JuLI™ Stage

Real-time live cell imaging system

User Manual

NanoEntek

JuLI™ Stage User Manual
Website: www.nanoentek.com
E-mail: sales@nanoentek.com

🏭 **Manufactured by**

NanoEntek, Inc.

851-14, Seohae-ro, Paltan-myeon, Hwaseong-si, Gyeonggi-do, 18531, Korea

Tel: +82-2-6220-7940

Fax: +82-2-6220-7999

NanoEntek America, Inc.

220 Bear Hill Road, Suite 102, Waltham, MA 02451, USA

Tel: +1-781-472-2558

Fax: +1-781-790-5649

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

Firmware and software changes and updates may change without prior consent or notification.

Documentation: **NESMU-JST-001E**

Revision history:

V.0.0	OCT	2014
V.0.5	MAY	2015
V.1.0	SEP	2015
V.1.5	MAR	2016
V.2.0	MAY	2017
V.2.5	MAR	2023
V.2.6	**OCT**	**2025**

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)
CE	This instrument and consumables conforms to the EC Declaration of Conformity.
FC	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. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. 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.

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.

JuLI™ Stage

1x

Control Box

2x

Objective lens (4x, 10x, 20x)

3x

Protective covers

2x

Connection Cable

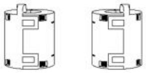
2x

Repeater, 5m

2x

Ferrite core

2x



Regional power cord & Adaptor

1 SET

JuLI™ Stage Installaion Guide

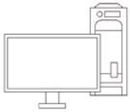
1x

JuLI™ Stage User manual

1x

JuLI™ Stage Desktop PC

1x



3 Description

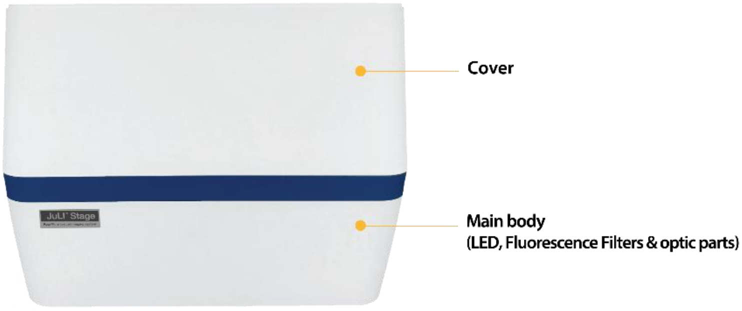
3.1 Front view

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.



[Front view]

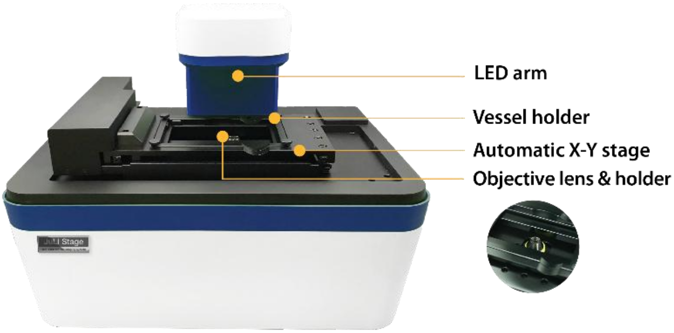
3.2 Side view

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

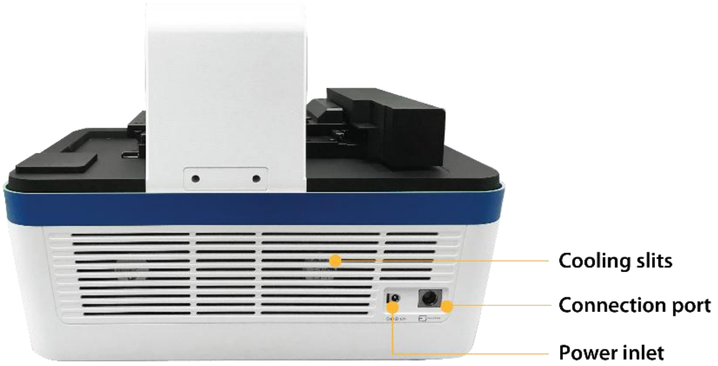
The JuLI™ Stage ventilates the internal parts using 2 fans behind the slits on the back side. Please make sure that the cooling system can operate freely.

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.



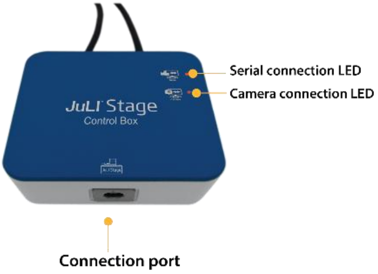
3.4 Control box

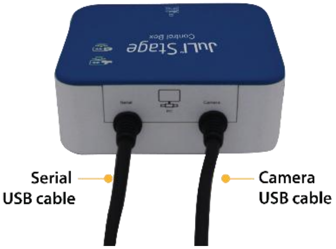
Serial and camera USB cables

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.

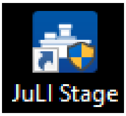
3. Plug the white connecting cable into the control box using the end of the cable *without* the ferrite core.

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.

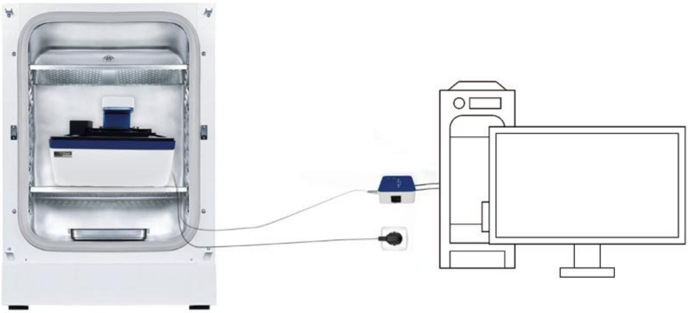


! **First use**: Before operating the JuLI™ Stage system for the first time, we strongly recommend allowing the instrument to equilibrate to room temperature for *20 minutes* outside the incubator while the device is turned on.

