFP have a set focus.



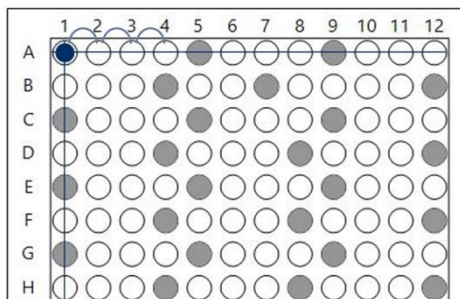
### Auto Focus

The image based auto focus is highly flexible and can be modified to focus in various different configurations. These options ensure that the user can save time, lower phototoxicity, correct for uneven plate bottoms, or moving samples while maintaining the optimal focus.



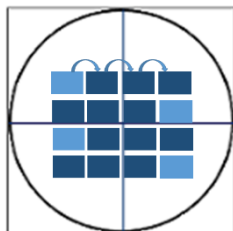
☞ Note: In case both manual focus and auto focus are activated, the Z-height of the manual focus will be used where applied, all other positions will be focused using the auto focus.

**Auto Focus:** You can choose how many steps to make until the next well that is supposed to be focused using the auto focus (1 = next well, 2 = second next well). Setting auto focus to 4 will image well 1, 5, and 9 and so on as shown below by the blue or grey wells.



**All Positions:** Similarly to the Auto Focus you can choose how many steps to make until the next position that is supposed to be focused using the auto focus (1 = next position, 2 = second next position). Setting All Positions to 4 will image position 1, 5, 9, and 13 as shown below by the light blue squares.

Auto Focus  ON 1 ▾ All Positions  ON 4 ▾



☞ Note: In case **All Positions** is turned off, auto focus will be activated in the first position of the well only.

**All Channel:** In order to focus on each channel individually **All Channel** can be used. If inactive, only brightfield will be used for the auto focus.

☞ Note: When **All Channel** is turned on, **Custom AF** is not activated.

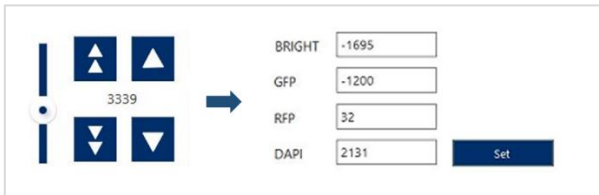
**Every Cycle AF:** Activate **Every Cycle AF** to use the auto focus in every cycle. This helps maintaining the correct focus, however, increases photobleaching/phototoxicity.



**Custom AF:** If the auto focus does not perform well you can use the Custom AF. This allows the user to set a specific range for the auto focus to look at, especially when there are differences between the different channels.



1. Focus on your sample manually or use the auto focus for the brightfield channel, and then click the **Custom AF** button (when the autofocus mode is turned on).
2. Click **Set** to confirm.



3. Select a channel using the channel selection.



4. Manually change the focus and click **Set**. The z-value will be displayed.
5. If necessary, repeat step 3 - 4 for all other channels.

⚠ Note: When **Custom AF** turned on, **All Channel** is deactivated.

**Extended Range:** The auto focus uses an extended search range and will take more time.



**Quick Mode:** The auto focus time can be reduced using the quick mode. However, the focus may not be as accurate in all images anymore.



### Image Output

The image output can be set to four different formats. **JPEG**, **BMP**, and **PNG** as 8-bit and **TIFF** as 16-bit image.



### Time lapse configuration

The time lapse can be configured using an end-point, interval and/or the cycle number.

- **Total time:** Depending on the time settings of the PC, the end point of the experiment can be defined using **Total Time**.

Total Time ◀ 000:01:00 | 02/21 22:31

▲	▲	▲	▲	▲
▲	▲	▲	▲	▲
2024	03	28	12	39
2023	02	27	11	38
Year	Month	Day	Hour	Min
▼	▼	▼	▼	▼
▼	▼	▼	▼	▼

Cycles: 0

Reset Cancel Apply

- **Interval time:** The interval time defines the duration between two time points. The red timer indicates the minimal duration for one imaging cycle.

Interval ◀ 000:01:00 | 000:01:00

▲	▲
▲	▲
01	02
00	01
Hour	Min
▼	▼
▼	▼

Reset Cancel Apply

- **Cycle:** The cycle is equivalent to time points.

Cycle ◀ 1

←	Clear	
7	8	9
4	5	6
1	2	3
0	OK	

⚠ Note: During imaging you can click **Pause** and modify the cycle number by double clicking either interval or cycle.

⚠ Note: In order to prevent phototoxicity an interval time of at least 15 minutes is recommended. However, the optimal time interval depends on cell lines.

### Start

After all parameters for the experiment are configured, click **Start**.



⚠ Note: The measurement starts only after the second confirmation by clicking **Play**.

### Cancel button

To reset all parameters click **Cancel**.



**⚠ IMPORTANT!**

- The JuLI™ Stage needs to be at a stable temperature. Before using the JuLI™ Stage in an incubator for the first time please follow the warm-up routine (30 minutes outside the incubator and 2 hours in the incubator while the JuLI™ Stage is turned on).
- The well plate's cover should be wetted with cell culture media before monitoring. This step is required to avoid condensation inside the well plate cover for monitoring.

**Experimental overview**

After clicking **Start** you can review all settings. Click **Play** to start the experiment.

The screenshot shows the JuLI Stage software interface, divided into three main vertical sections: Preview, Setup, and Data. The interface includes a well plate grid, a list of channels (BRIGHT, GFP, RFP, DAPI), camera settings, and a settings panel with controls for time and scale.

**Numbered Callouts:**

- ① Title: JuLIStage
- ② Well plate grid (rows A-H, columns 1-12)
- ③ Annotation List
- ④ Z-Stack
- ⑤ Channel selection buttons (BRIGHT, GFP, RFP, DAPI)
- ⑥ Camera Setting table
- ⑦ Total | Elapsed Time
- ⑧ Interval
- ⑨ Cycle
- ⑩ All Well (off), Scale Bar, Time Stamp
- ⑪ Start (Play) and Stop (Square) buttons

**Lettered Callouts:**

- a: Focus control (+/- buttons)
- b: All Well toggle
- c: Scale Bar toggle
- d: Time Stamp toggle

Camera Setting	Exposure / Brightness / LED Power /
BRIGHT	35 / 1 / 5 / 1
GFP	65 / 10 / 5 / 1
RFP	100 / 9 / 3 / 1
DAPI	90 / 6 / 2 / 1
Objective Lens	4X
Manual Focus	off
Auto Focus	off

- ① Title name
- ② Selected wells+
- ③ Annotation list
- ④ Z-Stack
- ⑤ Selected channels
- ⑥ Time lapse options
- ⑦ Total time
- ⑧ Interval time
- ⑨ Cycle numbers
- ⑩ View all wells
- Ⓜ Play button
- ⓐ Well navigation
- ⓑ Scale bar button
- ⓒ Time stamp button
- ⓓ Stop button

### Edit the cycle number

While the instrument operates, you can modify the cycle number.

1. Click cycle number display box.

Cycle	00001   00000
-------	---------------

2. Modify the cycle number and confirm by clicking **OK**.

Cycle	10
-------	----

←	Clear	
7	8	9
4	5	6
1	2	3
0	OK	

The cycle you entered will be applied.
<b>OK</b>

Cycle

00010 | 00001

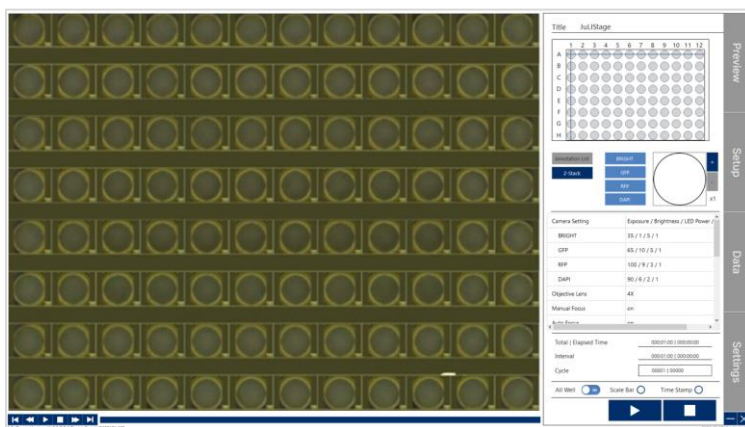
### Time lapse slider

While capturing time-lapse data all images already acquired (and stored) can be reviewed using the time lapse slider.



### All well

To review all wells at a glance use the toggle button **All Wells**.



### 7.3 Data menu

The data menu provides the function to review and export all datasets.

Once you selected the project, all test conditions including vessel type, layout, acquisition and time lapse settings are displayed.

The screenshot displays the JuLI Stage software interface with the following components and callouts:

- 1**: Title bar showing 'Title JuLIStage'.
- 2**: Table header with columns 'Name' and 'CreationTime'.
- 3**: Annotation List section with a 'Z-Stack' button.
- 4**: Z-Stack diagram showing a circular field of view with a central blue square.
- 5**: Grid of wells labeled A-H and 1-12.
- 6**: Well selection controls with '+' and '-' buttons and a 'x1' multiplier.
- 7**: Channel selection buttons: BRIGHT, GFP, RFP, DAPI.
- 8**: Camera Setting table:

Channel	Exposure / Brightness / LED Power / D
BRIGHT	35 / 1 / 5 / 1
GFP	65 / 10 / 5 / 1
RFP	100 / 9 / 3 / 1
DAPI	90 / 6 / 2 / 1
Objective Lens	4X
Total Time	000:00:00
Interval	000:01:00

- 9**: Settings section with 'All Well' (toggle), 'Scale Bar' (radio), and 'Time Stamp' (radio).
- 10**: 'Export' and 'Edit' buttons.
- a**: All Well toggle.
- b**: Scale Bar radio button.
- c**: Edit button.
- d**: Navigation icons (back, forward, search).



- ① Project name
- ② Project list
- ③ Annotation list
- ④ Z-Stack
- ⑤ Plate layout
- ⑥ Well navigation
- ⑦ Channel selection
- ⑧ Acquisition settings
- ⑨ View all wells
- ⑩ Export button
- Ⓐ Scale bar
- Ⓑ Time stamp button
- Ⓒ Edit button
- Ⓓ Minimize & close

### Project list

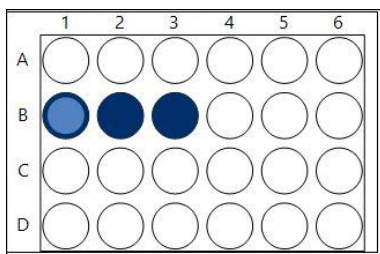
Selecting a project will mark it with a blue color and initialize the visualization of the vessel type, layout, acquisition settings, and time lapse settings.

Title JuLIStage

Name	CreationTime
JuLIStage	2023/02/27 11:49
20230227114322_	2023/02/27 11:43
20230224143504_	2023/02/24 14:38
20230224143149_	2023/02/24 14:32
20230224140925_	2023/02/24 14:14
20230224135744_	2023/02/24 14:00
20230224134146_	2023/02/24 13:46
20230224132620_	2023/02/24 13:28
20230224131933_	2023/02/24 13:21
20230224131714_	2023/02/24 13:19
20230224094820_	2023/02/24 09:40

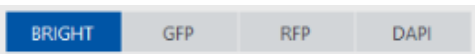
### Plate layout

All captured wells are highlighted in dark blue. The selected well will appear in light blue.



### Well navigation and channel selection

The well navigation shows all captured positions per well using blue squares (the selected positions is highlighted in light blue). All channels used during this experiment are blue (dark blue if deselected, light blue if selected) while channels that were not used are greyed out.



### Acquisition settings

All imaging settings are summarized in a table.

Camera Setting	Exposure / Brightness / LED Power / D-
BRIGHT	40 / 1 / 1 / 0
Objective Lens	4X
Total Time	000:00:22
Interval	000:01:00
Cycle	1
Manual Focus	off
Auto Focus	off

### Export

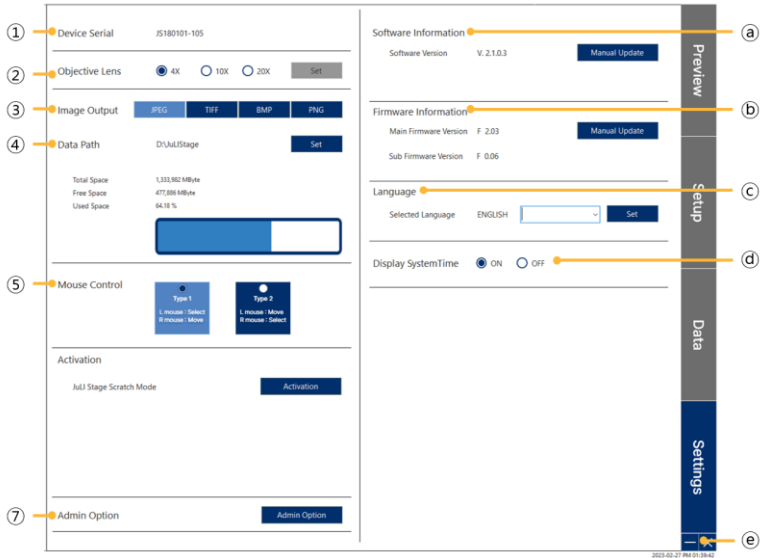
Click **Export** to export the dataset to another location.



**Note:** You can also access or copy all datasets in the JuLI™ Stage installation folder under “JuLIStage/TIMELAPSE”.

## 7.4 Settings menu

The **Settings** menu contains information about the JuLI™ Stage as well as several user preferences and other settings. Here you can change the objective, change the mouse button configuration, update the software, and display or hide the system time.



- |                                      |                           |
|--------------------------------------|---------------------------|
| ① Device serial number               | Ⓐ Software information    |
| ② Current objective lens             | Ⓑ Firmware information    |
| ③ Image output                       | Ⓒ Language information    |
| ④ Data path and hard drive info      | Ⓓ System time             |
| ⑤ Mouse button configuration         | Ⓔ Minimize & close button |
| ⑥ Activation information             |                           |
| ⑦ Admin option (service access only) |                           |

### Device Serial

The serial number of the JuLI™ Stage instrument.

Device Serial	JS180101-105
---------------	--------------

### Objective Lens

Provides the information which lens is currently used.



⚠ Note: The field of view for the 4x, 10x, and 20x objectives is 3.55 mm<sup>2</sup>, 0.58 mm<sup>2</sup>, and 0.14 mm<sup>2</sup>, respectively.

### How to change the objective

1. Select an objective by clicking the blue radio button. This will cause the stage to move to a neutral position.
2. Loosen and rotate the current objective counterclockwise to remove it from the holder.
3. Place the new objective on the holder and rotate clockwise. Be sure to rotate the objective until it is tight in the holder.
4. After replacing the objective, click **Set** to complete the routine.

⚠ Note: Changing the lens without modifying the settings will result in malfunctions of the device and the software.

⚠ Note: The objectives are especially prone to damage by excessive ethanol. Please always clean the objectives with ethanol-wetted objective cleaning paper.

### Image Output

The image output can be set to four different formats. **JPEG**, **BMP**, and **PNG** as 8-bit and **TIFF** as 16-bit image.



### Data Path

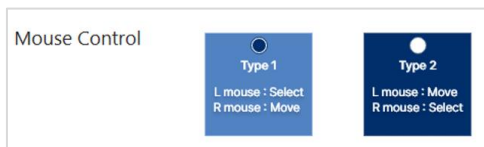
Allows users to assign a saving path and monitor the remaining hard drive volume.



## Mouse Control

Switches for mouse buttons: configurations 1 and 2.

☞ Note: Type 1 is the factory setting.



## Activation information

JuLI™ Stage Scratch and JuLI™ Stage Spheroid are optional modules that offer additional analyses as well as assay possibilities. These modules need to be purchased and activated. In this part you can see the activation status of each software.



☞ Note: To use this function, need to purchase a JuLI™ Stage Scratcher separately.

## Admin Option (service access only)

This menu enables service engineers to modify critical hardware parameters of the system.

## Software information

Gives information about the current software version. In order to update the software, click **Manual Update** select the provided .exe-file and follow further instructions on the screen.

## Firmware information

Gives information about the current firmware version. In order to update the firmware, click **Manual Update** select the provided hex-file and follow further instructions on the screen.

## Language

The standard language of the JuLI™ Stage is English. There are currently no other language options.

Language

Selected Language    ENGLISH       

### System Time

On the bottom right corner of the software the date and time is visible which can be hidden by clicking **Off**.

---

## 7.5 Quick Workflow (setting up an experiment)

1. Before the experiment determine the optimal objective and exchange the objective if necessary. See chapter 7.4 “ Settings menu” (How to change the objective).
2. Place the sample into the plate holder of the XY-stage
3. Open the control software and go to the **Setup** menu
4. Enter an appropriate experiment name
5. Turn on the brightfield mode and focus on the well border (some lids of round bottom plates have condensation rigs which can be used to calibrate plates as well).
6. Click **Calibration**, select a plate type and calibrate it. See chapter 7.1 “ Preview menu” (Vessel type).
7. In the plate layout select all wells that are supposed to be imaged and click **Add Position** to add the center field of all selected wells to the position list.  
  
Alternatively select all desired wells, then add positions to the currently shown well by clicking into the well navigation and click **Add Position** to add all positions to all wells.
8. Select a representative well and set up your channel settings below the image. All channels that are supposed to be captured have to be selected below the plate layout. See chapter 7.1 “ Preview menu” (Channel selection).
9. Activate stitching or z-stack, or the scratch-mode (if available). Stitching and z-stacks are applied to all positions
10. Choose the image output
11. Set the time lapse settings
12. Switch on **Save Protocol** if you want to re-use these settings
13. Start and review the time lapse settings
14. Confirm the experiment a second time by clicking **Play**.

## 8 **Cleaning and Maintenance**

Clean the surface of the JuLI™ Stage with a damp cloth. To clean the LCD screen, turn off the JuLI™ Stage, disconnect the power cable, and clean the LCD screen with a soft cloth lightly moistened with LCD cleansing detergent. Cleaning the screen with excessive force can damage the LCD screen.

If liquid spills on the JuLI™ Stage, turn off the power immediately and wipe dry.

The JuLI™ Stage does not need regular maintenance. To troubleshoot problems with the JuLI™ Stage, contact your local distributor or sales@nanoentek.com. Do not perform any repairs or service on JuLI™ Stage to avoid damaging the instrument.



### **IMPORTANT!**

Never disassemble or service the JuLI™ Stage by yourself.

Unauthorized repairs may damage the JuLI™ Stage or alter its functionality, which will void your warranty.

Contact your local distributor or sales@nanoentek.com to arrange for service.



### **IMPORTANT!**

The JuLI™ Stage is optimized for a standard size of cell culture incubator.

It is recommend that the temperature should be stabilized in case of a small size incubator before using the instrument.



### **IMPORTANT!**

Warm up the JuLI™ Stage for 2 hours before monitoring.

The power should be on when warming up the instrument (*20 minutes outside the incubator and 2 hours in the incubator*).



### **IMPORTANT!**

In an incubator, the JuLI™ Stage and PC must be turned on at all times to reduce the humidity.



 **IMPORTANT!**

The well plate's cover should be wetted by the cell culture media before monitoring.  
This step is required to avoid condensation inside the well plate cover for monitoring.

 **IMPORTANT!**

Avoid exposing the JuLI™ Stage to UV light.

UV light may degrade components, including plastics. Damage from UV exposure is not covered under the manufacturer's warranty.

 **IMPORTANT!**

Always wipe surfaces with ethanol-soaked paper towels.

☞ Note: The objectives are especially prone to damage by excessive ethanol. Please always clean the objectives with ethanol-wetted objective cleaning paper.

Do not directly spray ethanol anywhere on the JuLI™ Stage.

 **IMPORTANT!**

When the JuLI™ Stage is not used for long periods of time, it must be taken out from the incubator to reduce humidity and put in a clean and stable place.

## 9 Troubleshooting

<b>Installation</b>	
JuLI™ Stage does not power up	<p>Connect the device to the power outlet.</p> <p>Check the on/off switch on the side of the control box.</p> <p>Contact your distributor.</p>
Monitor is black	<p>Move the mouse.</p> <p>Check if the power supply is connected and the monitor is switched on.</p> <p>Check if the monitor is connected to the PC.</p> <p>Turn the monitor off and on again.</p>

<b>Monitoring</b>	
Poor image (Too dark and too bright)	<p>Re-optimize the brightness and exposure settings.</p> <p>Restart the device.</p>
Dirty image	<p>Wipe the stage carefully.</p> <p>Eliminate any dust on the device and culture dish.</p> <p>Check for and remove any condensation on the lid or the bottom of the vessel.</p> <p>Let the sample warm up before imaging.</p>
Time-lapse images become dark or bright	<p>Warm up the instrument for 2 hours before monitoring (power should be on when warming up the instrument).</p> <p>Cell culture flask should be wetted with culture media before imaging.</p> <p>Eliminate any dust from the culture dish.</p> <p>Remove any condensation from the lid or bottom of the vessel.</p> <p>To prevent any problem such as shaking of culture dish or inflowing light, pay special attention when you open or close the incubator door during monitoring.</p>
Problem saving movie file	<p>Check the available volume of the hard disk.</p>

# 10 Specifications

## 10.1 Product specifications

Items	Specification
Light source	Blue, Green, UV LED (Intensity adjustable)
Objective Lens	4X, 10X, 20X + Digital zoom Inter-changeable objective lens
Fluorescence	3 fluorescences DAPI: Excitation 378/52, Emission 447/60 GFP: Excitation 466/40, Emission 525/50 RFP: Excitation 543/22, Emission 580LP
Camera	High-sensitivity monochrome CCD (Sony sensor 2/3") 1,936 x 1,456 pixels (2.8M), 53 FPS, 14 bit
Stage	Automated, motorized, X-Y-Z stage Ex-changeable vessel holders (optional)
Exported formats	Image: JPEG; TIFF; BMP, PNG Video: AVI Raw data: CSV
PC	Desktop computer, Desktop monitor 24-in. LCD CPU: Intel i5, 9 generation or over spec. OS: Windows® 10 Pro 64 bit RAM: 16 GB Hard drive: 2 TB Network: Gigabit Ethernet, WiFi  <i>*PC specifications may change without notice</i>
Operating power	100 - 240 V, 1.5 A, 50/60 Hz
Electronic input	12 VDC, 2.0 A
Operating environment	5 - 40 °C, 20 - 95%
Dimensions	429 (W) X 310 (D) X 324 (H) mm
Weight	18.5 kg / 41 lbs

## 10.2 Ordering information

Cat. No.	Product	Description
JS1000S	JuLI™ Stage, Stater Pack	JuLI™ Stage basic set (JS1000), Desktop computer (JP0200), 3 Objective lenses (4X, 10X & 20X)
JS1000	JuLI™ Stage, Real-Time live cell imaging system	Main device, power supply, control box
JP0100	Desktop Computer	CPU: Intel i5, 9 generation or over spec. OS: Windows® 10 Pro 64 bit RAM: 16 GB Hard drive: 2 TB Network: Gigabit Ethernet,
JMO100	Desktop Monitor	24" Full HD (1920 x 1080) monitor
JP0150	External Hard Disk Drive (Optional)	Total 8 TB (4 TB x 2 ea)
JO0004	Objective Lens (4X)	Magnification: 4X, NA: 0.16
JO0010	Objective Lens (10X)	Magnification: 10X, NA: 0.3
JO0020	Objective Lens (20X)	Magnification: 20X, NA: 0.45
JVH001	Vessel Holder (Optional)	Micro Slide (26 x 76 mm)
JVH002	Vessel Holder (Optional)	Petri Dish (35 mm)
JVH003	Vessel Holder (Optional)	Petri Dish (60 mm)
JVH004	Vessel Holder (Optional)	Petri Dish (100 mm)
JVH005	Vessel Holder (Optional)	T-Flask (25 & 75 cm <sup>2</sup> )
JSCT100	JuLI Analysis Software (Scratch)	JuLI Scratch STAT JuLI Scratcher
JSPT100	JuLI Analysis Software (Spheroid)	JuLI Spheroid STAT

## 10.3 **Warranty**

The warranty lasts for 1 year for defects of material and workmanship.

If any defects occur in the JuLI™ Stage, free repair service for the defective parts will be provided at its discretion. The following defects, however, are specifically excluded:

- Defects caused by improper operation
- Repair or modification done by anyone other than NanoEntek or an authorized agent
- Damage caused by substituting alternative parts
- Use of fittings or spare parts supplied by anyone other than NanoEntek
- Damage caused by accident or misuse
- Damage caused by disaster
- Corrosion caused by improper solvent or sample

For your protection, the JuLI™ Stage units being returned must be insured against possible damage or loss. NanoEntek cannot be responsible for damage incurred during shipment of a defective instrument. It is recommend that you save the original packing material in which the instrument was shipped. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service, contact your local distributor or [sales@nanoentek.com](mailto:sales@nanoentek.com)

# 11 JuLI™ Stage EDIT Software

## 11.1 Software overview

With JuLI™ Stage EDIT, measurement data sets can be reviewed, brightness and contrast settings of single or all images of a project can be edited, and a variety of movies can be created from time lapse measurements.

For this, two image tools are available:

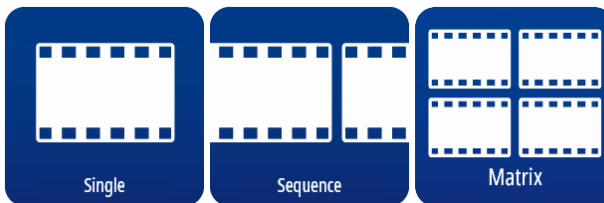


### Image Editor

With the **Image Editor**, brightness and contrast settings of selected or all images of a measurement can be optimized and saved under a new project name. Image editing can also be done on single images which are then saved in .jpg, .tiff, .bmp or .png-format for e.g. reports or presentations.

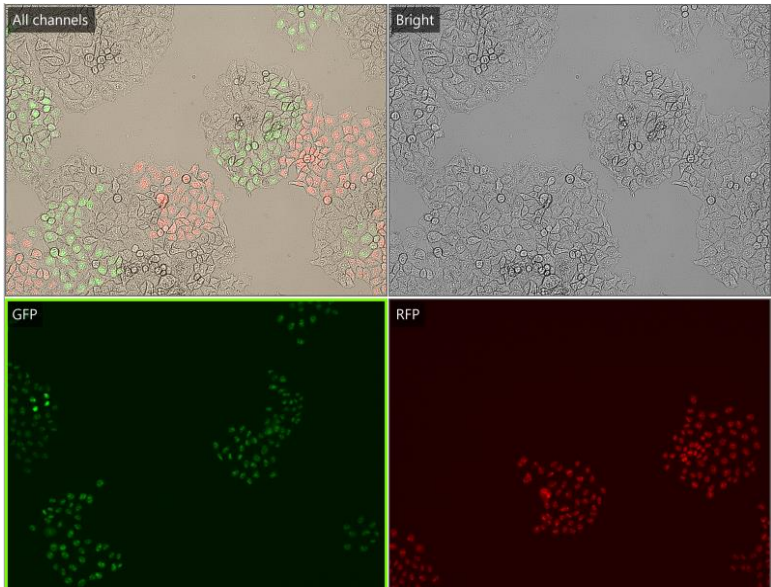
### Movie Maker

The **Movie Maker** offers a variety of movie types to be created from time lapse measurements, named **Single**, **Sequence** and **Matrix**.



- The frame rate for all movies can be selected between 1 and 30 (fps, frames per second) and all movies are saved in an .avi-format.
- The **Single** movie function allows you to convert a complete time lapse of a single well or only selected time points thereof into a single movie file.
- Using the **Sequence** movie function, you can concatenate different time windows, positions, wells or even projects to a sequentially played, single movie.

- The **Matrix** movie function allows you to combine several movies from one or different projects, or channels, or movie sequences, next to each other in a grid of 1x1 up to 9x9. Here for example a 2x2 grid:

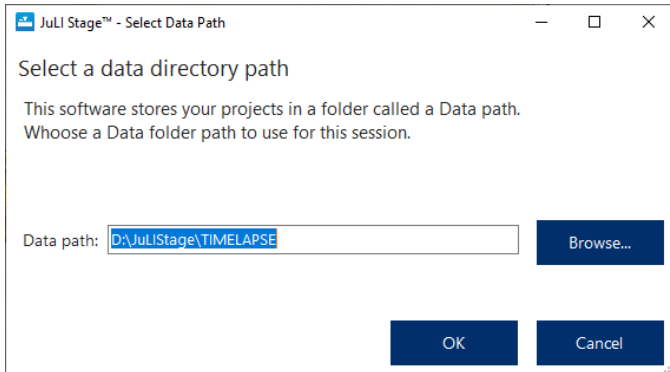


## 11.2 Operation

Double click **JuLI EDIT** on the desktop to run the program.



Click **File** and **Data Path**. Enter a directory or click **Browse...** to choose a directory. Then press **OK**.

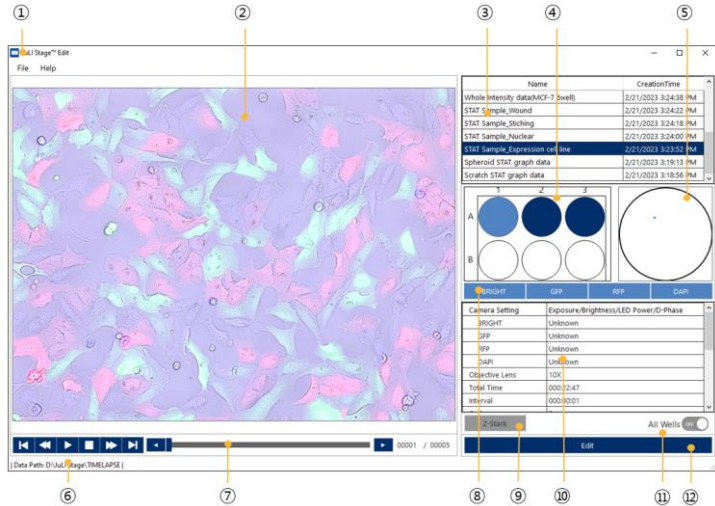


☞ Note: Selecting a single project folder directly will not work, always select a parent folder.



## 11.2.1 Main viewer

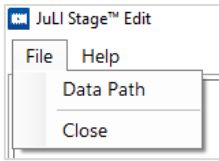
The main viewer is the starting interface for reviewing and selecting image data sets from the JuLI™ Stage for further editing and movie creation.



- ① Menu bar
- ② Image view
- ③ Project list
- ④ Vessel display
- ⑤ Well navigation
- ⑥ Time lapse buttons and slider
- ⑦ Data path
- ⑧ Channel selection
- ⑨ Z-stack
- ⑩ Project information
- ⑪ Display all wells
- ⑫ Edit

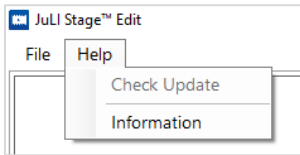
① **Menu bar**

• **File menu**



- **Data Path:** Click **Data Path** to change the originally chosen or enter a new data path: remember to always select a parent folder containing the projects / measurements.
- **Close:** Click **Close** to close the **JuLI™ Stage EDIT** software.

• **Help menu**

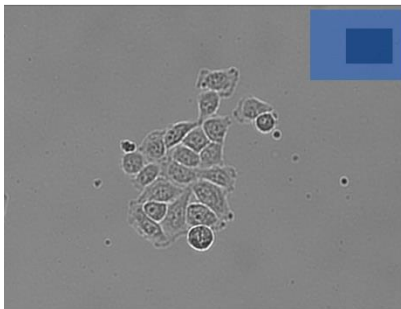


- **Information:** Software version and copyright information of **JuLI™ Stage EDIT** are displayed.

② **Image view**

The **Image view** section displays the current image of a selected well, field and timepoint of a measurement.

- **Zoom-in:** Using the mouse wheel scroll up in the image view to zoom in on the image. A navigation screen will be displayed in blue on the top right corner. You can drag the small dark blue box in the navigation screen or click in the image itself with the mouse button held down to move the zoomed area.
- **Zoom-out:** Scroll down the mouse wheel to zoom out again.



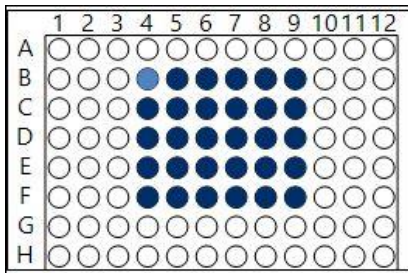
### ③ Project list

All available measurements / projects belonging to the selected data path are listed and sorted by date. By clicking a line in the table, a measurement is selected and thereby marked dark blue. By clicking the header field "Creation Time", the order of the list can be reversed.

### ④ Vessel display

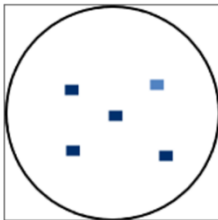
Of the selected measurement project, all wells of the plate type are displayed:

- White wells are not imaged wells,
- Dark blue wells are imaged but not selected and
- Light blue wells are selected and currently displayed on the screen.



### ⑤ Well navigation

All imaged positions in a well are displayed as blue squares. By clicking a blue square it is selected to be displayed and turns light blue.



### ⑥ Time lapse buttons and slider



Click any position within the time lapse slider bar to view an image at different time points. The cycle number appears as mouse-over on the time lapse slider. Click the **Play** button to see the full sequence played.

⑦ **Data path**

Display of the data path in use.



Use **File > Data Path** to change the data path.

⑧ **Channel selection and overlay**

Display of all 4 channels available on the JuLIStage: grey channels were not measured in the selected project while light blue channels were acquired and are displayed in image view automatically as an overlay, e.g. brightfield and RFP channel:



Clicking a light blue channel will deactivate it in the overlay and change its button to dark blue.

E.g. display of brightfield channel only:



⑨ **Z-stack**

If a measurement / project was acquired in Z-stack mode, the **Z-Stack** button will appear in light blue (instead of grey) together with a **Z-Stack** window showing stack planes and options to select the best plane:



Note: Only one Z-plane of a multi-plane stack can be processed at a time.

⑩ **Project information**

Once a measurement / project is selected in the list, acquisition details such as objective lens used, total time, number of cycles and interval time are displayed in this window. Also, channel settings of the measurement (exposure time, brightness and LED power) are detailed:

Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	30 / 5 / 2 / 1
GFP	75 / 15 / 8 / 1
RFP	80 / 20 / 8 / 1
Objective Lens	10X
Total Time	000:05:11
Interval	000:01:00
Cycle	1

⑪ **Display All Wells**

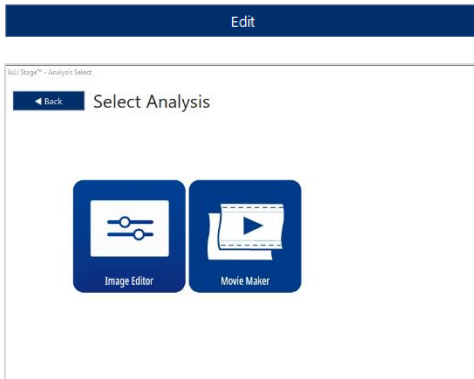
Switching the toggle switch of **All Wells** to **ON** will display all wells of the selected plate at a glance – measured ones as well as not measured ones.

In this example, the upper 3 wells of a 6-well plate images were acquired, while the second row was not part of this measurement:

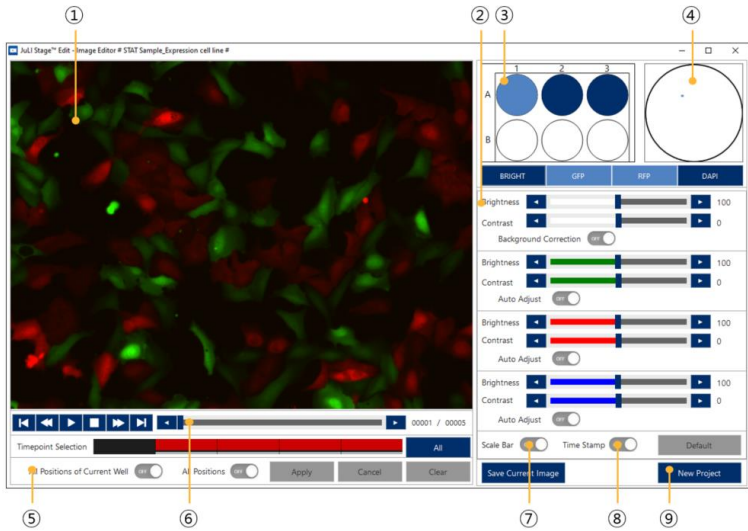


⑫ **Edit**

After a project / Z-plane was selected, clicking **Edit** will open the next window, where two different editing options are offered.



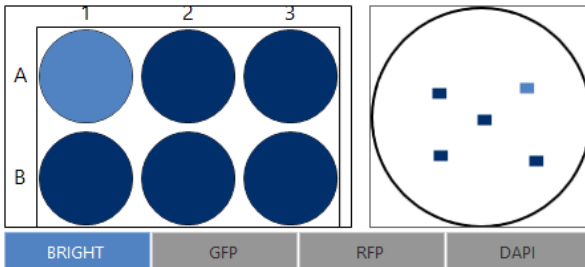
## 11.2.2 Image editor



- ① Image view
- ② Edit image
- ③ Vessel display
- ④ Well navigation
- ⑤ Edit panel
- ⑥ Time lapse slider
- ⑦ Scale bar
- ⑧ Time stamp
- ⑨ New project

### Vessel display & navigation

Select well, channel and position inside the well to review and edit image brightness and contrast.

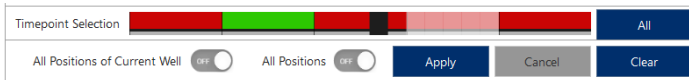


### Time lapse slider

The time lapse slider can be used to navigate through timepoints (cycles) of the displayed data set using the buttons on the left or by dragging the slider bar.



### Time-point selection



Enables the selection of different timepoints or time windows.

- **Black bar:** Representation of the time point currently being displayed (analogue to the time lapse slider bar)
- **Red:** Time points available for selection
- **Grey:** Selected time points
- **Green:** Timepoints turn green after editing of the images and pressing **Apply**

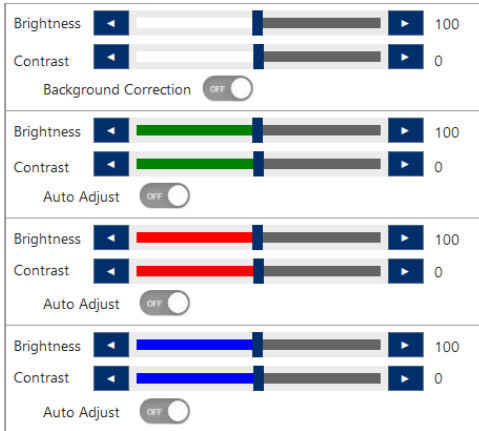
### Edit panel

- **All positions of current well:** Apply the same edits to every position in the selected well
- **All positions:** Apply the same edits to all positions in all wells
- **Apply:** Apply the changes to the selected time points (grey changes to blue after pressing **Apply**)
- **Cancel:** Cancel the edits in the selected image
- **Clear:** Cancel all edits in the selected positions

### Edit images

Each channel can be corrected for brightness and contrast manually as well as automatically. Additionally, a background correction is available for the brightfield channel to correct for shadows or other artifacts of the background.