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.

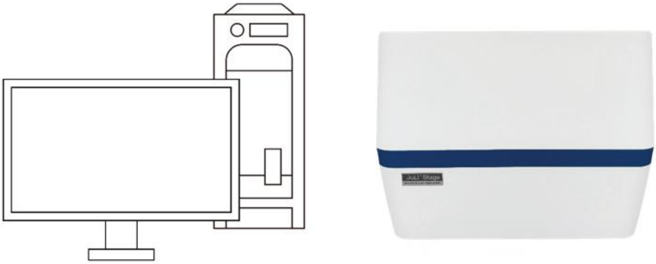
⚠ IMPORTANT!

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℃ to 25℃. Temperature outside this range may affect the performance of the JuLI™ Stage.

The lid of the well plate should be wetted with cell culture media before monitoring.

This step is recommended to prevent condensation from forming inside the lid of the well plate during 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

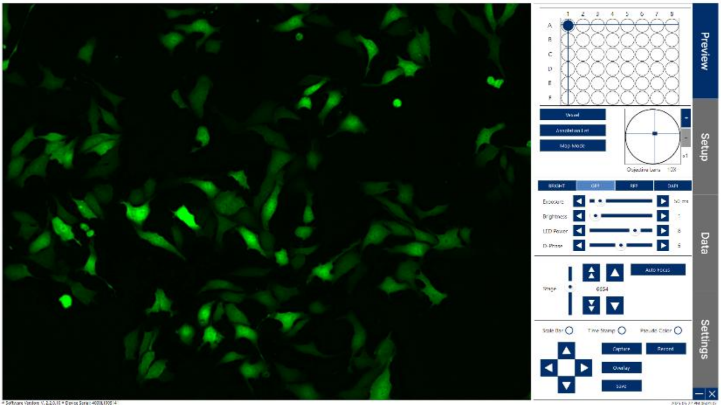
If the control PC is connected to the internet, the Windows® operating system is automatically updated (and rebooted if necessary). In order to avoid errors during operation, we recommend:

- 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

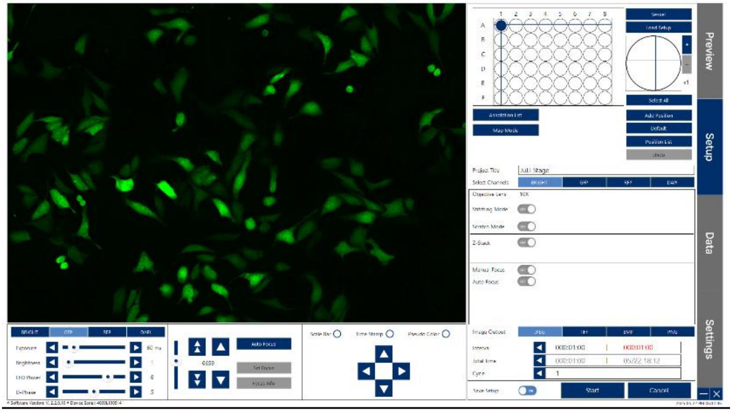
Preview menu

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



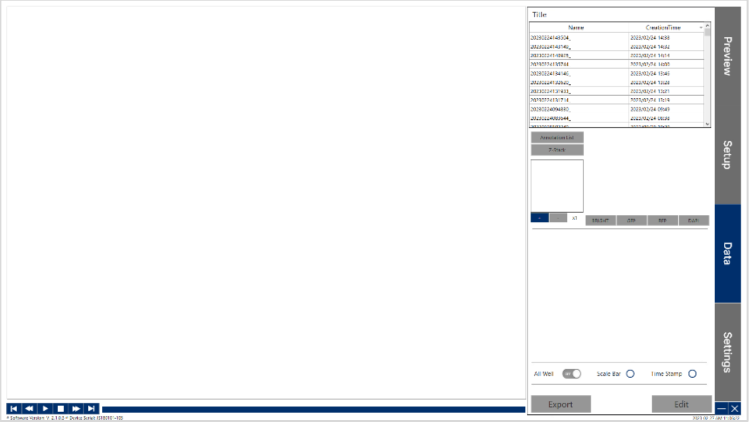
Setup menu

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.



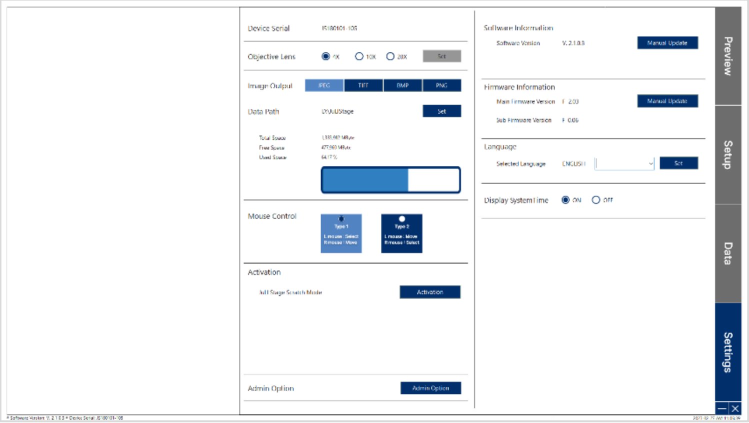
Data menu

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).



Device Serial IS180101-106

Objective Lens ● 4X ○ 10X ○ 20X

Data Path D:\JuLIStage

Total Space 1,333,982 MByte
Free Space 477,960 MByte
Used Space 64.17 %

Software Version V. 2.1.0.3

Main Firmware Version F 2.03

Sub Firmware Version F 0.06

Selected Language ENGLISH

Display SystemTime ● ON ○ OFF

6 *Basic operation*

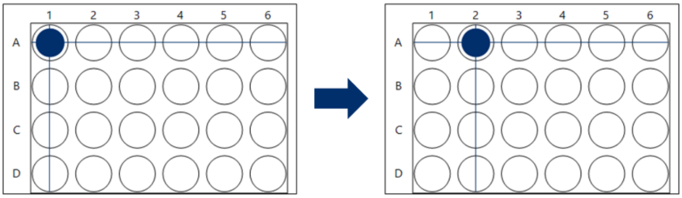
The JuLI™ Stage is controlled by a PC (monitor resolution 1920x1080) via wireless mouse and keyboard.