- **Background Correction:** Uniformly calibrates the brightness of the background of brightfield images
- **Auto Adjust:** Automatically calibrates brightness and contrast
- **Default:** Reverse all changes

### Save current image

Clicking **Save Current Image** the image currently shown in the image view can be saved as jpeg, tiff, bmp, or png.

☞ Note: **Scale Bar** and **Time Stamp** are only displayed in image view and will neither be part of a saved current image, nor will they be saved in **New Project**-images.

### New Project

After applying brightness, background and/or contrast changes to all or selected images, the complete data set can be saved under a new project name containing all changes.

☞ Note: All data of this project will be duplicated and saved, even if only one or a few images were modified.

☞ Note: The image format (e.g. .tiff) of the edited / duplicated project will be identical to the originally selected acquisition format.

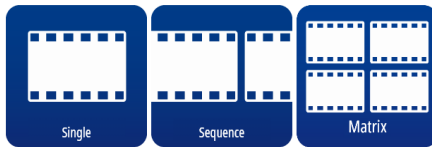
☞ Note: To apply changes to the images and save them in a New Project, clicking one of the two **All Positions** buttons is mandatory

### Quick Workflow to export a modified data set

1. Select a well and position
2. Choose either all positions of the data set, the current whole well, or only the current position will receive modifications
3. Select one or all time-points or a time window
4. Edit the channels
5. Press **Apply**
6. Press **New Project** and select a directory, confirm

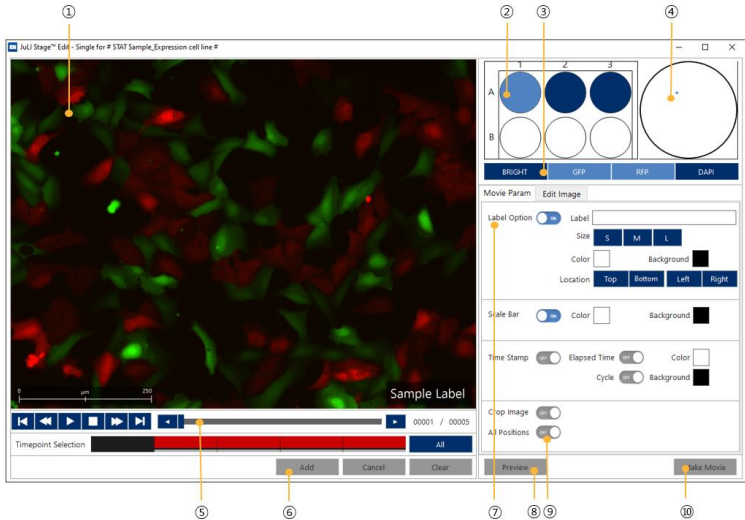
### 11.2.3 Movie Maker

The movie maker allows to convert time-lapse images into movies using three different methods: **Single**, **Sequence**, and **Matrix**.



## 11.2.4 Movie Maker – Single

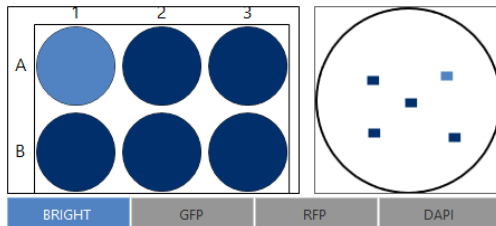
The **Movie Maker – Single** creates movies from all or selected time-points of the whole or a cropped single position.



- |                        |                 |
|------------------------|-----------------|
| ① Image view           | ⑥ Edit panel    |
| ② Vessel display       | ⑦ Label options |
| ③ Channel selection    | ⑧ Preview       |
| ④ Well navigation      | ⑨ All positions |
| ⑤ Time point selection | ⑩ Make movie    |

### Vessel display & well navigation

Select the well and position to use for the movie



### Channel selection

Activate all channels you want to be shown in the movie.



### Position slider

The time lapse slider can be used to navigate through timepoints (cycles) of the displayed data set using the buttons on the left or by dragging the slider bar.



### Timepoint Selection

Enables the selection of different time points or even time windows.

- **Black bar:** Representation of the time point currently being displayed (analogue to the time lapse slider bar)
- **Red:** Time points available for selection
- **Grey:** Selected time points
- **Green:** Timepoints turn green after pressing **Add**

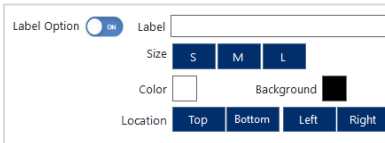


- **All:** Select all time points
- **Cancel:** Remove images you have added
- **Clear:** Cancel all settings for the selected position

### Label Option

Insert a label into the movie.

- **Size:** Select small, medium, or large font size
- **Color:** Change the color of the text
- **Background:** Change the background color of the label
- **Location:** Choose the corner of the image for the label



## Scale Bar

Insert a scale bar into the movie

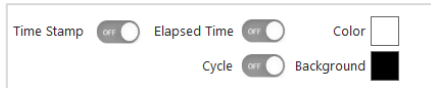
- Color: Change the color of the scale bar
- Background: Change the background color of the scale bar



## Time Stamp

To display a Time Stamp in the movie:

- Elapsed Time: Time displayed for captured image
- Cycle: Display cycle of image
- Color: Change color of Time Stamp
- Background: Change the background color of Time Stamp



## Crop Image

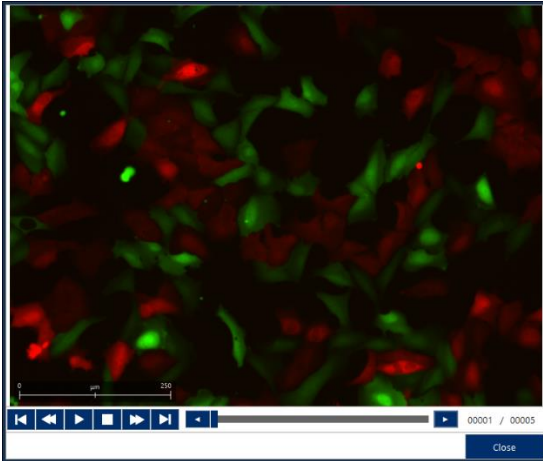
Crop the image to adjust the displayed size of your movie.



⚠ Note: The Scale Bar is only partially visible in cropped images.

## Preview

View a preview of the edited movie.



## All Positions

Apply all settings to all positions.

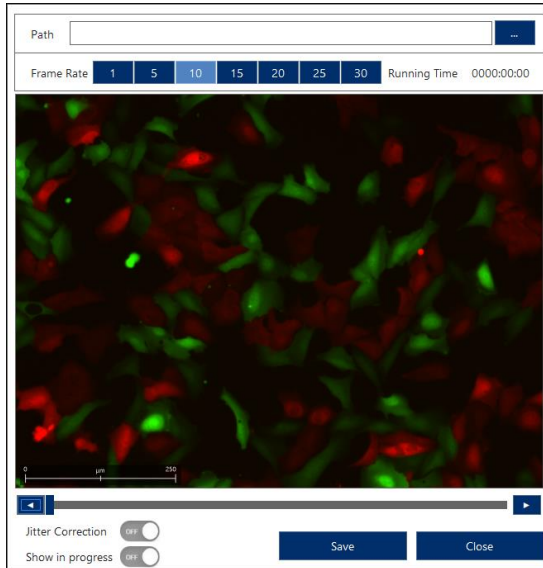
☞ Note: If **All Positions** is **ON**, movies for all wells and all positions in a well will be created with automatically given, individual names. E.g. a measurement containing 6 wells and 5 positions within a well will create 30 movies named for example B02\_00005.avi for well B2 and position 5 in well B2.

☞ Note: If the area within a position is cropped via **Crop Image**, and **All Positions** is **ON**, the same cropping area and location in the image will be applied to all wells (and all positions in a well) of the measurement.

## Make Movie

Final setting for the movie.

- **Path** : Specify the name and location to save the movie
- **Frame rate** : Set the playback speed [frames per second]
- **Running Time** : Shows the movie duration time
- **Show in Progress** : See images in real time during movie making
- **Save**: Save the movie
- **Close**: Close the window



### Quick Workflow to create a Single movie

1. Select a well, position, and channels
2. Add labels, size bar, or time stamp
3. Crop image
4. Choose time window
5. Click **Add**
6. Decide if all positions or not
7. Click **Make Movie**
8. Set a path, file name if required, and a frame rate
9. Click **Save**

## 11.2.5 Movie Maker – Sequence

In order to show a series of movies in one sequence you can concatenate movies using **Movie Maker - Sequence**.



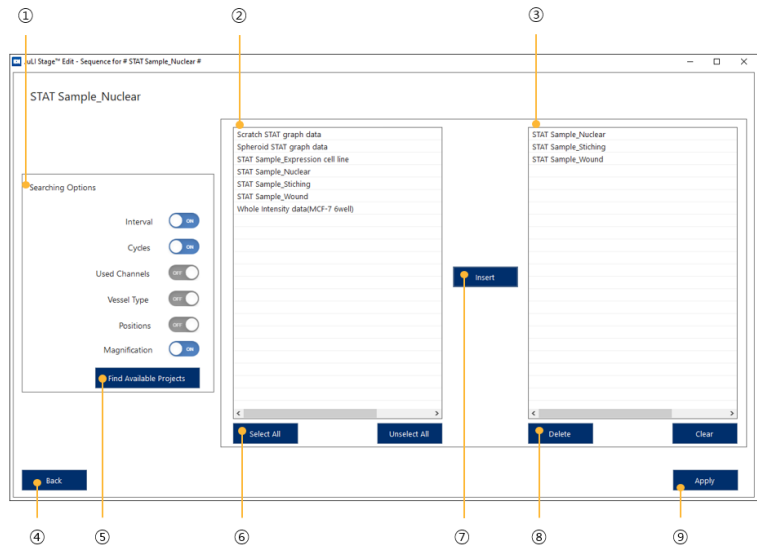
- ① Image view
- ② Project list
- ③ Find projects
- ④ Sequence list
- ⑤ Add to sequence

- ⑥ Time lapse slider
- ⑦ Edit sequence panel
- ⑧ Preview
- ⑨ Make movie



## Find Projects

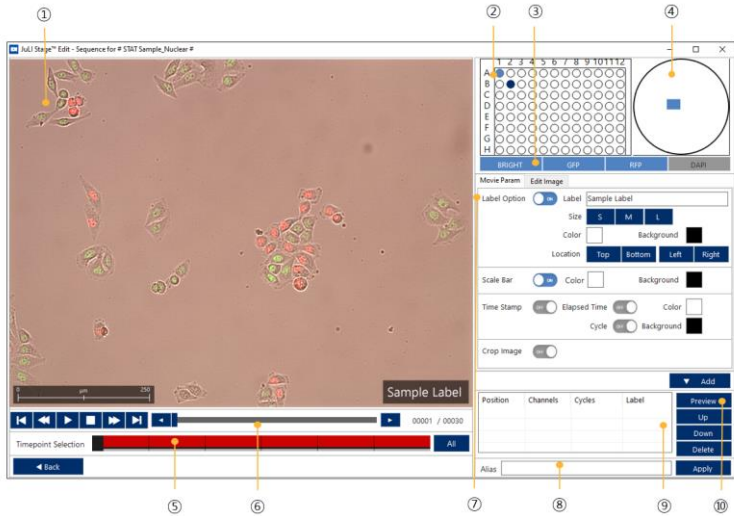
Here you can add additional data sets to the project list.



- |                           |                         |
|---------------------------|-------------------------|
| ① Searching options       | ⑥ Project select option |
| ② Project list            | ⑦ Insert                |
| ③ Selected project list   | ⑧ Delete projects       |
| ④ Back                    | ⑨ Apply                 |
| ⑤ Find available projects |                         |

### Add to Sequence

After selecting a project click **Add to Sequence** to go to the edit movie window. Here you can put together a movie series in order to assign it to the sequence list.



- ① Image view
- ② Vessel display
- ③ Channel selection
- ④ Well navigation
- ⑤ Time point selection
- ⑥ Time lapse slider
- ⑦ Label options
- ⑧ Alias
- ⑨ Movie series
- ⑩ Preview

### Alias

The selected position and time window can be given a meaningful name in order to be able to distinguish the movies in the Sequence List.

Alias

### Sequence List

The sequence list will display the order and alias names of depicted movies of wells/positions or projects which will be used to assemble the sequence movie.

- **Preview** : Preview the full movie sequence
- **Up**: Moves selected movie up in the sequence list
- **Down**: Moves selected movie down in the sequence list
- **Delete** : Remove selected movie from the list

### Make Movie

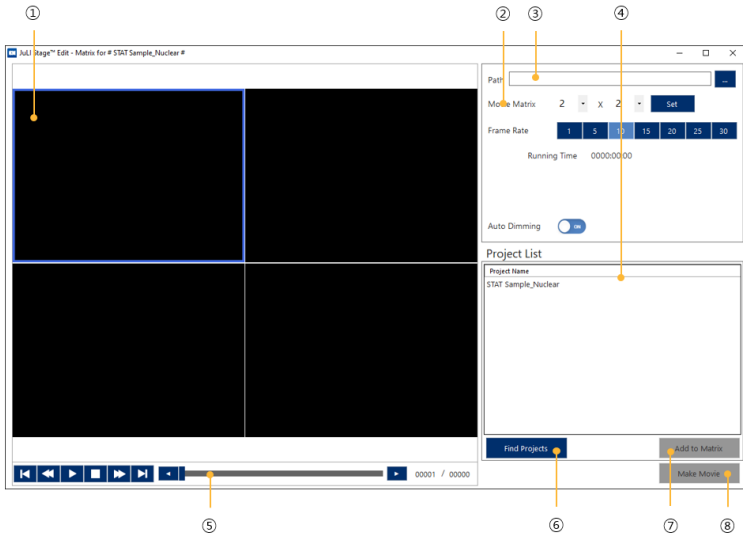
Apply final settings and save the movie sequence.

### Quick Workflow to save a sequence movie

1. Select a project in the Project List by clicking on the line (add another project to the list using **Find Projects** if required)
2. Press **Add to Sequence**
3. Select a well, position, and channels
4. Add labels, size bar, or time stamp
5. Crop image
6. Choose time window
7. Click **Add**
8. Assign an **Alias**
9. Click **Apply**
10. (repeat for all additional movies in this series)
11. Arrange the **Sequence List** and click **Make Movie**
12. Set a path, filename, and a frame rate
13. Click **Save**

## 11.2.6 Movie Maker – Matrix

The **Movie Maker – Matrix** can combine different movies in a tiled overview to better compare conditions with each other. Each tile can belong to one or can be a sequence of more than one time lapse measurement.



① Matrix view

② Movie matrix options

③ Data path

④ Project List

⑤ Time lapse slider

⑥ Find Projects

⑦ Add to Matrix

⑧ Make Movie

### Movie Matrix

Choose the number of tiles of the movie matrix from 1x1 and up to 9x9

### Auto Dimming

If activated, movies that are shorter than others will turn black after they finished to better indicate tiles with movies that finished earlier.

  ON

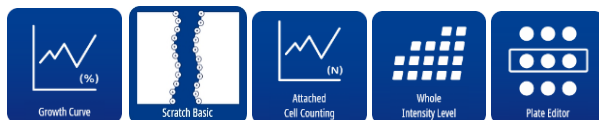
### Quick Workflow to save a matrix movie

1. Set a path, file name, movie matrix type (confirm with Set), and select a frame rate
2. Activate **Auto Dimming**
3. Click on a black tile in the matrix
4. Double click on the Project Name in the Project List (use **Find Projects** to add more projects)
5. Click **Add to Matrix**
6. Select a well, position, and channels
7. Add labels, size bar, or time stamp
8. Choose time window
9. Press **Add**
10. (repeat for all additional movies in this series)
11. Press **Apply**
12. Select another tile and repeat step 4 – 11 for all tiles
13. Press **Make Movie**

## 12 JuLI™ Stage STAT Software

### 12.1 Software overview

With JuLI™ Stage STAT, projects containing measurement data are selected and analyzed with one of four evaluations: **Growth Curve**, **Scratch Basic**, **Attached Cell Counting** and **Whole Intensity Level**.



With **Plate Editor**, detailed experiment conditions of the selected project can be defined and saved in plate maps: each well can be assigned to specific assay conditions (such as cell type and number, compound concentration etc.) in order to calculate e.g. mean values of triplicates and their standard deviations.

In addition, the **View Results** section of each evaluation module also offers to create or load a saved experiment layout.

All four evaluation modules lead to tables and ready-to-use graphs which can be copied and pasted. Numerical analysis results are presented in an exportable .csv format for further processing with external software such as Microsoft Excel® or other spread sheet software.

Evaluation module	Input channel	Output metrics	Example assays
<b>Growth Curve</b>	BRIGHT GFP, RFP, DAPI	Confluence = Growth rate [%] or [ $\mu\text{m}^2/\text{image}$ ], [ $\mu\text{m}^2/\text{well}$ ] [ $\text{mm}^2/\text{image}$ ] [ $\text{mm}^2/\text{well}$ ]  Mean fluorescence intensity per well [-] (only for fluorescence channels)	Cell health and viability assays, cytotoxicity, transfection rate, cell culture quality control
<b>Scratch Basic</b>	BRIGHT GFP, RFP, DAPI	Confluence in wound = Growth rate (in wound) [%] or [ $\mu\text{m}^2/\text{image}$ ], [ $\mu\text{m}^2/\text{well}$ ] [ $\text{mm}^2/\text{image}$ ] [ $\text{mm}^2/\text{well}$ ]  Mean fluorescence intensity per well [-] (only for fluorescence channels)	Scratch wound assay, migration and invasion, different magnifications
<b>Attached Cell Counting</b>	BRIGHT	Counting result [n/image], [n/ $\text{mm}^2$ ] and [n/well], n=number of adherent cells	Cell health and viability assays, cytotoxicity, transfection rate

	GFP, RFP, DAPI	Mean fluorescence intensity per well [-] (only for fluorescence channels)	
<b>Whole Intensity Level</b>	GFP, RFP, DAPI	Mean fluorescence intensity per well [-] (only for fluorescence channels)	Calcium-flux

☞ Note: Only measurements based on multi-well plates (6, 12, 24, 48, 96 or 384 well plates) can undergo further analysis. Workaround: If image analysis is required for other vessel types such as flasks, dishes or slides, please set up the measurement with one of the plate types instead.

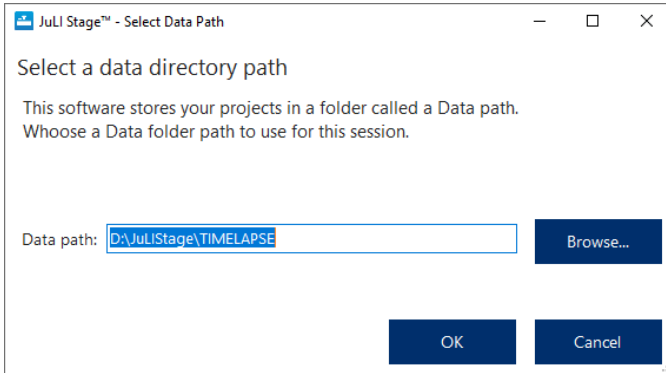
☞ Note: **Attached Cell Counting** and **Whole Intensity Level** are only displayed as an analysis option if a fluorescence channel was acquired during the measurement.

## 12.2 Operation

Double click the 'JuLI STAT' icon on the desktop to run the program.