☞ 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

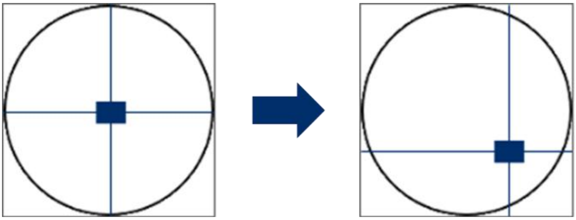
In both the **Preview** and **Setup** menu you can move to the center of a well by clicking it using the right mouse button.



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.

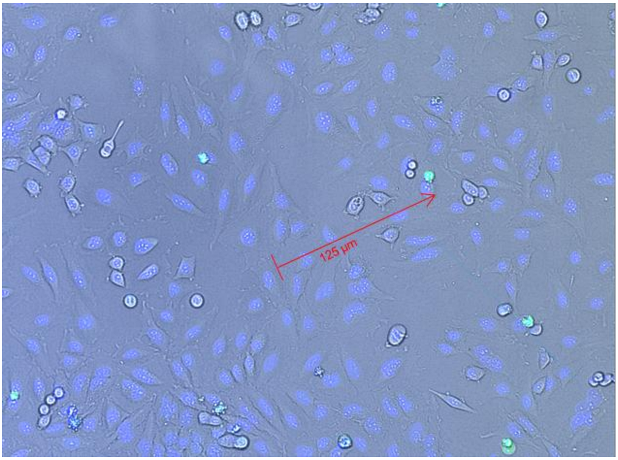


The selected position is marked by a blue square and is equivalent to the field of view.

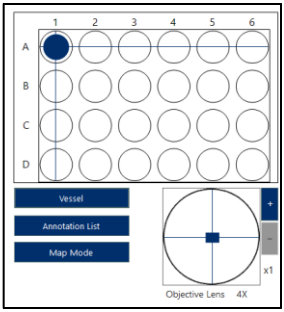
☞ Note: The field of view for the 4x, 10x, and 20x objectives is 3.55 mm^2, 0.58 mm^2, and 0.14 mm^2, respectively.

Well navigation using the image

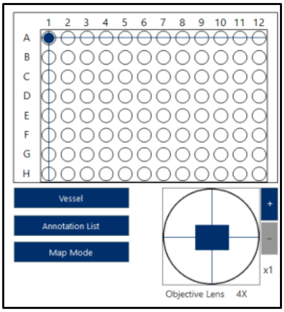
Clicking the right mouse button and dragging over the image will move the current position towards the direction of the arrow in the **Preview** and **Setup** mode. The moving distance is indicated beside a red line as shown below.



☞ 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.

Please wait while the stage is moving.

6.2 Selection

Well selection

Left mouse

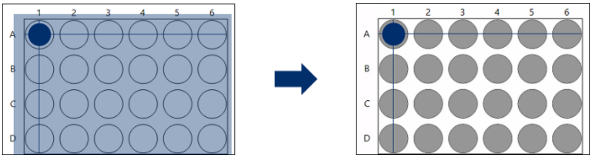
A well can be selected by left click which will highlight the well with a grey color. Clicking the well a second time will de-select it.

☞ 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.



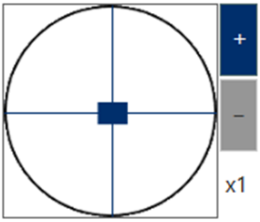
Selecting additional positions

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.

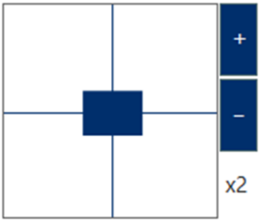
☞ Note: Selecting a well and positions within this well without clicking **Add Position** will result in these positions <u>not</u> being included in the imaging experiment.

6.3 Position zoom

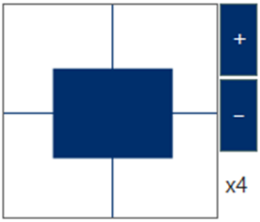
You can zoom in or out of the position clicking **+** or **–** on the right side of the well-representation.



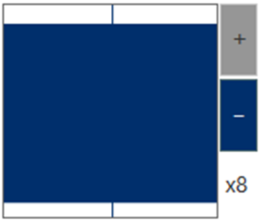
1X zoom in



2X zoom in



4X zoom in



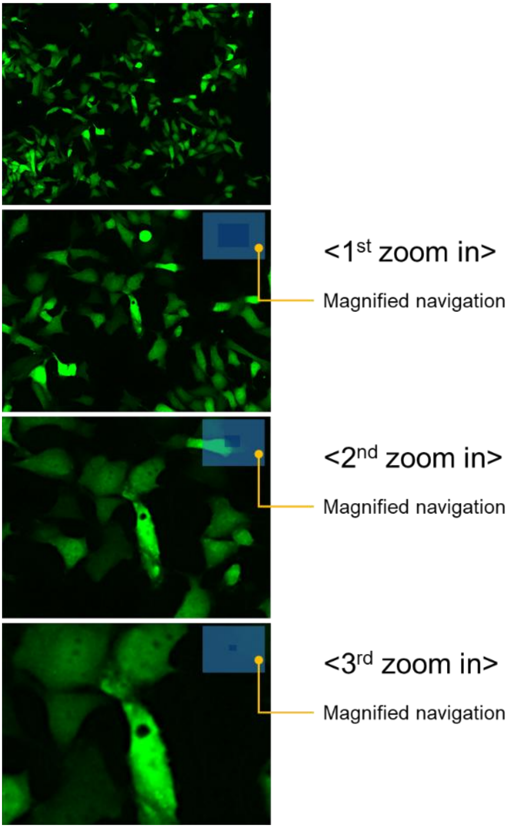
8X zoom in

6.4 Mouse wheel

Mouse wheel
(Up and down)

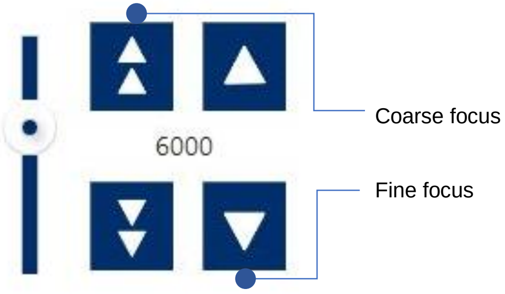
Zoom-in and out

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



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.