Select a data directory path. Click **Browse...** to select the parent folder (located locally or in the network) containing measurement projects. Then click **OK**.

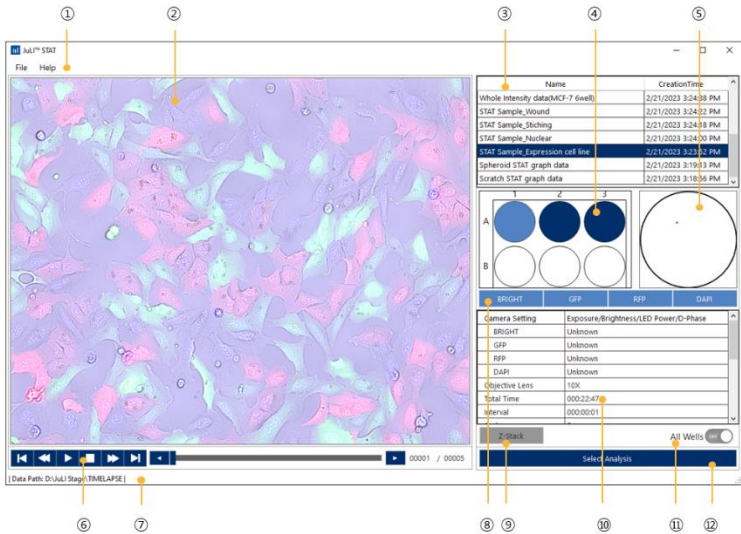


☞ Note: Selecting a single project folder directly will not work.



## 12.2.1 Main viewer

The main viewer is the starting interface for reviewing and selecting measurement data from the JuLI™ Stage for further analysis.

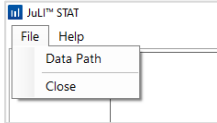


- |                                 |                       |
|---------------------------------|-----------------------|
| ① Menu bar                      | ⑦ Data path           |
| ② Image view                    | ⑧ Channel selection   |
| ③ Project list                  | ⑨ Z-stack             |
| ④ Vessel display                | ⑩ Project information |
| ⑤ Well navigation               | ⑪ Display all wells   |
| ⑥ Time lapse buttons and slider | ⑫ Select analysis     |

① **Menu bar**

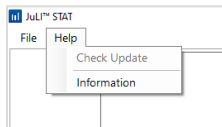
• **File menu**

- **Data Path:** Click **Data Path** to change the originally chosen data path: remember to always select a parent folder containing the projects / measurements.
- **Close:** Click **Close** to close the JuLI™ STAT Analysis software.



• **Help menu**

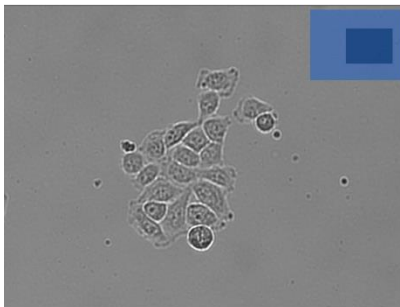
- **Information:** Software version and copyright information of JuLI™ STAT are displayed.



② **Image view**

The image view section displays the current image of a selected well, field and time point of a measurement.

- **Zoom-in:** Scroll up the mouse wheel in the view field to zoom in on the image. A navigation screen will be displayed in blue on the top right side. You can drag the small dark blue box in the navigation screen or click in the image itself with the mouse button held down to move the zoomed area.
- **Zoom-out:** Scroll down the mouse wheel to zoom out again.



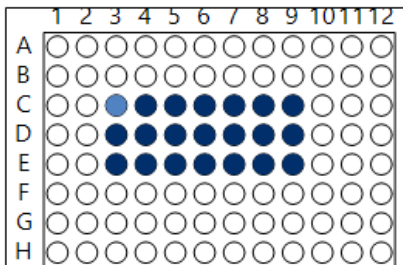
### ③ Project list

All available measurements / projects belonging to the selected data path are listed and sorted by date. By clicking a line in the table, a measurement is selected and thereby marked dark blue. By clicking the header field "Creation Time", the order of the list can be reversed.

### ④ Vessel display

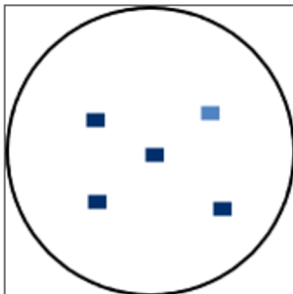
Of the selected measurement project, all wells of the plate type are displayed:

- white wells are not imaged wells,
- dark blue wells are imaged but not selected and
- light blue wells are selected and currently displayed on the screen.



### ⑤ Well navigation

All imaged positions in a well are displayed as blue squares. By clicking a blue square it is selected to be displayed and turns light blue.



### ⑥ Time lapse buttons and slider



Click any position within the time lapse slider bar to view an image at different time points. The cycle number appears as mouse-over on the time lapse slider. Click the **Play** button to see the full sequence played.

### ⑦ Data path

Display of the data path in use.



Use **File > Data Path** to change the data path.

### ⑧ Channel selection and overlay

Display of all 4 channels available on the JuLI™ Stage: Gray channels were not measured in the selected project while light blue channels were acquired and are displayed in image view automatically as an overlay, e.g. brightfield and RFP channel:



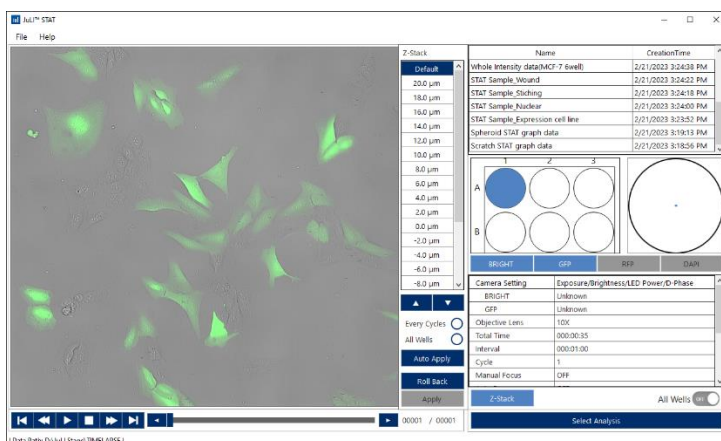
Clicking a light blue channel will deactivate it in the overlay and change its button to dark blue.

E.g. display of brightfield channel only:



### ⑨ Z-stack

If a measurement / project was acquired in Z-stack mode, the **Z-Stack** button will appear in light blue (instead of grey) together with a **Z-Stack** window showing stack planes and options to select the best plane:

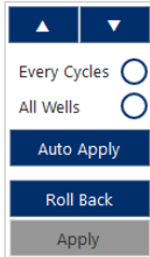


**Note:** Only one Z-plane of a multi-plane stack can be processed in an analysis.

The initial image view always displays the **Default** Z-plane, which is 0,0 µm.

Z-Stack	
Default	
10.5 µm	
0.0 µm	
-10.5 µm	

In order to pick one Z-plane regarded as optimal, select one channel and scroll through the stack using the arrow buttons.



- Checking the circles **Every Cycles** and **All Wells** followed by **Apply** will use only this plane of the selected channel in further analyses.
- Clicking **Auto Apply** will automatically search for the best plane based on the contrast of the image in the selected channel.
- **Roll Back** will reset changes back to the 0,0 µm plane.
- If no plane was selected the analysis will be executed on the 0,0 µm plane.
- Clicking **Z-Stack** will hide the **Z-Stack** window and turn the button dark blue.

☞ Note: The information which plane was selected will not be accessible later on in results tables or anywhere else.

### ⑩ Project information

Once a measurement / project is selected in the list, acquisition details such as objective lens used, total time, number of cycles and interval time are displayed in this window. Also, channel settings of the measurement (exposure time, brightness and LED power) are detailed:

Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	Unknown
GFP	Unknown
Objective Lens	10X
Total Time	000:00:35
Interval	000:01:00
Cycle	1
Manual Focus	OFF

11 Display All Wells

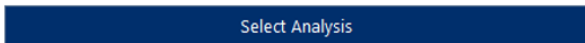
Switching the toggle switch of **All Wells** to **ON** will display all wells of the selected plate at a glance – measured ones as well as not measured ones.

In this example, in the upper 3 wells of a 6-well plate images were acquired, while the second row was not part of this measurement:



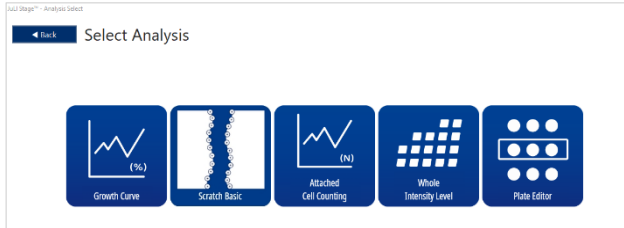
12 Select Analysis


After a project / Z-plane was selected, clicking **Select Analysis** will open the next window, where different analysis modules are offered.



## 12.2.2 Analysis modules

Clicking **Select Analysis** will direct you to the next window where four different analyses – **Growth Curve**, **Scratch Basic**, **Attached Cell Counting** and **Whole Intensity Level** - next to a **Plate Editor** are available:



Clicking  brings you back to the main viewer.

⇒ Note: Only measurements based on multi-well plates (6, 12, 24, 48, 96 or 384 well plates) can undergo further analysis.

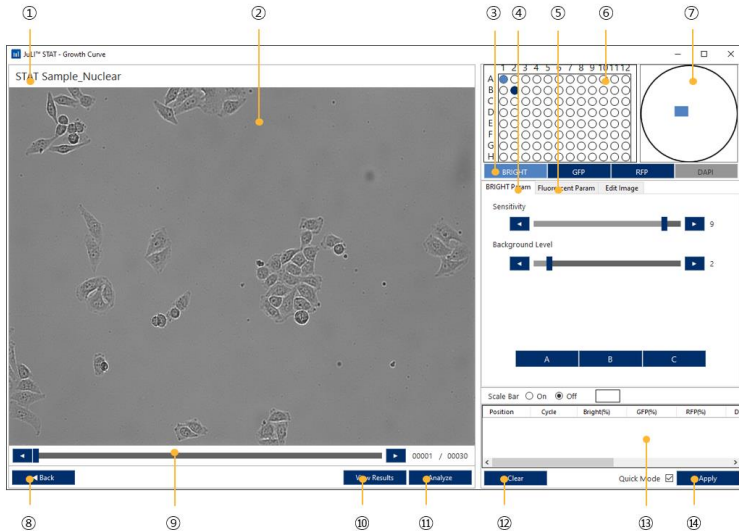
Workaround: If image analysis is required for other vessel types such as flasks, dishes or slides, please set up the measurement with one of the plate types instead.

⇒ Note: **Attached Cell Counting** and **Whole Intensity Level** are only displayed as an analysis option if a fluorescence channel was acquired during the measurement and if the measurement project does not contain stitched images.

### 12.2.2.1 Growth Curve



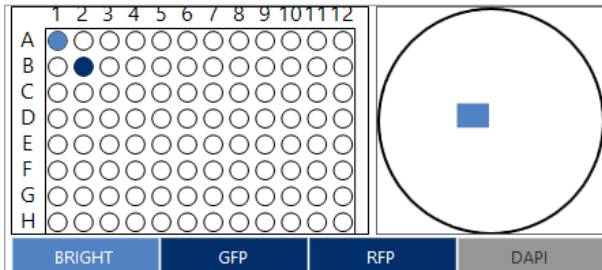
The **Growth Curve** analysis is used to quantify the cell confluence in images over time, based on both brightfield or fluorescence images. In addition, the mean fluorescence intensity per well is calculated for fluorescence channels automatically.



- |                               |                          |
|-------------------------------|--------------------------|
| ① Project / measurement name  | ⑧ Back (to main viewer)  |
| ② Image view                  | ⑨ Time lapse slider      |
| ③ Channel selection           | ⑩ View results           |
| ④ Brightfield channel param.  | ⑪ Analyze                |
| ⑤ Fluorescence channel param. | ⑫ Clear results list     |
| ⑥ Vessel display              | ⑬ Confluence result list |
| ⑦ Well navigation             | ⑭ Apply                  |



## Navigation



The default setting of the selected measurement always displays the brightfield image and therefore this channel is presented in light blue. Any other acquired channel of the measurement is shown in dark blue, not measured channels in grey.

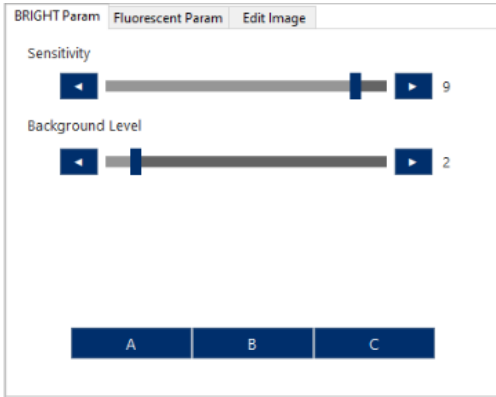
☞ Note: Only one channel can be selected at a time, no other acquired channel can be co-selected to create overlays.

- Positions in the well are light blue when selected, otherwise are shown in dark blue.
- Using the time lapse slider bar, different time points of a selected well can be viewed.
- Confluence analysis can be calculated on a brightfield image as well as on a fluorescence image, both having different sliders and parameters to optimize segmentation results.

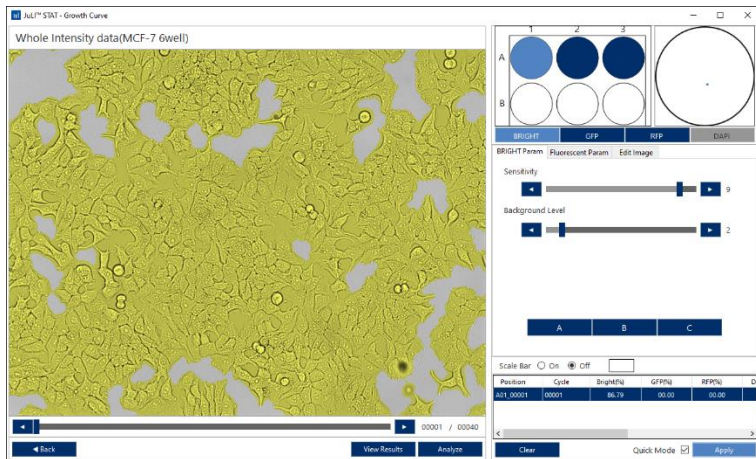
### Optimizing confluence analysis on brightfield images

There are two parameters on the **BRIGHT Param** tab to influence the segmentation result of brightfield images: **Sensitivity** and **Background Level**. Under **A**, **B** and **C** different settings for these two parameters are preconfigured.

Depending on the brightness and contrast of the image, they lead to different segmentation results.



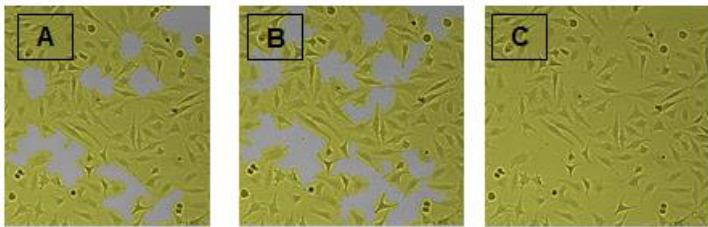
To view the segmentation and confluence result of an image, click e.g. **A** and then **Apply**:



The yellow colored area outlines the segmentation result of the latest sensitivity and background settings on the image view, and the table shows the numerical results of the selected well, position in well and time point. Changing the time point or well will add a line to the table, while different settings on the same image will overwrite the values of the former analysis.

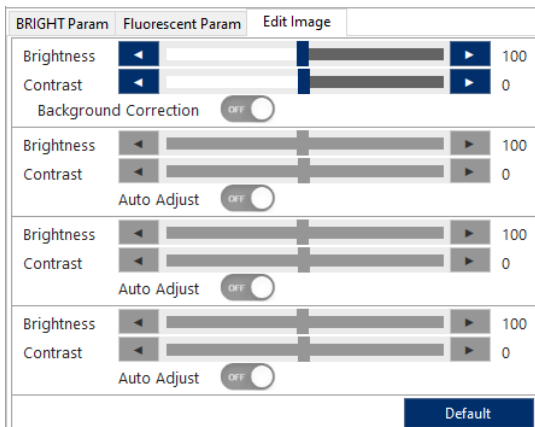
Position	Cycle	Bright(%)	GFP(%)	RFP(%)	D
A01_00001	00001	86.79	00.00	00.00	
A02_00001	00001	81.98	00.00	00.00	
A03_00001	00001	64.71	00.00	00.00	
A03_00001	00002	64.49	00.00	00.00	
A03_00001	00003	64.58	00.00	00.00	

Example results for preconfigured sensitivity and background level settings **A**, **B** and **C**, with **B** delivering the best separation of foreground and background:



Note: **Quick Mode** has no effect on the analysis of brightfield images.

If the segmentation results of **A**, **B** and **C** turn out unsatisfactory, **Edit Image** gives more options to optimize brightness and contrast of the original image:



- By moving the **Brightness** and **Contrast** sliders followed by clicking **Apply**, the confluence analysis results can be viewed instantly and the best settings can subsequently be applied to the entire measurement.
- Clicking **Default** reverts any changes.

Note: For a more selective optimization of brightness and contrast image settings of e.g. only distinct wells or time points of a measurement, open the

measurement project in **JuLI™ EDIT** and save the original measurement under a new name. For more details, see chapter 11 “JuLI™ EDIT Software”

☞ Note: Parameters to optimize confluence segmentation on fluorescent images (**Fluorescent Param**) are deactivated for the brightfield channel.

☞ Note: How to analyze the whole measurement project, create graphs and export result tables once all settings are optimal, see later in this chapter.

### Optimizing confluence analysis on fluorescent images

In order to analyze the percentage of confluence on fluorescence images, first a fluorescence channel needs to be selected by clicking it.

There are many settings available to optimize the confluence analysis: **Background Control**, **Default Gating**, **Tolerance Factor**, **Maximum intensity zero**, manually gating min and max intensities, **Quick Mode** and the optimization of **Brightness** and **Contrast** on the images in **Edit Image**.

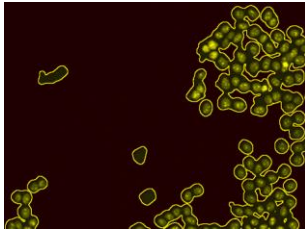
The screenshot displays the 'Edit Image' configuration window for a fluorescent channel. At the top, there are tabs for 'BRIGHT', 'GFP', 'RFP', and 'DAPI', with 'GFP' currently selected. Below these are sub-tabs for 'BRIGHT Param', 'Fluorescent Param', and 'Edit Image', with 'Fluorescent Param' active. The 'Fluorescent Param' section includes three checkboxes: 'Background Control' (unchecked), 'Default Gating' (unchecked), and 'Maximum intensity zero' (checked). A 'Tolerance Factor' input field is set to '25'. A histogram shows 'Count(n)' on the y-axis (ranging from 64054 to 323270) and 'Fluorescence Intensity' on the x-axis (ranging from 0 to 255). Below the histogram is a 'Reset' button and a 'Set' button with a slider bar and input fields for '0' and '255'. The 'Scale Bar' is set to 'Off'. A table with columns 'Position', 'Cycle', 'Bright(%)', 'GFP(%)', 'RFP(%)', and 'DAPI(%)' is shown below. At the bottom, there is a 'Clear' button, a 'Quick Mode' checkbox (checked), and an 'Apply' button.

Start to optimize the confluence analysis by selecting a representative well, time point and field in a well and then click **Apply** using the default settings shown above.

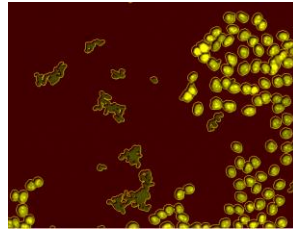
☞ Note: Finding the best combination of settings is an iterative process and should always be tested on representative images (different time points, positive and negative controls, etc.).

Continue comparing results with **Quick Mode** turned off (then Click **Apply** twice). In **Quick mode**, pixels of an image are binned, thereby reducing the processing time of large data sets. Binning means that neighboring pixels are aggregated to a larger single pixel representing the mean intensity of the binned pixels.

**Quick Mode: on**



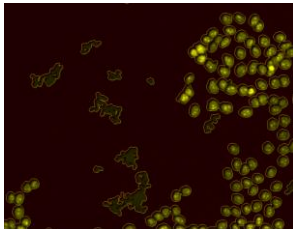
**Quick Mode: off**



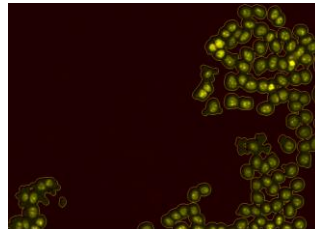
*In this example, the Quick mode improved segmentation, but some background is still falsely detected in both cases.*

Then compare the segmentation result with **Background Control** (automatic background brightness correction) turned on or off.

**Background Control: off**



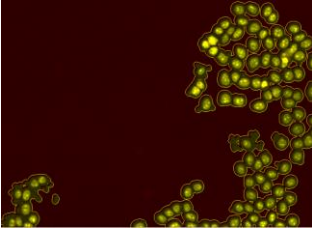
**Background Control: on**



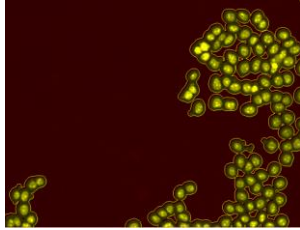
*This greatly improved the separation of background and foreground in this example image.*

Another value to influence the quality of the segmentation is called **Default Gating**. It automatically gates the intensity graph (manual min/max intensity inputs are disabled then).

**Default Gating: off**



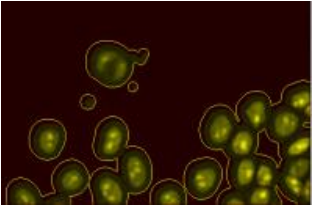
**Default Gating: on**



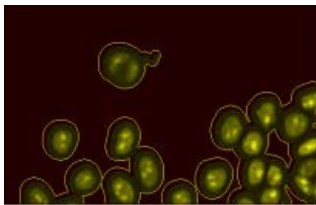
Here, in the lower left corner a difference can be seen.

Also, the **Tolerance Factor** can be changed to check its influence on the segmentation:

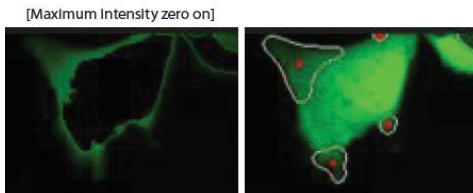
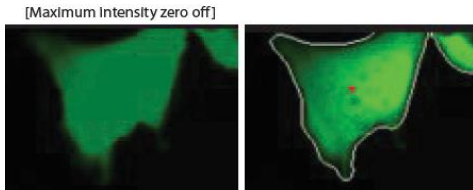
**Tolerance Factor: 1**



**Tolerance Factor: 25**



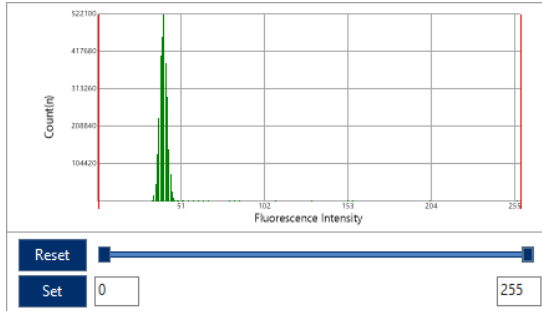
Check the **Maximum intensity zero** box to exclude areas exceeding the maximum intensity (option when **Default Gating** is unchecked):




## Manual intensity gating

Manual intensity gating can be useful to e.g. exclude objects with low fluorescence.

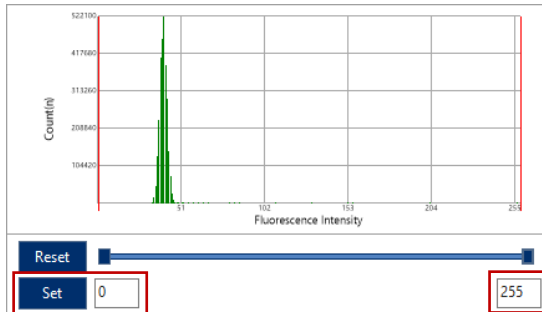
The intensity plot shows the intensity distribution of pixels in an image, with fluorescence intensity plotted in x and number of pixels on the y-axis.



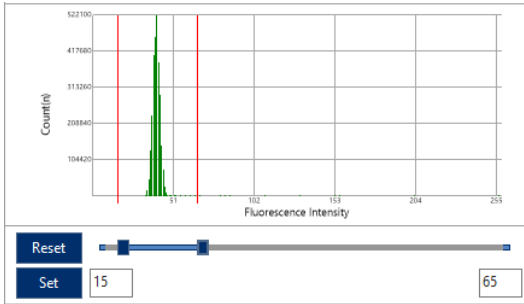
 Note: The intensity graph is not available for the brightfield channel.

Pointing the mouse to a position in the image will show the intensity of the pixels as a tooltip to support the decision on the intensity values to be used for the gating.

To zoom the graph, enter a minimum and/or maximum intensity value or use the slider, then Click **Set**.



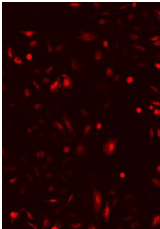
Red lines indicate the new gating window.



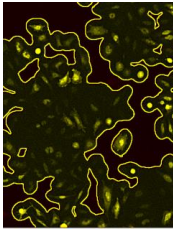
Click **Reset** to return to the initial graph.

Example for excluding low expressing cells from the confluence analysis: here, only cells with intensities above 50 are included in the segmentation mask:

**Original**

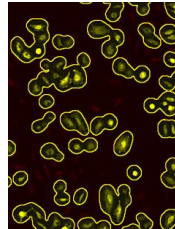


**Default gating**



54 % confluence

**Manual gating: 50 to 255**



28 % confluence



## Edit Image

The segmentation results for fluorescence images can be further improved using **Edit Image**:

- By moving the **Brightness** and **Contrast** sliders followed by clicking **Apply** the confluence analysis results can be viewed instantly and the best settings can subsequently be applied to the entire measurement.
- Clicking **Default** resets any changes.

BRIGHT Param	Fluorescent Param	Edit Image	
Brightness		<input type="checkbox"/>	100
Contrast		<input type="checkbox"/>	0
Background Correction <input type="checkbox"/>			
Brightness		<input type="checkbox"/>	100
Contrast		<input type="checkbox"/>	0
Auto Adjust <input type="checkbox"/>			
Brightness		<input type="checkbox"/>	100
Contrast		<input type="checkbox"/>	0
Auto Adjust <input type="checkbox"/>			
Brightness		<input type="checkbox"/>	100
Contrast		<input type="checkbox"/>	0
Auto Adjust <input type="checkbox"/>			
			<b>Default</b>

☞ Note: For a more selective optimization of **Brightness** and **Contrast** settings of only distinct wells or time points of a measurement, open the measurement project in **JuLI™ EDIT** and save the original measurement under a new name. For more details, see chapter 11 “JuLI™ EDIT Software”

### Confluence result list: Numerical results

Whenever the **Apply** button is clicked, the numerical results of the covered cell area, i.e. cell confluence in % (as well as an extrapolated area in  $\mu\text{m}^2$ ) is shown in a table for the selected channel, well, position in well and time point. Changing the time point or well will add a line to the table, while different analysis settings on the same image will overwrite the values of the former analysis.

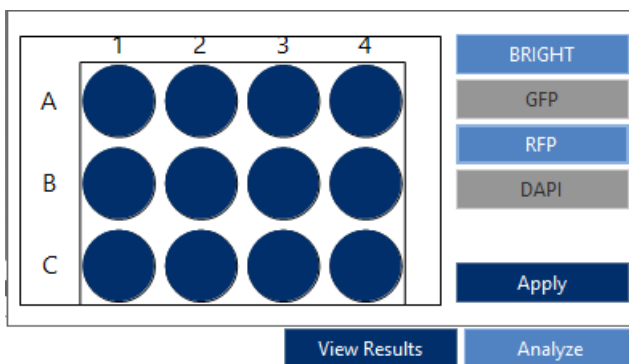
Position	Cycle	Bright(%)	GFP(%)	RFP(%)	D
A01_00001	00001	00.00	00.00	78.80	
A01_00001	00011	00.00	00.00	76.95	
A02_00001	00001	00.00	00.00	61.55	
A02_00001	00011	00.00	00.00	64.09	
A03_00001	00001	00.00	00.00	45.99	
A03_00001	00011	00.00	00.00	41.42	

Clicking **Clear** will delete the complete result list.

⚠ Note: This results table can neither be saved nor exported to an external program.

### Analyze: Create exportable graphs and result tables

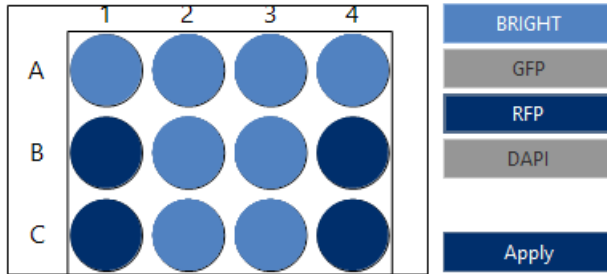
Once analysis settings and parameters for all channels are finalized, exportable graphs and result tables can be created by clicking **Analyze**:



A window will appear, where all or only some wells (but not time points or positions in a well) as well as channels can be selected: hold the mouse button down and draw a rectangle from the upper left to the lower right corner to select multiple wells or click single wells to subject them to the analysis: selected wells turn light blue now. Clicking light blue wells one more time will cause their deselection.

The analysis will be performed on all channels by default (light blue color code), clicking a light blue channel will remove this channel from the analysis (and turning it dark blue).

In this example, the brightfield-based confluence in 8 selected wells will be calculated:

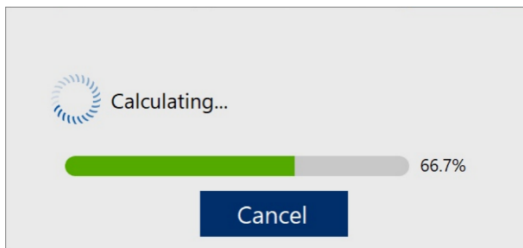


Click **Apply** to start the analysis (and creation of graphs and tables) or click once more on **Analyze** to go one step back.

☞ Note: The final settings of an applied analysis (such as Quick Mode on, Background control checked etc.) are not saved.

☞ Note: Whenever a new analysis is applied to single, multiple or all wells or channels, former analysis results will be overwritten.


After clicking **Apply**, a window will display the progress of the analysis. Click **Cancel** if you want to stop it.

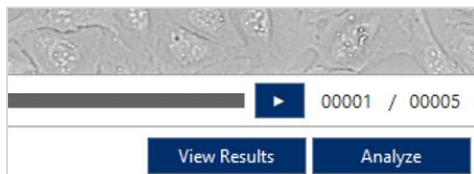


When the analysis is finished, it can be saved or discarded:



### View Results

Click **View Results**  to proceed to different display options of result tables and graphs:

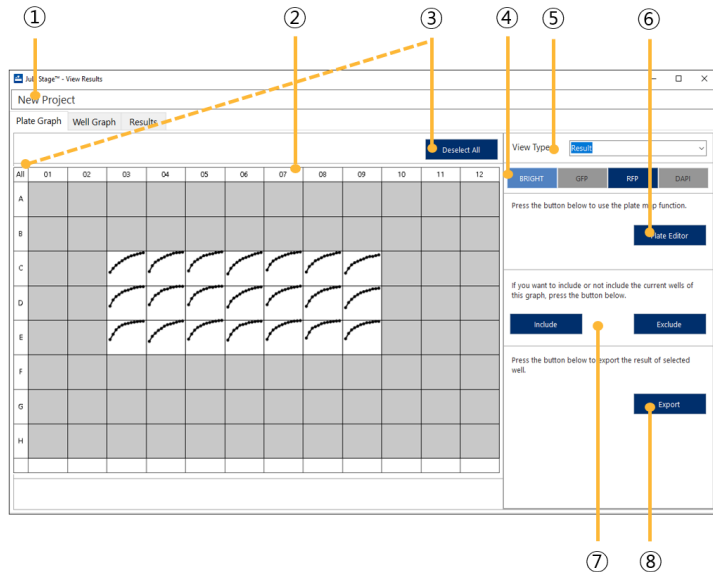


A new window will be opened, divided into three tabs offering different result presentation options: **Plate Graph**, **Well Graph** and **Results**.



## Plate Graph tab

In the **Plate Graph** tab, a plate overview for all analyzed wells can be visualized depending on channel and readout parameter. Single or multiple wells can be excluded or included into the final results to be compiled as graphs and tables using **Export**.



- |                                  |                         |
|----------------------------------|-------------------------|
| ① Selected project / measurement | ⑤ Readout selection     |
| ② Plate overview                 | ⑥ Link to plate editor  |
| ③ Select and deselect All        | ⑦ Include/exclude wells |
| ④ Channel selection              | ⑧ Export                |

In the plate overview ②, curves of analyzed wells are presented for a selected channel ④ (only one channel at a time, no overlay available) and a selected readout ⑤.

☞ Note: If more than one image was acquired in a well the mean value of all images in the well will be given out as numerical read out. However, in the **Well Graph** tab results can be reviewed and exported for single positions inside wells, but only well by well.

- To mark wells, click **All** in the upper left corner of the plate view, or on single wells or draw a rectangle over multiple wells with the mouse.
- Click **Include** or **Exclude** ⑦ to insert or remove all readouts of all channels belonging to these wells from the graph and results presentation generated via **Export** ⑧. Reverse your selection by clicking **Deselect All** ③.

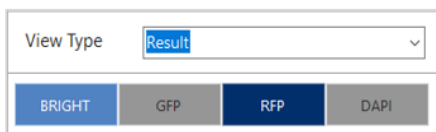
- A direct link to the **Plate Editor** ⑥ can be used to create a detailed experiment layout for the selected measurement project: assign names, colors or compound concentrations to each well in order to change the colors of curves in graphs or to calculate mean values of e.g. triplicates.

☞ Note: More details on how to create a plate map using **Plate Editor** please refer to chapter 12.2.3 “Plate Editor”.

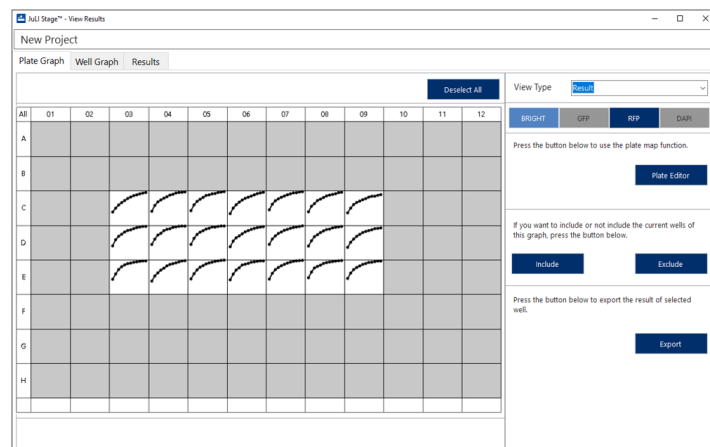
### How to create, edit and export graphs and result tables step by step:

In this example, a time series of 21 wells on a 96 well plate were measured with BRIGHT and RFP channel and both channels were analyzed in the **Growth Curve** module.

The default screen will display the results of the latest analysis and the BRIGHT channel:

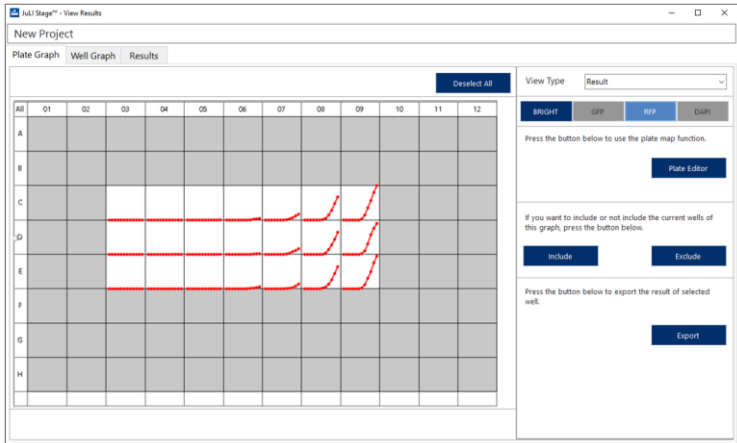


Under **View Type** ⑥ the readout Result is displayed, which is always the main readout of the present analysis module: in **Growth Curve** it is Growth rate [%] over time:

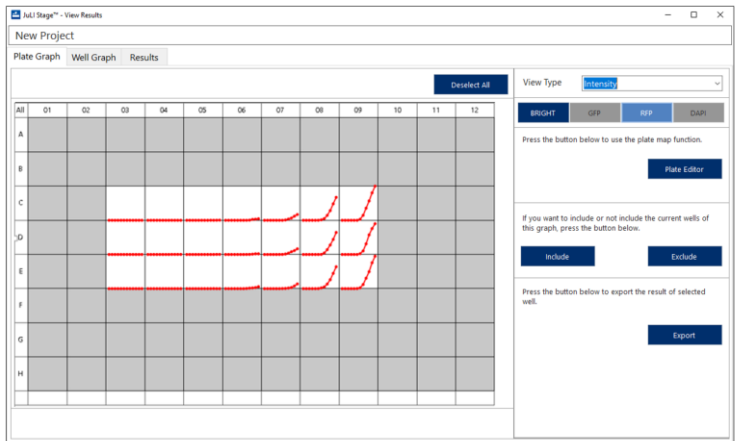


☞ Note: When the BRIGHT channel is active, **View Type** can be changed via the drop down arrow to **Intensity**, however, the intensity of wells acquired in brightfield will not be quantified and therefore be displayed in a zero line.

Changing the channel selection to **RFP**, will display the **View Type Result** of the Growth Rate [%] analyzed on the basis of red fluorescing cells. The color of the curves will match the color of the selected channel.

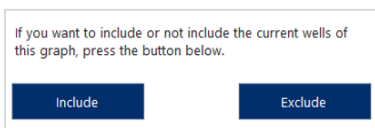


Note: Depending on which part of a cell is stained by the fluorescence dye, the confluence results might differ from the brightfield values as a smaller area of each cell might be subjected to segmentation.