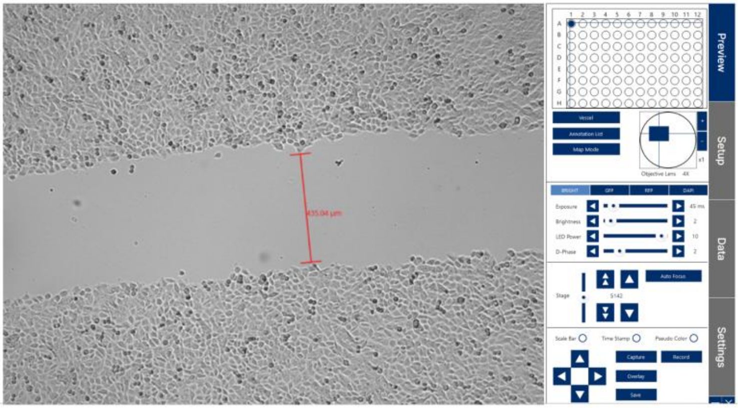


☞ Note: The coarse focus is not supported by the mouse wheel.

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.

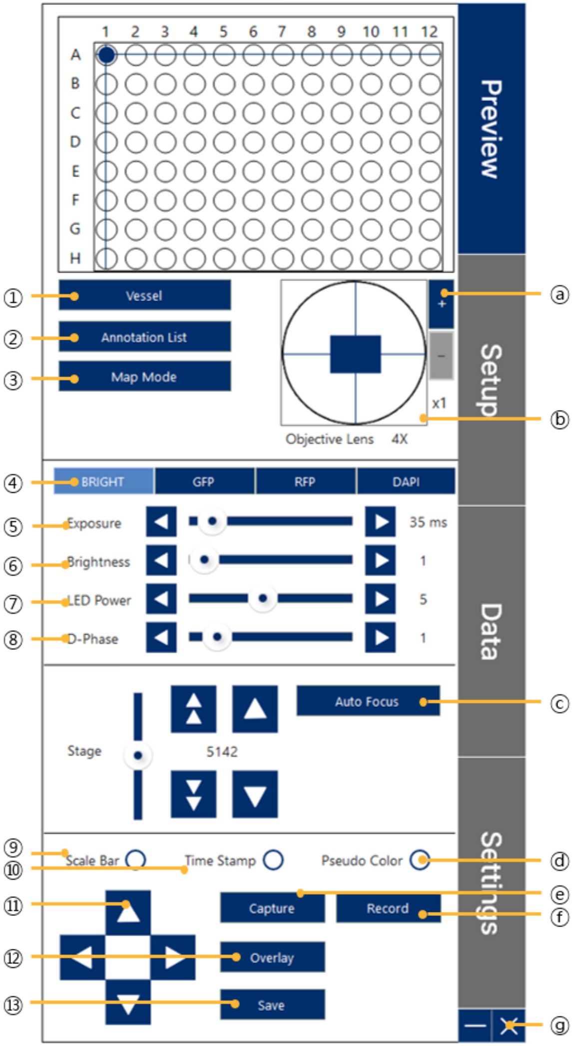
☞ Note: The size measurement function can be used in both the **Preview** and **Setup** menu.



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

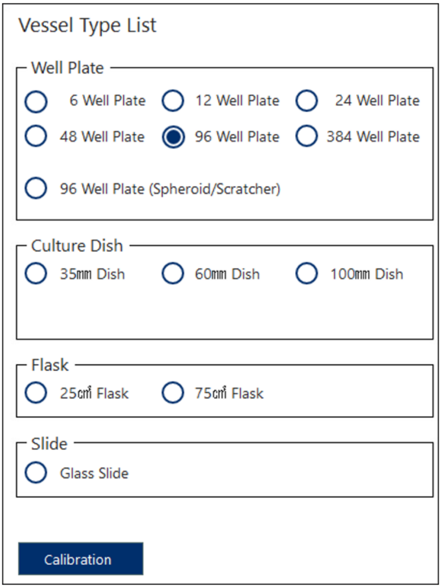
⑪ XY direction control
⑫ Image overlay button
⑬ Image save button
ⓐ Well zoom in & out
ⓑ Well navigation
ⓒ Auto focus button
ⓓ Pseudo color button
ⓔ Capture image
ⓕ Record short movie
ⓖ Minimize & close buttons

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

○ 35㎜ Dish ○ 60㎜ Dish ○ 100㎜ Dish

Flask

○ 25㎠ Flask ○ 75㎠ Flask

Slide

○ Glass Slide

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.

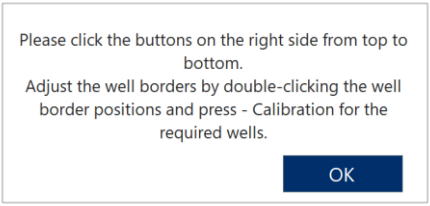
Vessel calibration

1. Click **Calibration**.

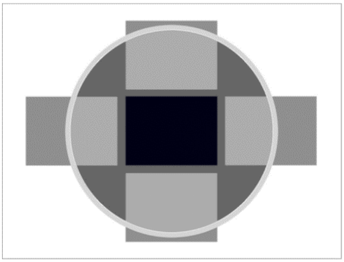
2. Follow the calibration guide on the right from top to bottom.
   a) Click **Move to A01 (or B02)** and **Check A01 (or B02).** After the well borders were captured set the red calibration lines onto the well borders by double clicking. Then click **Calibration A01 (or B02)** to confirm the new definition.
   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.



Please click the buttons on the right side from top to bottom.
Adjust the well borders by double-clicking the well border positions and press - Calibration for the required wells.



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

☞ 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.

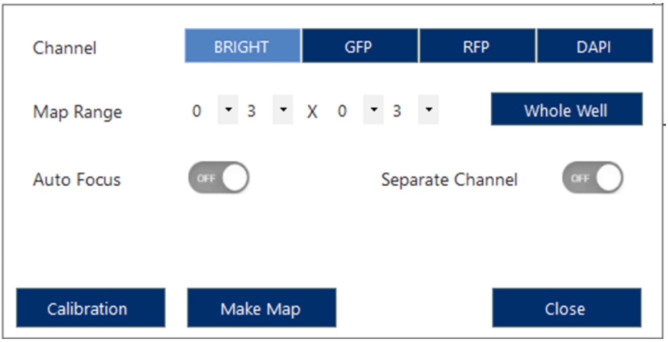
5. Save or load the plate information that has been calibrated with the **Import** and **Export** button.

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



Map Range 0 3 X 0 3

Auto Focus OFF

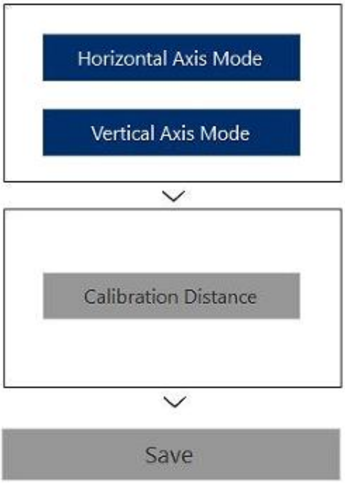
Separate Channel OFF

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. Select **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 : 1 - 40
- LED power range : 1 - 10
- D-Phase range: 0 - 10

Exposure 35 ms
Brightness 1
LED Power 5
D-Phase 1

☞ 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.


Scale Bar ●

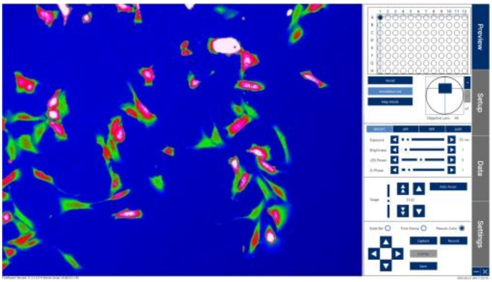
Time Stamp

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.


Time Stamp ●

Pseudo color

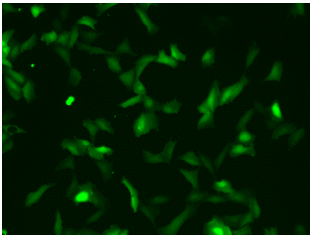
During the adjustment of acquisition conditions, the option **Pseudo Color** can help to visualize image illumination. Dark pixels are blue which change to green, red, and finally white for saturated pixels.




Pseudo Color ●

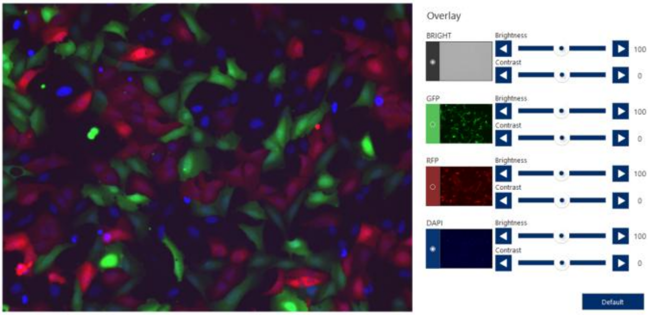
Image snapshots

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



2. Repeat for all desired channels.

3. Click **Overlay** and edit your images if necessary



4. Activate any combination of channels by clicking the white dot on the left side of the small images.
5. 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.


JuLI Stage™

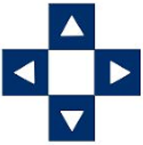
05 Min 00 Sec

Elapsed Time: 000:00:00

☞ 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.



☞ 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

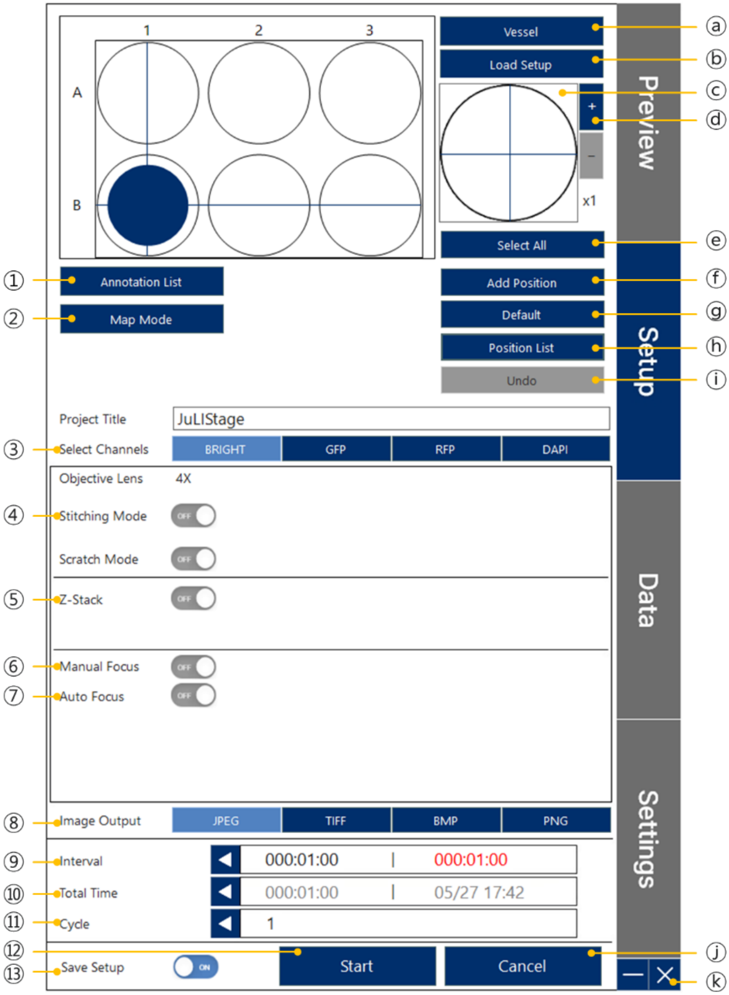
Minimize or close the control software.