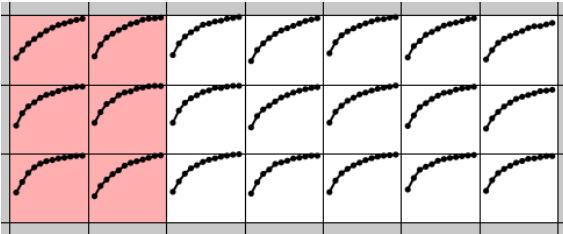


The readout Growth Rate can be changed to (mean) **Intensity** per well by selecting **Intensity** in the drop down menu of **View Type**:

To remove outliers or unwanted wells from the final graphs and result tables, they can be marked and excluded by clicking **Exclude**:

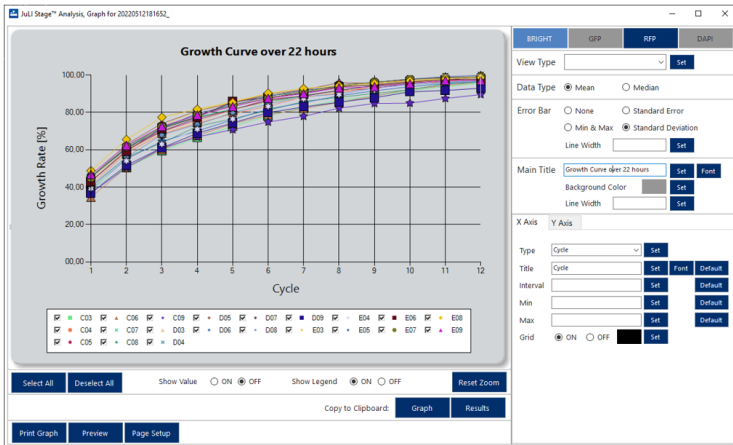


Excluded wells appear in red and all results (in all channels) belonging to these wells won't be shown in graphs and result tables compiled under **Export**:



This selection can be reversed via **Include**.

Finally, clicking **Export** will create a graph showing the results of all 21 wells and all time points in individually colored lines:



As before, clicking another channel or selecting another readout under **View Type** will display other available readouts. Here for example the mean intensity per well in the **RFP** channel:



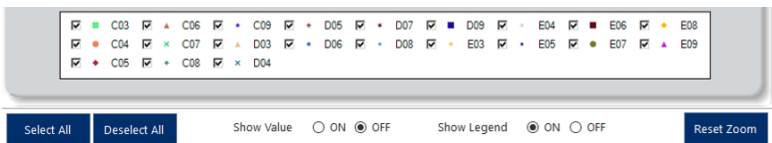


There are plenty options to change the appearance of the graphs, most of which are self-explaining and easy to apply.

For example, the **Main Title**, which is the project name by default, can be changed to a more meaningful one, and also **Background Color** can be selected. Most changes require **Set** to be clicked to become active.

Main Title	<input type="text" value="Well intensity changes over 22 hours"/>	<input type="button" value="Set"/>	<input type="button" value="Font"/>
Background Color	<input type="color" value="#808080"/>	<input type="button" value="Set"/>	
Line Width	<input type="text" value=""/>	<input type="button" value="Set"/>	

Lines can be removed from the graph by unchecking their box in the legend, numerical values can be included in the graph (**Show Value**), and a zoomed version of the graph (draw a rectangle over the region to be zoomed with the mouse or click the graph and turn the mouse wheel) can be copied to clipboard. The legend can be part of the final layout or left out with **Show Legend ON** or **OFF**.



Optional changes of the X- and Y-Axis are **Type**, **Title**, **Interval**, **Min** and **Max Grid ON** or **OFF**:

X Axis	Y Axis		
Type	Cycle	Set	
Title		Set	Font Default
Interval		Set	Default
Min		Set	Default
Max		Set	Default
Grid	<input checked="" type="radio"/> ON <input type="radio"/> OFF	Set	

X Axis	Y Axis		
Type	%	Set	
Title	Growth Rate (%)	Set	Font Default
Interval		Set	Default
Min		Set	Default
Max		Set	Default
Grid	<input checked="" type="radio"/> ON <input type="radio"/> OFF	Set	
Exponent	<input type="radio"/> ON <input checked="" type="radio"/> OFF		

☞ Note: The **Interval** of the **X-Axis** requires the entry of a cycle number, independent of the **Type** selected.

☞ Note: On the **X-Axis** only timelapse describing entries are selectable, not compound dilutions or any other entries defined in a plate map.

Alternative units to express the confluence are also offered:

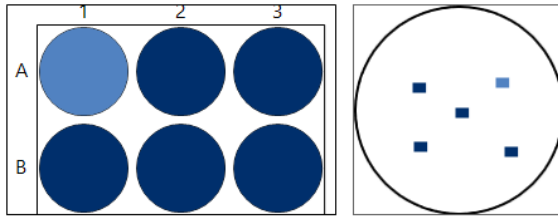
Type	%
Title	µm <sup>2</sup> / Image µm <sup>2</sup> / Well
Interval	mm <sup>2</sup> / Image mm <sup>2</sup> / Well

☞ Note: The units µm<sup>2</sup>/well and mm<sup>2</sup> /well are theoretical values: based on the detected cell-covered area the confluence in the whole well is extrapolated.

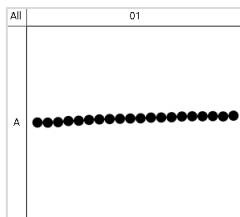
The display variants **Mean** and **Median** in **Data Type** as well as **Error Bar** options are only selectable under specific conditions:

Data Type	<input checked="" type="radio"/> Mean	<input type="radio"/> Median
Error Bar	<input type="radio"/> None	<input type="radio"/> Standard Error
	<input type="radio"/> Min & Max	<input checked="" type="radio"/> Standard Deviation
Line Width	<input type="text"/>	Set

One condition is when more than one field was acquired in an experiment as shown in this example: 5 positions inside the wells were measured:



In the **Plate Graph** tab automatically only one curve per well is displayed and only the selected numerical readouts (mean or median, together with standard deviation or standard error etc.) are exported to the Results table.



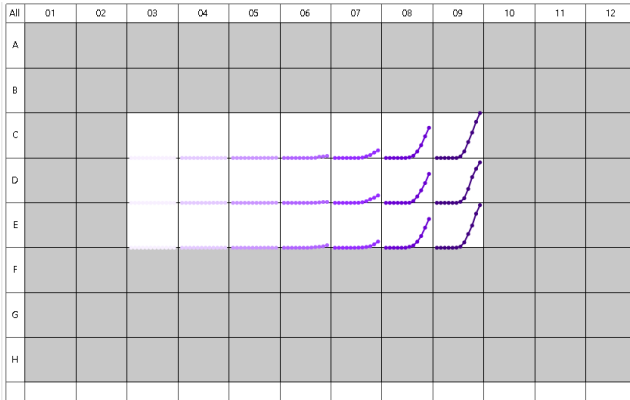
⇒ Note: The results for each position in a well will be accessible in the **Well Graph** tab and can be exported as Graph or numerical readouts there. See later in this chapter.

⇒ Note: It is not possible to exclude single positions in a well from neither the analysis nor the calculation of mean / standard deviation etc. They can be excluded from graphs, though.

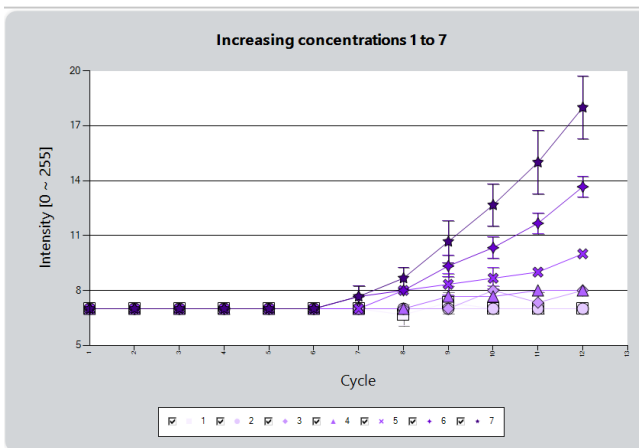
The other case, where **Data Type Mean** or **Median**, as well as all **Error Bar** options are accessible is when a plate map (created in **Plate Editor**) is available for the measurement project. Here, the software needs to know where well repeats are located in order to calculate mean values and their standard deviation.

⇒ Note: More details on how to create a plate map using **Plate Editor** please refer to chapter 12.2.3 "Plate Editor".

In our example, triplicates were located column-wise and 7 concentrations were tested. A plate map was created naming the concentrations 1 to 7 and assigning different colors to triplicates:



Instead of the former 21 curves, now only 7 curves are shown displaying e.g. **Mean** and **Standard Deviation** of the whole well **Intensity** of triplicates assigned the names 1 to 7:



Once the edits to the graph are finalized they can be either **printed** directly or **Copied** to the **Clipboard** to be pasted in an external program such as Word or Powerpoint:

Copy to Clipboard: Graph Results

Print Graph
Preview
Page Setup

Being transferred as bitmaps, graphs cannot be edited after being pasted into an external program.

However, copying **Results** to the clipboard, exports the actual results data table (csv-format) (of the selected channel and readout), which can be opened and further

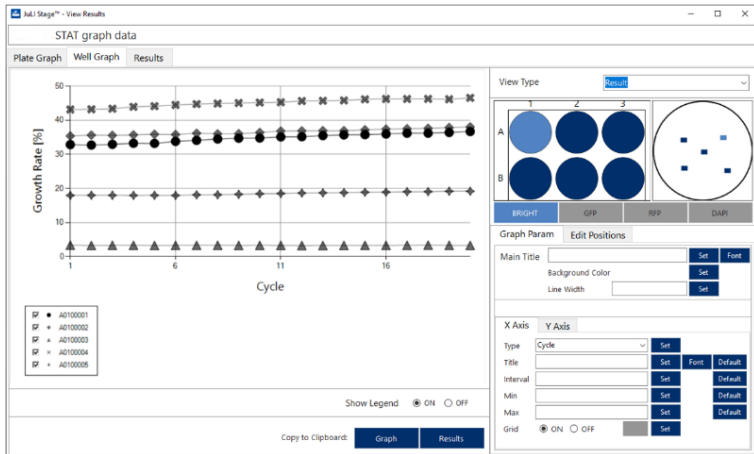
processed with external software such as Excel or other spreadsheets. In case of mean values and standard deviations being available, both data will be exported.

☞ Note: All available readouts of an analysed measurement (and all available units for all readouts) for each channel need to be exported separately, there is no overall export containing all numerical results of an analysis.

☞ Note: Exports of result data tables contain header lines which need to be removed if data are pasted into certain external software programs such as GraphPad Prism.

## Well Graph tab

This tab is especially good for a more detailed look on measurements which contain more than one position within a well. In **Well Graph**, analysis results for all positions in a well are presented in separate curves.



Also, analysis results for each position within a well can be exported in this tab together with their mean values, their standard errors and standard deviations by clicking **Results**.

While the configuration options regarding graphs using **Graph Param** is mostly identical to the ones already described in detail in this chapter, one more option is available here, called **Edit Positions**. In **Edit Positions** each position in a well can be assigned a new legend name after selecting it, which is then confirmed by clicking **Change**.

Graph Param
Edit Positions

You can change the legend of the current position. Use the controls below to make changes.

Change

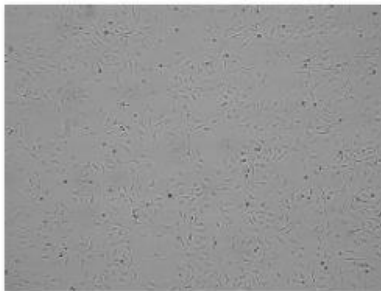
If a measurement is only containing one position per well, **Edit Positions** will assign the new legend name to the well.

## Results tab

The **Results** tab is designed for a quick overview of all numerical data available for a plate and its channels together with a small and instant view on the segmented image belonging to the selected value in the table. Inspecting segmented images can quickly confirm the validity of a value in the table. And they can easily be copied as jpegs into presentations via **Open Location**.

Date Time (file index)	Cycle	Elapsed Time	D06	D07	D08	D09	E04
01/23/2018 14:33 (00000)	00001	00000000	0	0	0	0	0
01/23/2018 15:33 (00001)	00002	00100000	5.21	4.57	6.45	4.84	3.59
01/23/2018 16:33 (00002)	00003	00200000	12.00	10.99	14.44	11.42	10.14
01/23/2018 17:33 (00003)	00004	00300000	19.03	17.17	22.47	17.95	16.97
01/23/2018 18:33 (00004)	00005	00400000	27.93	24.65	32.09	23.65	24.64
01/23/2018 19:33 (00005)	00006	00500000	36.87	33.74	41.63	31.59	33.64
01/23/2018 20:33 (00006)	00007	00600000	46.05	43.56	54.18	39.21	43.15
01/23/2018 21:33 (00007)	00008	00700000	54.91	51.58	65.25	48.01	52.73
01/23/2018 22:33 (00008)	00009	00800000	63.05	60.36	75.16	55.28	61.67
01/23/2018 23:33 (00009)	00010	00900000	70.41	70.22	86.36	62.2	67.52
01/24/2018 00:33 (00010)	00011	01000000	77.66	78.65	95.29	70.59	73.06
01/24/2018 01:33 (00011)	00012	01100000	85.7	86.56	100	75.62	81.43
01/24/2018 02:33 (00012)	00013	01200000	90.01	92.96	100	82.48	85.65
01/24/2018 03:33 (00013)	00014	01300000	93.01	97.46	100	85.99	90.34
01/24/2018 04:33 (00014)	00015	01400000	97.62	98.8	100	91.53	94.81
01/24/2018 05:33 (00015)	00016	01500000	98.15	100	100	95.62	98.03
01/24/2018 06:33 (00016)	00017	01600000	98.81	100	100	96.94	100
01/24/2018 07:33 (00017)	00018	01700000	100	100	100	97.79	100
01/24/2018 08:33 (00018)	00019	01800000	100	100	100	98.78	100

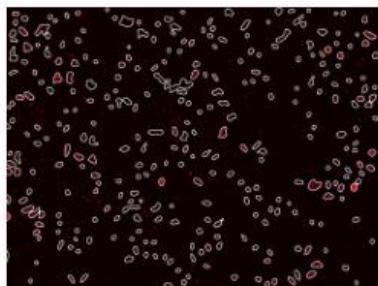
Clicking **Open Location** will open the folder containing the original image and segmented images (saved as .jpg) belonging to one well. These images have abbreviation tags following their name depending on the analysis module used to generate them, i.e. GCL for **Growth Curve**, and ACC for **Attached Cell Counting** and WHL (wound healing) for **Scratch Basic**.



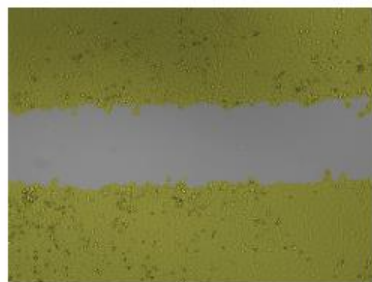
00002.JPG



00002\_GCL.JPG



00011\_ACC.JPG



00000\_WHL.JPG

☞ Note: The enumeration of images in their folders differs from the cycle enumeration displayed in the analysis result tables: if for example 12 cycles were acquired, image one is stored under number 0, while images belonging to the 12th cycle are stored under number 11.

Clicking **Copy Results** will copy the analysis results from the whole table (of the channel and readout selected before) to the clipboard, from where it can be pasted into external spreadsheets.



### 12.2.2.2 Scratch Basic

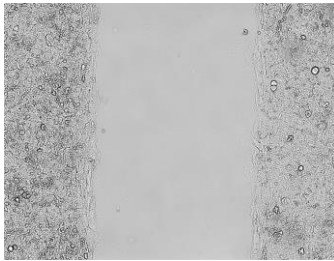


The **Scratch Basic** analysis can be used to quantify the wound confluence in images over time - based on both brightfield or fluorescence images.

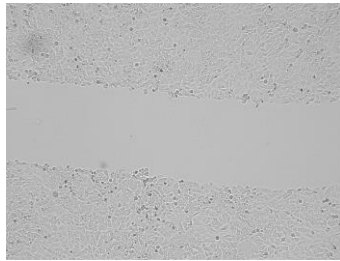
The main differences to the optionally available software **JuLI™ Stage Scratch**, are that there is only one readout – wound confluence over time – (instead of 6 readouts such as relative wound density and speed of wound closure) and that it can be applied to all magnifications (instead of only 4x).

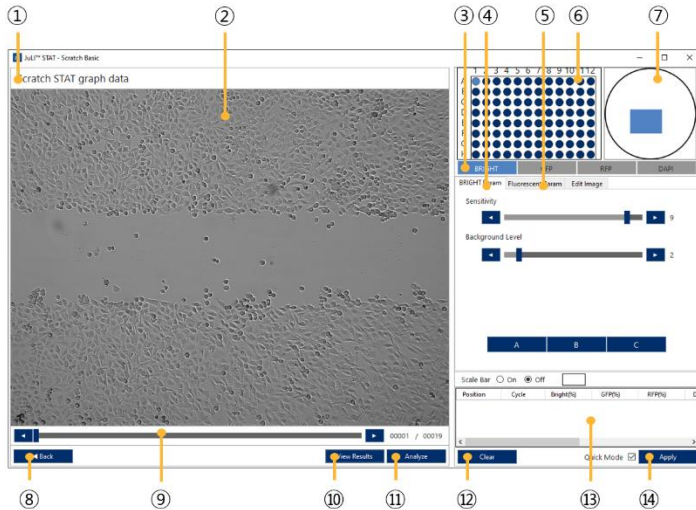
The wound can be oriented horizontally or vertically

**10x, horizontal orientation**



**4x, vertical orientation**

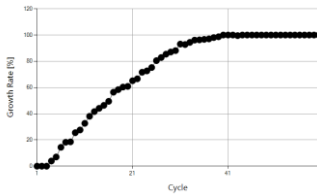
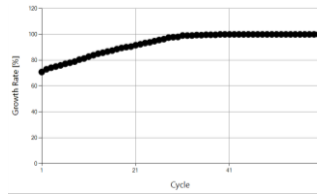




- |                             |                          |
|-----------------------------|--------------------------|
| ① Project name              | ⑧ Back                   |
| ② Image view                | ⑨ Time lapse slider      |
| ③ Channel selection         | ⑩ View results           |
| ④ Bright chan. param.       | ⑪ Analyze                |
| ⑤ Fluorescence chan. param. | ⑫ Clear results list     |
| ⑥ Vessel display            | ⑬ Confluence result list |
| ⑦ Well navigation           | ⑭ Apply                  |

In principal, the interface for optimizing the analysis parameters and to analyze and view the results, is identical to the **Growth Curve** analysis. Therefore please refer to chapter 0 “12.2.2.1 Growth Curve” for further details.

The main function of **Scratch Basic** in comparison to **Growth Curve** is the confluence readout: in **Scratch Basic** the Growth Rate [%] is wound related, starting at 0 % at time point 0, as it detects the closing wound area over time (while in **Growth Curve** the graph starts at an initial value reflecting the cell confluence in the image at time point 0), as illustrated here:

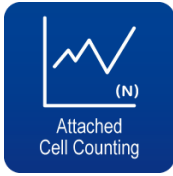
Well A1, **Scratch Basic**Well A1, **Growth Curve**

☞ Note: When configuring the optimal analysis settings in **Scratch Basic**, the instantly created result list displays Growth Rate values per image (not wound). However, once an analysis is applied via **Analyze**, wound-related confluence results will be displayed.

☞ Note: The confluence unit Growth Rate [%] reflects the closing wound over time. Changing this [%]-unit to e.g. [ $\mu\text{m}^2/\text{image}$ ] will falsely display confluence values of the whole image.

The remaining functions of the **Scratch Basic** module (**Analyze, View Results**) are identical to **Growth Curve** and described in chapter 0 “12.2.2.1 Growth Curve” in detail.

### 12.2.2.3 Attached Cell Counting



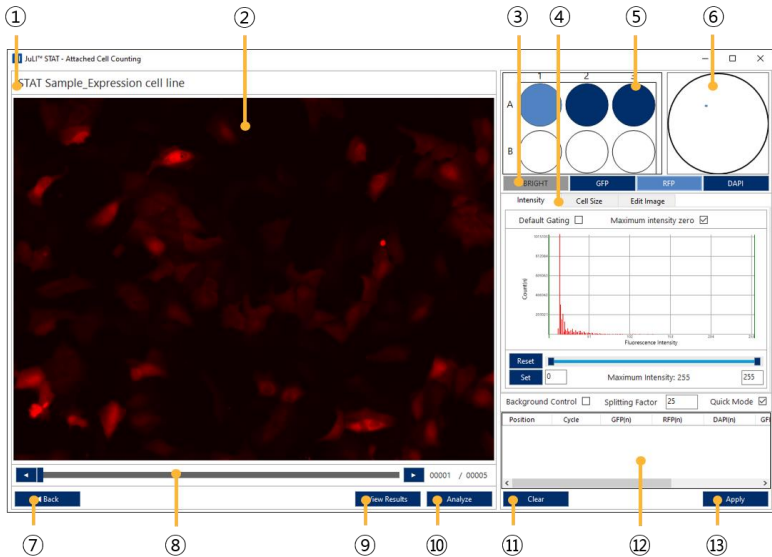
With the **Attached Cell Counting** evaluation module, adherent, fluorescence stained cells can be counted. Intensity and cell size thresholds next to other parameters can be changed to improve segmentation results.

☞ Note: **Attached Cell Counting** is only displayed as an analysis option if a fluorescence channel was acquired during the measurement.

☞ Note: **Attached Cell Counting** is not usable on BRIGHT images; it will be inactivated automatically (grey channel color).

☞ Note: **Attached Cell Counting** is not available for stitched images.

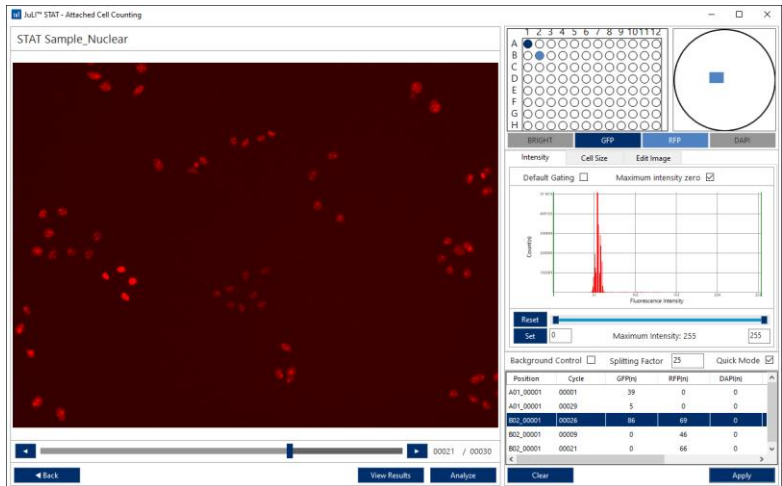
As the initial interface after clicking the **Attached Cell Counting** icon has many similarities to the **Growth Curve** module, only main differences will be explained on the following pages.



- ① Project / measurement name
- ② Image view
- ③ Channel selection
- ④ Counting parameter
- ⑤ Vessel display
- ⑥ Well navigation
- ⑦ Back
- ⑧ Time lapse slider
- ⑨ View results
- ⑩ Analyze
- ⑪ Clear
- ⑫ Cell count result list
- ⑬ Apply

**Optimizing cell counting on fluorescence images**

There are two tabs - **Intensity** and **Cell Size** - offered for optimization of the segmentation.



Start by selecting a representative well and time point and click **Apply** to apply the default settings of the **Intensity** tab.

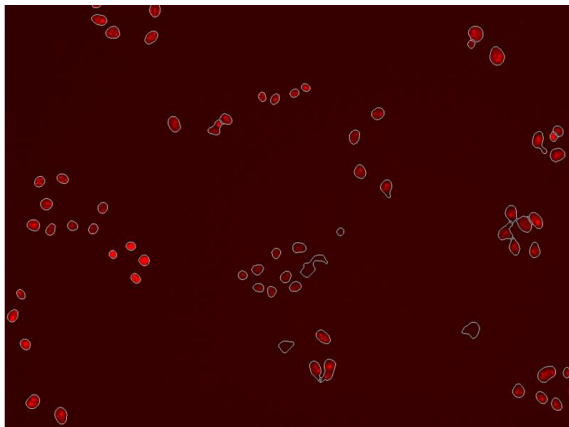
The numerical result will be displayed in the cell counting results table (changing the cycle number of a well will add one line, while applying different settings on the same well and cycle number will overwrite the numbers in the table):

Position	Cycle	GFP(n)	RFP(n)	DAPI(n)	GFI
A01_00001	00001	39	0	0	
A01_00001	00029	5	0	0	
B02_00001	00026	86	69	0	
B02_00001	00009	0	42	0	

Scrolling in the results table to the right will display the result of counted cells per mm<sup>2</sup>. The size of the imaged position in a well = one Field of View (FOV) depends on the objective used in the measurement: for

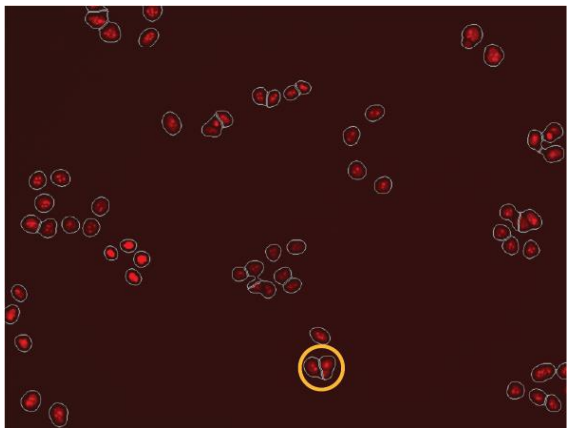
- 4x the size of one FOV is 3.55 mm<sup>2</sup>
- 10x the size of one FOV is 0.58 mm<sup>2</sup>
- 20x the size of one FOV is 0.14 mm<sup>2</sup>

Default settings, **Apply**

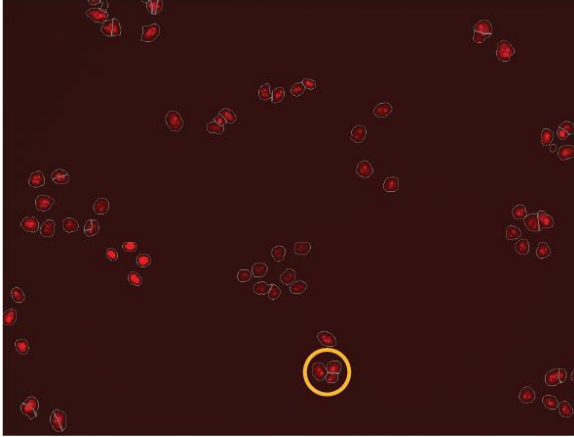


Applying the default settings to this image results in dim cells not being segmented and some falsely selected background signals:

**Background Control** checked

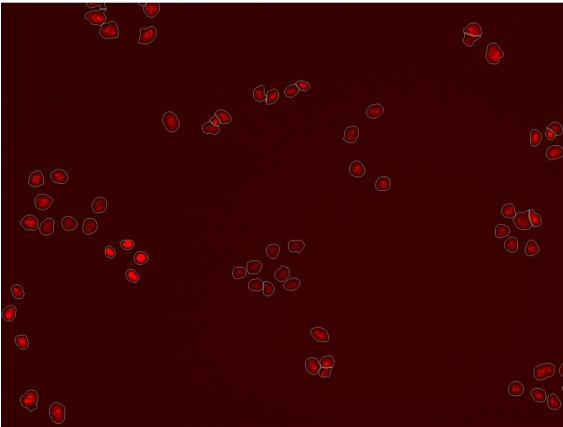


Using the **Background Control** (effecting an automatic background brightness correction) results in the false signal being excluded, but still some falsely fused (see yellow circle for example).

**Quick Mode** unchecked

Turning off **Quick mode** improved the cell separation, then falsely fused cell were separated (yellow circle). But in the example image, some cells were overseparated. (In **Quick mode**, pixels of an image are binned, thereby reducing the processing time of large data sets. Binning means that neighboring pixels are aggregated to a larger single pixel representing the mean intensity of the binned pixels.)

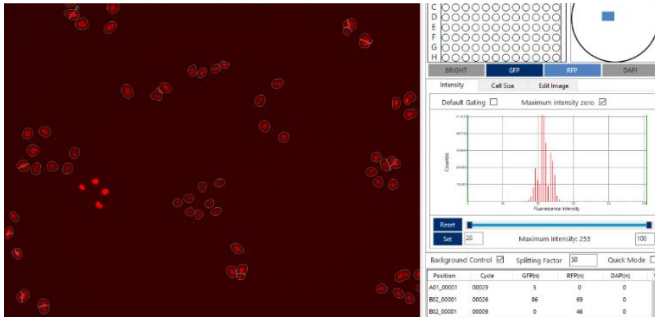
**Splitting Factor** changed from 25 to 50



Now, the splitting of cells in the overseparated area improved. The number of detected cells decreased with increased splitting factor.

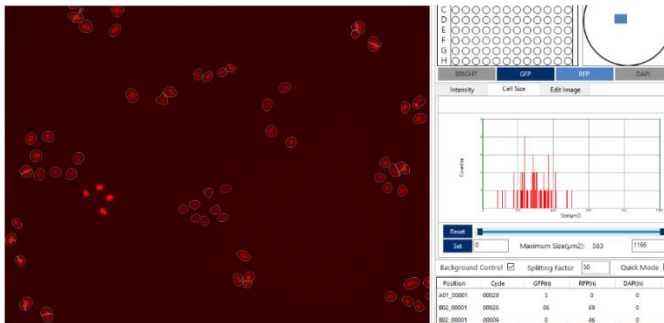
In order to exclude cells with low or high intensities from counting, an intensity gating/thresholding can be applied. A histogram (x-axis: fluorescence intensity, y-axis: number of objects) as well as a mouse hover information on cells pointed at with the mouse is available to support the decision process.

**Intensity** gating: Minimum intensity **Set** to 20, maximum intensity set to 100, then **Apply**



In the **Cell Size** tab, additional options to influence segmentation are available: thresholds to exclude certain cell sizes can be set. There is a mouse hover in image view which displays the cell size of the cell pointed at to support this decision process, next to a histogram display (x-axis: size in  $\mu\text{m}^2$ , y-axis: number of objects).

**Cell Size** set minimum and maximum, apply new value by clicking **Set** and **Apply** (In the example below, no gating value was applied).



Note: Finding the best combination of settings is an iterative process and should always be tested on representative images (different time points, positive and negative controls, etc.).

The remaining functions of the **Attached Cell Counting** module (**Analyze, View Results**) are identical to **Growth Curve** and described in chapter 0 “12.2.2.1 Growth Curve” in detail.

Note: If more than one position was acquired in a well, the readout [n/well] is equal to the sum of all cells counted in all positions in a well. For example: 3 positions were acquired with a 10x objective (0.58  $\text{mm}^2$  per FOV) in a 384 well plate. 180, 126 and 85 cells/image were counted. Then the mean would be 130 cells/image, and based on the mean there are 224 cells/ $\text{mm}^2$ . The sum would be 391 cells/well (=180+126+85).



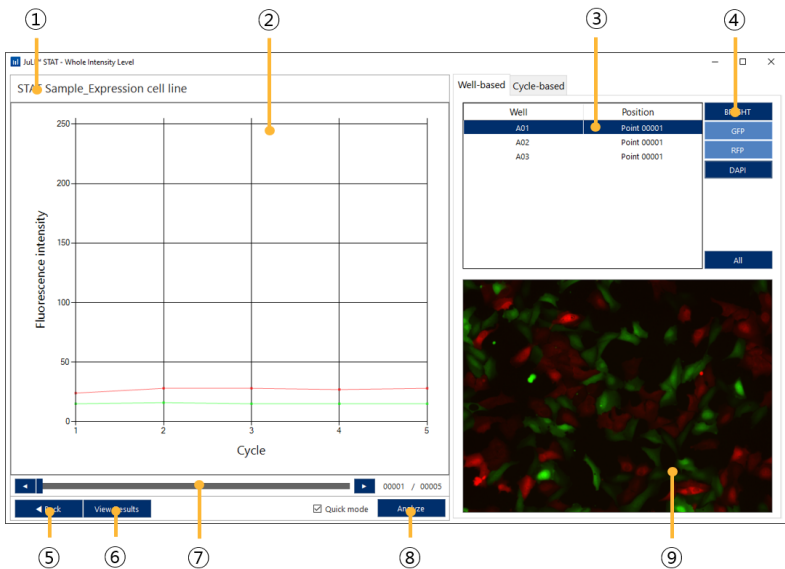
### 12.2.2.4 Whole Intensity Level



With the **Whole Intensity Level** analysis, the mean intensity of images in the GFP, RFP and DAPI channel can be quantified. The interface of this analysis module differs from the others as it displays results in the starting window and there are no parameters to influence the analysis.

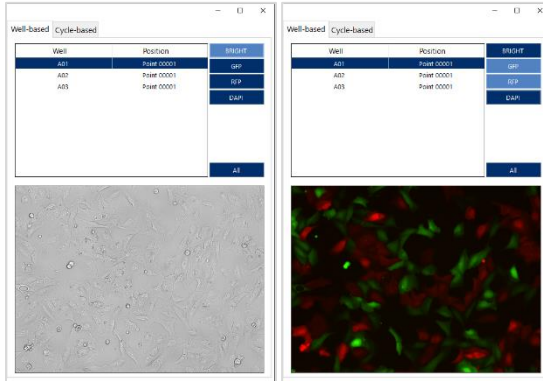
☞ Note: **Whole Intensity Level** is only displayed as an analysis option if a fluorescence channel was acquired during the measurement.

☞ Note: **Whole Intensity Level** cannot be calculated for the BRIGHT channel.

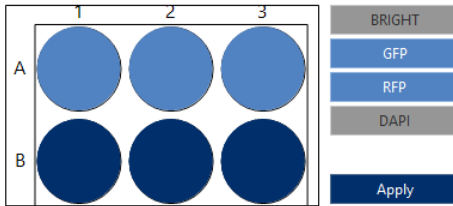


- ① Project / measurement name
- ② Result graph
- ③ Well list display
- ④ Channel selection
- ⑤ Back
- ⑥ View results
- ⑦ Time lapse slider
- ⑧ Analyze
- ⑨ Image view

Start by selecting a project/measurement and click **Select Analysis - Whole Intensity Level**: in the initial screen, images of all channels acquired (light blue) can be inspected separately or as overlay by selecting rows in the Well list ③ display in the **Well-based** tab and channels in the ④ Channels selection.



In order to analyze the mean fluorescence intensity per well, click **Analyze**, select wells and fluorescence channels followed by **Apply**.

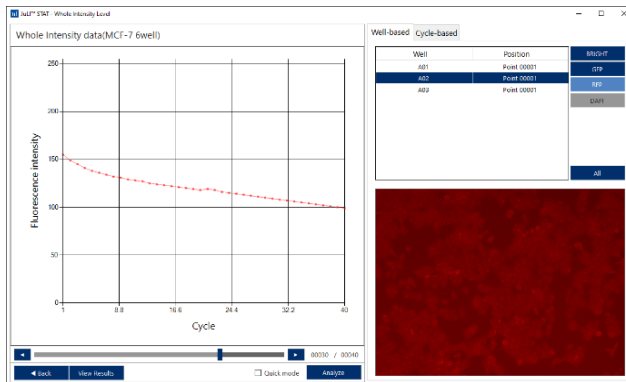


⚠ Note: Measurements acquired with more than one position per well can be displayed (named Point 00001, Point 00002, etc.) in the initial screen, but the analysis will only calculate mean values per well.

⚠ Note: **Quick mode** has neither influence on the analysis results nor the analysis speed.

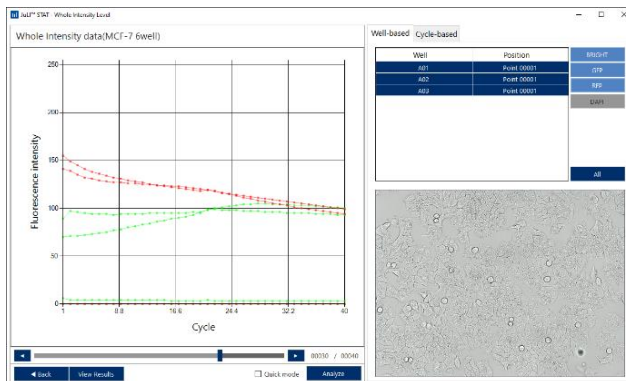
## Well-based result display

The analysis results can be viewed instantly by selecting a well in the well list (dark blue) and one or more channels (dark blue).



When the time lapse slider is moved, the image of the selected channel(s) at exactly this time point will be displayed.

Also, multiple rows in the well list can be selected and displayed by holding the control or shift key while clicking the rows or clicking **All**:



A mouse hover will display the well coordinate, cycle number and mean intensity per well and channel.

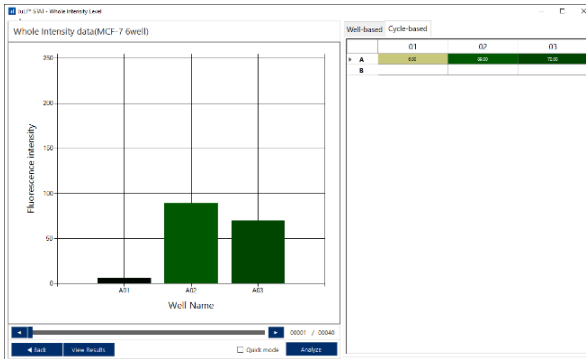
Note: When scrolling through single wells on the well list using the arrow keys of the keyboard, the intensity plots will not be refreshed accordingly. Please click the rows in the list with the mouse button for a correct display of the curves.

## Cycle-based results display

In the **Cycle-based** display, graphs and numerical data are displayed as bar chart next to a well-based display, using heatmap coloring: the darker the color the lower

the value. By moving the time lapse slider, different time points can be displayed, but only one channel at a time:

GFP channel, time point 0



GFP channel, time point 40



[RFP channel, time point 0]



The export option to display results in ready-to-use graphs and exportable numerical results are found under **View Results**. Please refer to chapter 0 “12.2.2.1 Growth Curve” to see details about the **View Results** functionalities.

## 12.2.3 Plate Editor

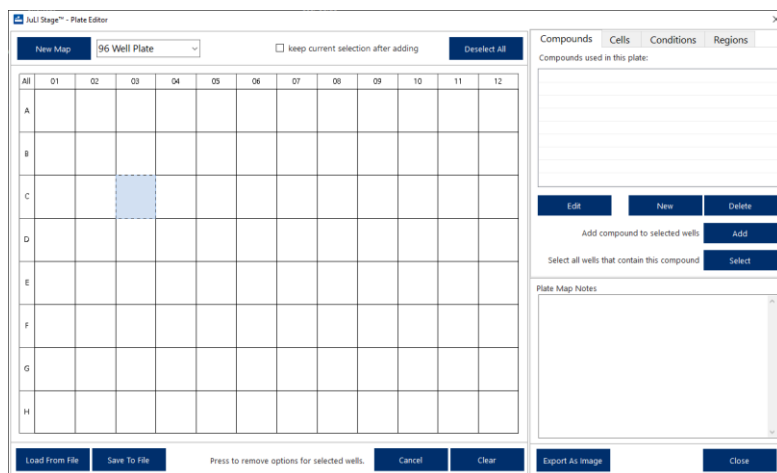


With **Plate Editor**, detailed experiment conditions of the selected measurement project can be defined and saved in plate maps: each well can be assigned to specific assay conditions (such as cell type and number, compound concentration etc.) in order to calculate e.g. mean values of triplicates and their standard deviations.