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.



Project Title: JuLIStage

Objective Lens: 4X

Interval: 000:01:00 | 000:01:00

Total Time: 000:01:00 | 05/27 17:42

Cycle: 1

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 Interval time (time lapse)

⑩ Total time (time lapse)

⑪ Cycle number (time lapse)

⑫ Start experiment

⑬ Save setup toggle button

ⓐ Vessel calibration (P)

ⓑ Load experimental setup

ⓒ Well navigation (P)

ⓓ Well zoom in & out (P)

ⓔ Select all wells button

ⓕ Add position button

ⓖ Default button

ⓗ Position button

ⓘ Undo button

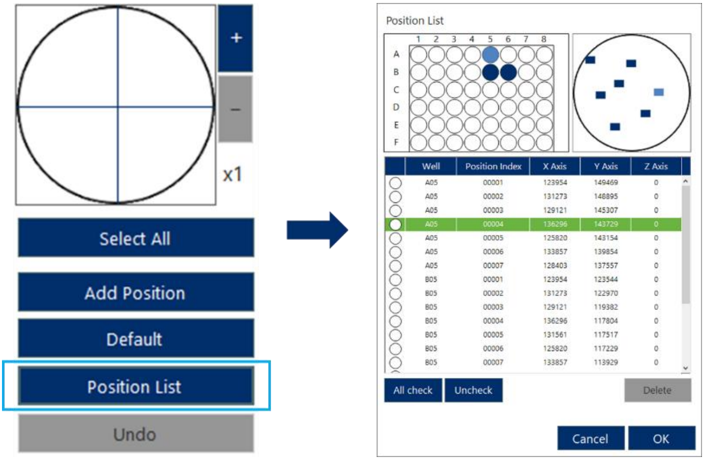
ⓙ Cancel button

ⓚ Minimize & close button (P)

Navigation position set

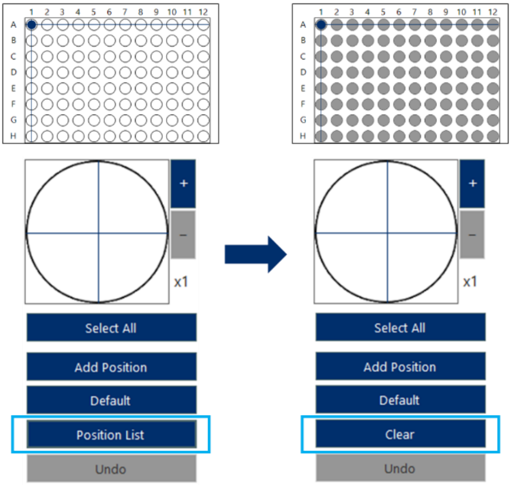
- To select all wells click **Select All**.
- After selecting one or multiple wells, clicking **Add Position** will add the current position of each selected well to the **Position list**.
- There are two ways to proceed if more than one position inside a well is supposed to be measured in only selected 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.
  - Positions can be selected from the Plate and Well navigation views or from the Position Table below, and the selected position is highlighted in both.
  - To delete specific positions, check the circles next to the positions you want to remove, then click the **Delete**.



	Well	Position Index	X Axis	Y Axis	Z Axis
○	A05	00001	123954	149469	0
○	A05	00002	131273	148895	0
○	A05	00003	129121	145307	0
○	A05	00004	136296	143729	0
○	A05	00005	125820	143154	0
○	A05	00006	133857	139854	0
○	A05	00007	128403	137557	0
○	B05	00001	123954	123544	0
○	B05	00002	131273	122970	0
○	B05	00003	129121	119382	0
○	B05	00004	136296	117804	0
○	B05	00005	131561	117517	0
○	B05	00006	125820	117229	0
○	B05	00007	133857	113929	0

- **Clear** will delete all positions from all selected wells (enabled only when wells are selected).

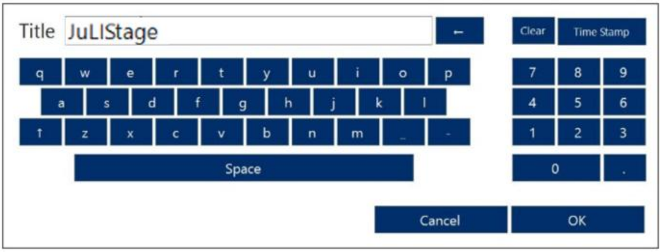


Project Title

Project Title: JuLIStage

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.

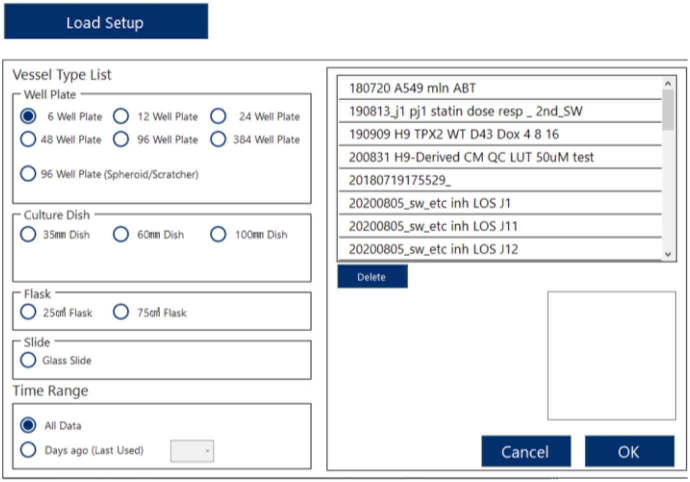


Title: JuLIStage

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.