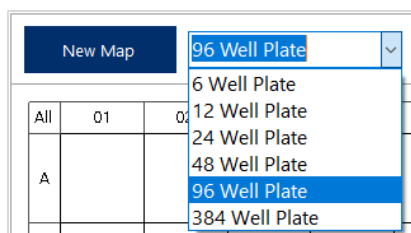
There are two ways to create and save plate maps: either via the **Plate Editor** icon or in the **Plate Graph** tab of the **View Results** section of each analysis module.

### Creating a plate map step-by-step:

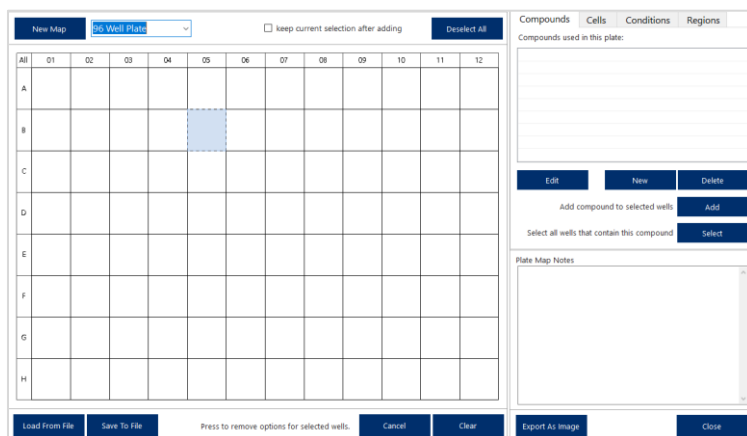
Start by clicking the **Plate Editor** icon to open it:



The first step is selecting a plate type out of 6 options via a drop down menu, followed by clicking **New Map**:



For example, a 96 well plate was selected:



There are 4 tabs available to allocate and describe detailed conditions for each well of a plate: **Compounds**, **Cells**, **Conditions** or **Regions**. (Headers of these tabs cannot be changed.)

Compounds   Cells   Conditions   Regions

- **Compounds:** Name, concentration and dilution series of compounds can be entered and assigned to wells.
- **Cells:** Cell types, passage number, seeding density and dilution series can be entered to wells.
- **Conditions:** Can be used to assign any other important information to wells.
- **Regions:** Wells can be bundled to regions, which will ease the selection of wells in the other tabs.

The first 2 tabs offer additional sub-menus in separate windows to support and ease filling in concentrations or cell numbers, however, there are two important facts to consider:

- Firstly, all entries of all tabs will appear in the header of result tables and on the legends of graphs.
- And secondly, concentrations of compounds are neither selectable on the x-axis of graphs nor can they be exported and accessed in secondary analyses such as IC<sub>50</sub> calculations.

All plate map entries are only aiming at

- localizing well repeats in order to calculate mean values and standard deviations or
- the detailed description of conditions of certain wells.

☞ Note: In order to allocate well-repeats in a plate layout (e.g. to calculate mean values of triplicates) it is not necessary to fill in all 4 tabs, one tab (compounds, cells or conditions) is already sufficient.

Therefore, we start creating an example plate map with one tab only and then inspect its influence on a graph.

Click on **New** in the **Conditions** tab and enter the description Negative control into both fields – **Display Name** (will appear in the wells) and **Description** (will appear in the list). Select a dilution color and click **Apply** to confirm your entries:

Add Condition ✖

Display Name

Description

Dilution Color

Apply

The new entry appears in the list of the **Conditions** tab:

Compounds Cells Conditions Regions

Media, coatings and other growth conditions:

Neg. Ctrl.

Continue by clicking **New** once more and enter the name Compound A with a different color to the list:

Add Condition ✖

Display Name

Description

Dilution Color

Apply

Now, two entries are displayed:

Compounds Cells Conditions Regions

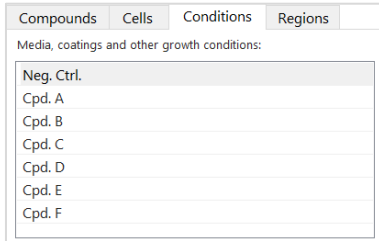
Media, coatings and other growth conditions:

Neg. Ctrl.

Cpd. A

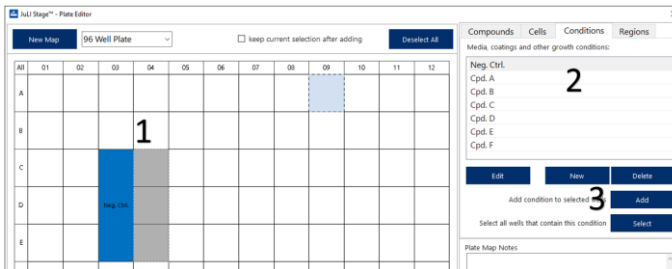


Continue, until 7 conditions are defined using different colors:

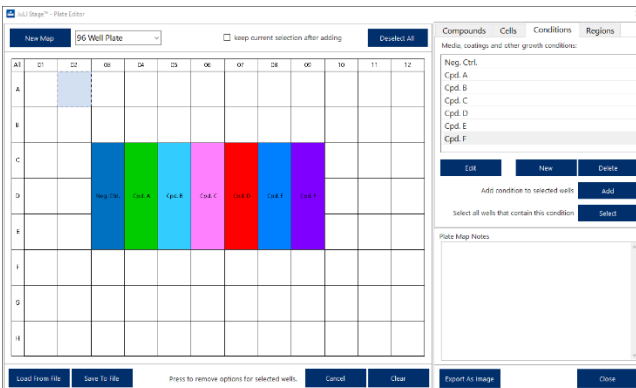


In order to assign each condition to three selected wells each:

1. Mark the wells belonging to a condition by either clicking on single wells or drawing a rectangle with the mouse to mark multiple wells. Selected wells turn gray.
2. Then mark the line of e.g. Compound A.
3. Click **Add**.

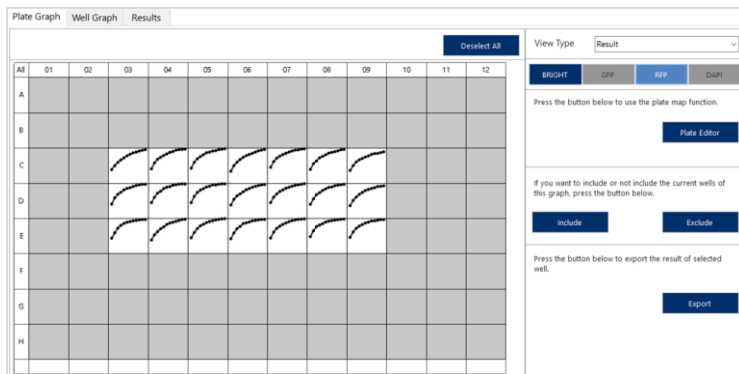


Repeat these 3 steps, this time assigning the remaining compounds to 3 wells each:

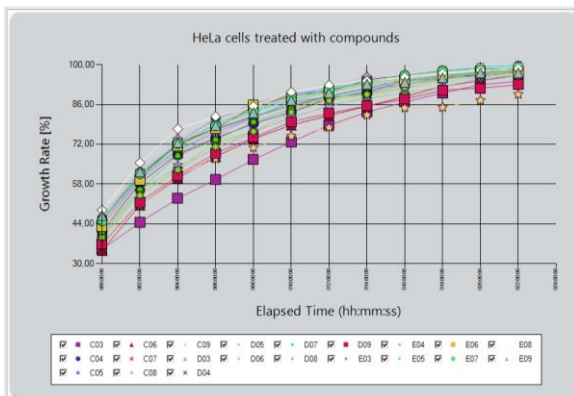


Click **Save To File** to save the plate map to a folder of your choice.

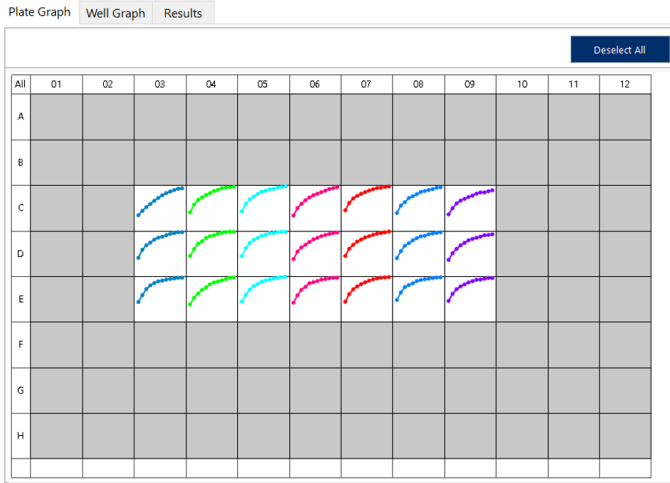
Now, we can compare the results of an existing analysis with and without plate map: Via **Select Analysis** and **View Results** open a **Plate Graph**. Graphs of the analysis will be displayed in one color:



Click on **Export**: 21 curves belonging to 21 wells are displayed each in an individual color:

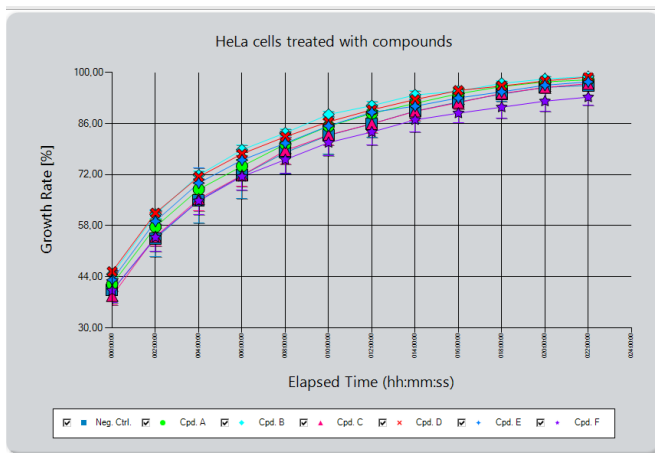


In order to apply the created plate map, close the **Export** window again, and in the **Plate Graph** tab click **Plate Editor – Load From File**. Open the **.StageMap** file and click **Apply**:



The colors of the displayed curves instantly change according to the position of triplicates.

Click **Export** again: Now, instead of 21 curves only 7 curves are displayed, showing the mean of triplicates and their standard error; also, the legend entries display the compound names from the plate map.



Note: The plate map belonging to a measurement is not attached permanently to a measurement but needs to be loaded again every time the analysis is opened.

Up until now we only used 1 of totally 4 tabs in the Plate Editor, the **Conditions** tab.

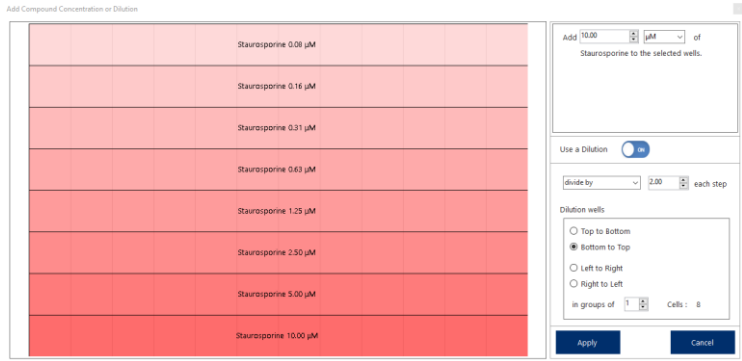
There are 3 more tabs which can be useful to set up a plate map: **Compounds**, **Cells** and **Regions**.

Entries to the **Compounds** tab need to follow the same series of clicks as described for **Conditions**, however, there is an additional sub-menu in a separate window which assists the setup of a compound dilutions:

1. Click **New** to enter the compound name followed by **Apply**.

2. Mark wells belonging to the compound by either clicking on single wells or drawing a rectangle with the mouse to mark multiple wells. Or click **All** in the upper left corner to select all well (reverse or delete selections with **Deselect All**). Selected wells turn grey.
3. Then click Staurosporine in the compound list:

4. Click **Add**. This will open the compound sub-menu window where the concentration, it's unit and a dilution series (Dilution-toggle button switched **ON**) can be entered. The dilution series can be defined row- or column-wise. Here for example a bottom to top dilution, with the highest concentration of 10  $\mu\text{M}$  diluted step wise by a factor 2, one row per concentration, was defined:



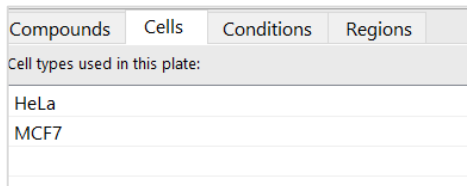
Another layout option would be to reduce the concentration by a factor of 10, from left to right, combining 3 columns into a group (in groups of 3). (The column- or row-wise grouping can only result in groups with integer numbers.)



5. By clicking **Apply** the new layout will be applied to the wells.

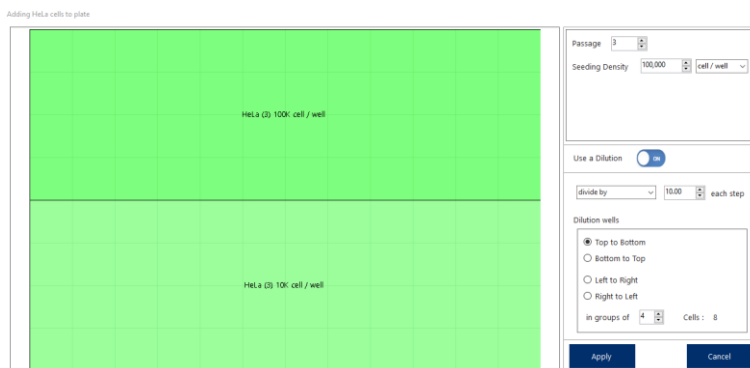
Furthermore, different cell types can be used in this layout. For that, the **Cells** tab offers layout options in a sub-menu as well.

1. Open the **Cells** tab, click **New** to enter e.g. two new cell types, followed by **Apply**.



2. Mark wells belonging to cell type HeLa by either clicking on single wells or drawing a rectangle with the mouse to mark multiple wells. Or click **All** in the upper left corner to select all well (reverse or delete selections with **Deselect All**). Selected wells turn grey.

- Then click HeLa in the cell list and click **Add**: this will open a new window - the **Cells** sub-menu. Here the Passage number, the Seeding Density and it's unit, as well as a dilution similar to the **Compounds** tab can be used to enter details of the plate map:

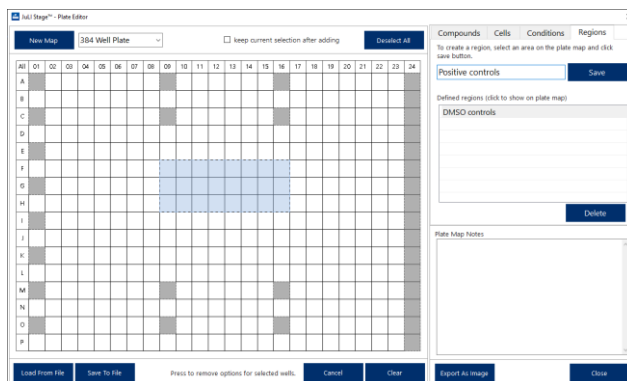


- This can be repeated for all 3 tabs and the final layout will contain all information at a glance: compound name, concentration, cell type, seeding density, and condition.

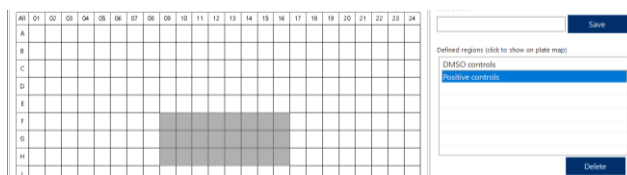
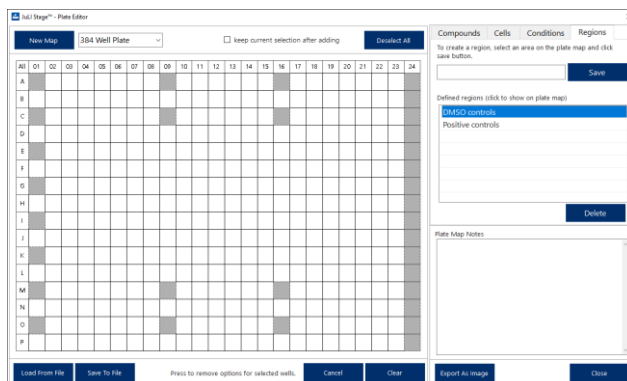
All	01	02	03	04	05	06	07	08	09	10	11	12
A	Staurosporine 0.01 µM HeLa (3) 100k cell / well						Staurosporine 0.01 µM MCF7 (3) 10k cell / well					
B												
C	Staurosporine 0.10 µM HeLa (3) 100k cell / well						Staurosporine 0.10 µM MCF7 (3) 10k cell / well					
D												
E	Staurosporine 1.00 µM HeLa (3) 10k cell / well						Staurosporine 1.00 µM MCF7 (3) 100k cell / well					
F												
G	Staurosporine 10.00 µM HeLa (3) 10k cell / well						Staurosporine 10.00 µM MCF7 (3) 100k cell / well					
H												

The tab **Regions** can be used to mark specific wells to bundle them into regions, which will ease the selection of wells in the other tabs.

For example, a 384 well plate contains DMSO and positive controls at defined positions. In order to easily mark them and use them in the other tabs, mark all wells belonging to a group and **Save** them under a name:



Then click on one region in the regions list and wells belonging to the regions will be marked accordingly.



When the tab is changed to e.g. **Compounds**, the wells belonging to a region can be edited more easily.

**The Select button** can be used to quickly visualize compound/cells or conditions areas in more complex plate maps: clicking on a row in the cell/compound/condition list and then **Select** will mark all wells belonging to the selection.

Select all wells that contain this compound Select

Select all wells that contain these cells Select

Once the tab is changed exactly these marked wells can be assigned a different layout information. However, after clicking **Add** the well marks will disappear unless the box **keep current selection after adding** was checked: then the formerly marked wells will be available after a further tab change:

keep current selection after adding

Button **Export As Image**:

Export As Image

- A plate map can be saved as an image in .jpg-format to be pasted in presentations or lab protocols.

Buttons **Cancel** and **Clear**:

Cancel Clear

- **Cancel** will delete all labels of selected wells.
- **Clear** will delete all labels of a plate.

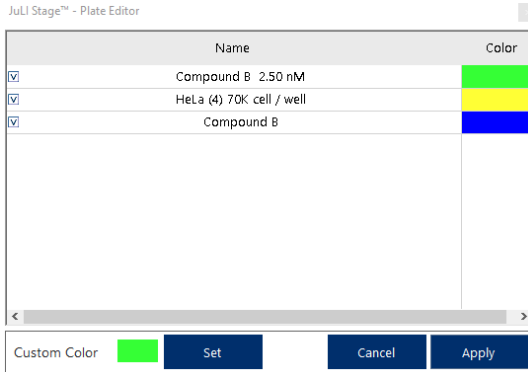
Buttons **Edit** and **Delete**:

Edit Delete

- **Edit** will enable the change of an entry before it was added to selected wells via **Add**. Once the entry was added to a plate map, any changes via **Edit** are ineffective.
- **Delete** will remove a selected entry from the list. However, once the entry was added to a plate map, Delete will not remove the entry from the plate layout.



Right clicking a specific well will open a window which enables the color change of single wells.



# JuLI™ Stage

Real-time live cell imaging system

NESMU-JST-001E (V.2.5)



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