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

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 3 x 0 3

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

☞ Note: Stitching and Z-stack acquisition cannot be used at the same time. Selecting one will automatically disable the other.

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 µm – 20 µm.

Z-Stack ON

# of Images 3

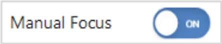
Depth 10.5 µm

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

☞ Note: Stitching and Z-stack acquisition cannot be used at the same time. Selecting one will automatically disable the other.

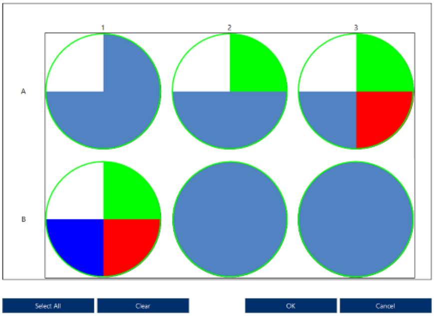
Manual Focus

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



Manual Focus ON

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.
    - A4: Brightfield, GFP, RFP and DAPI have a set focus.



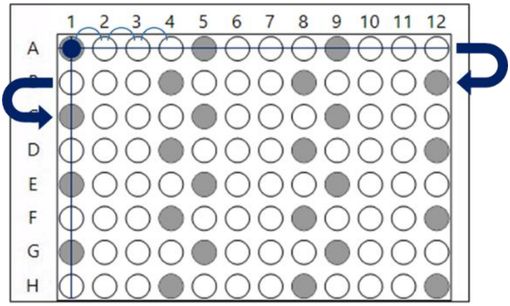
Auto Focus

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.

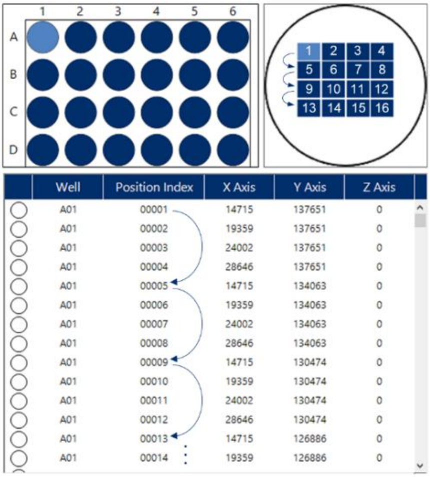
Auto Focus ON 4



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.

☞ Note: The position index is assigned based on the X and Y coordinates.

All Positions ON 4



	Well	Position Index	X Axis	Y Axis	Z Axis
○	A01	00001	14715	137651	0
○	A01	00002	19359	137651	0
○	A01	00003	24002	137651	0
○	A01	00004	28646	137651	0
○	A01	00005	14715	134063	0
○	A01	00006	19359	134063	0
○	A01	00007	24002	134063	0
○	A01	00008	28646	134063	0
○	A01	00009	14715	130474	0
○	A01	00010	19359	130474	0
○	A01	00011	24002	130474	0
○	A01	00012	28646	130474	0
○	A01	00013	14715	126886	0
○	A01	00014	19359	126886	0

☞ 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.

Every Cycle AF: Activate **Every Cycle AF** to use the auto focus in each cycle. This helps maintain proper focus, but may increase photobleaching or phototoxicity. It may also result in incorrect focus, so use it only when focus shifts are expected in each cycle.

Every Cycle ON

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

Extended Range OFF

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.

Quick Mode OFF

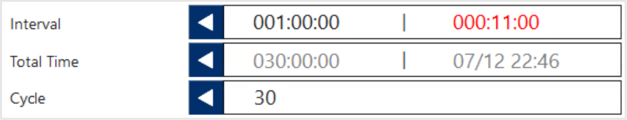
Image Output

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

Image Output JPEG TIFF BMP PNG

Time lapse configuration

The time lapse can be configured by setting the interval first, followed by either the total time or the cycle number, with the remaining value being automatically determined based on the entered parameters.

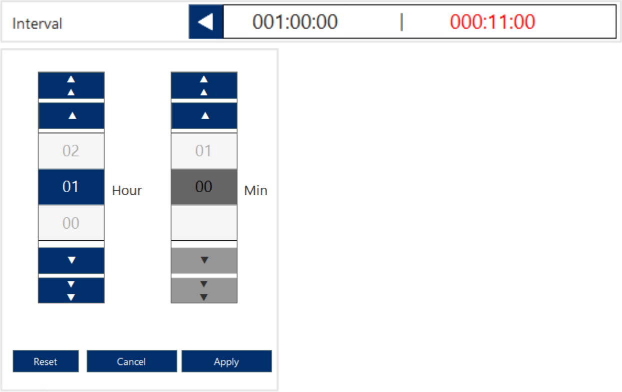


Interval 001:00:00 | 000:11:00

Total Time 030:00:00 | 07/12 22:46

Cycle 30

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

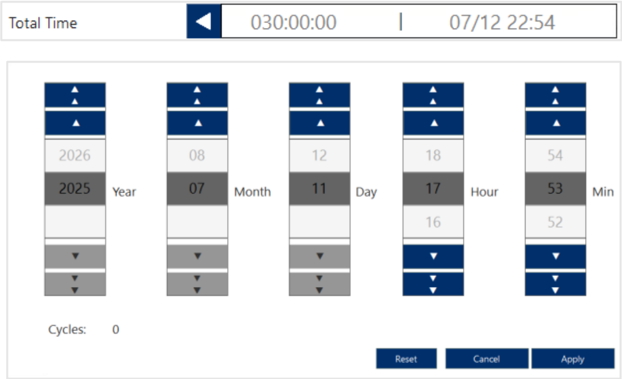


Interval 001:00:00 | 000:11:00

Hour: 02, 01, 00

Min: 01, 00

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



Total Time 030:00:00 | 07/12 22:54

Year: 2026, 2025

Month: 08, 07

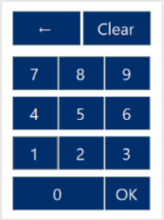
Day: 12, 11

Hour: 18, 17, 16

Min: 54, 53, 52

Cycles: 0

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



☞ Note: While the instrument operates, you can modify the cycle number by click cycle number display box. Please refer to page 50 for more details.

☞ 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

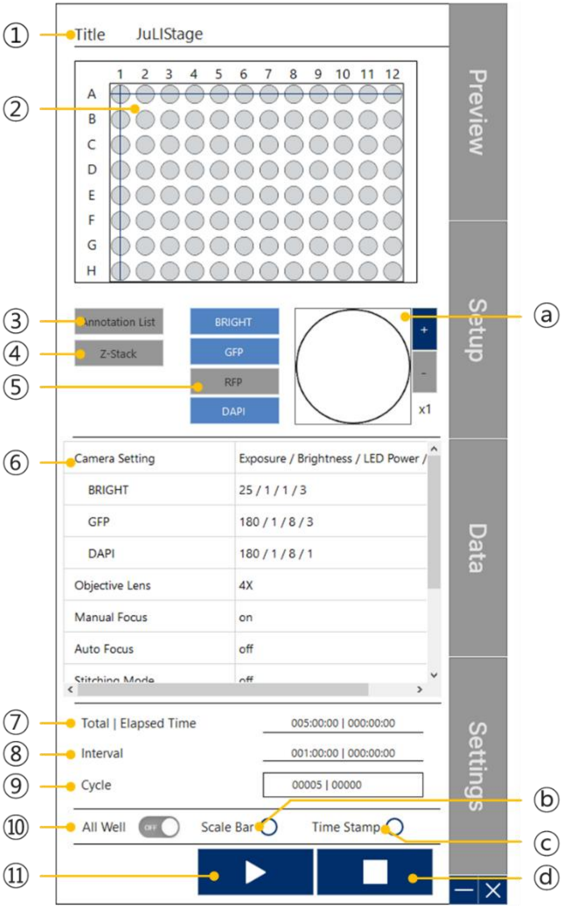
To reset all parameters click **Cancel**.

⚠ 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 lid of the well plate should be wetted with cell culture media before monitoring. This step is recommended to prevent condensation from forming inside the lid of the well plate during monitoring.

Experimental overview

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



Title JuLIStage

Camera Setting	Exposure / Brightness / LED Power /
BRIGHT	25 / 1 / 1 / 3
GFP	180 / 1 / 8 / 3
DAPI	180 / 1 / 8 / 1
Objective Lens	4X
Manual Focus	on
Auto Focus	off
Stitching Mode	off

Total | Elapsed Time 005:00:00 | 000:00:00

Interval 001:00:00 | 000:00:00

Cycle 00005 | 00000

All Well OFF Scale Bar ○ Time Stamp ○

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

⑪ Start button

ⓐ Well navigation

ⓑ Scale bar button

ⓒ Time stamp button

ⓓ Stop button

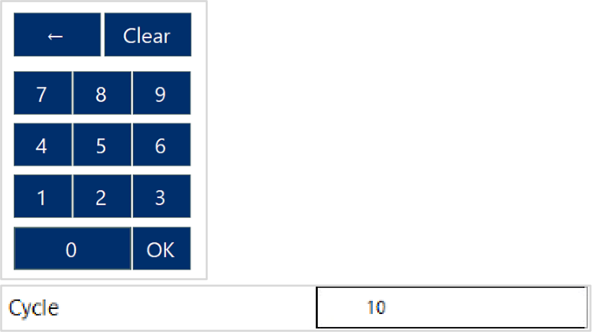
Edit the cycle number

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

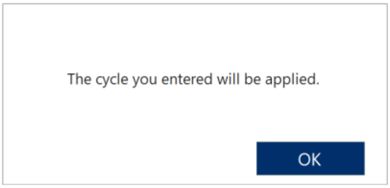
1. Click cycle number display box.

Cycle 00001 | 00000

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



Cycle 10



The cycle you entered will be applied.

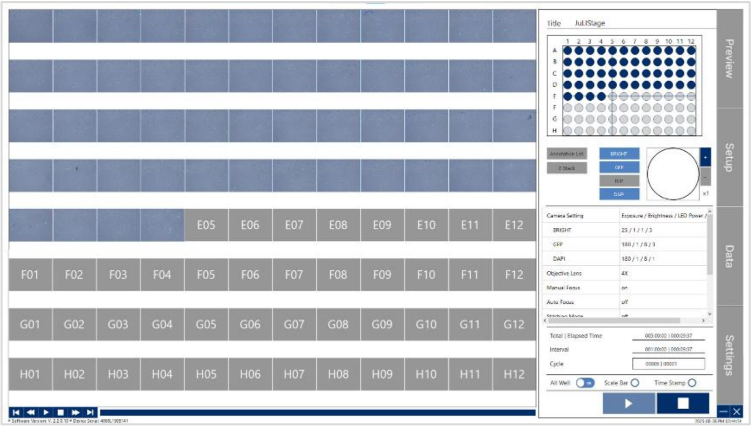
Cycle 00010 | 00001

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**.



Title JuLIStage

Camera Setting	Exposure / Brightness / LED Power /
BRIGHT	25 / 1 / 1 / 3
GFP	180 / 1 / 8 / 3
DAPI	180 / 1 / 8 / 1
Objective Lens	4X
Manual Focus	on
Auto Focus	off
Stitching Mode	off

Total | Elapsed Time 005:00:00 | 000:09:37

Interval 001:00:00 | 000:09:37

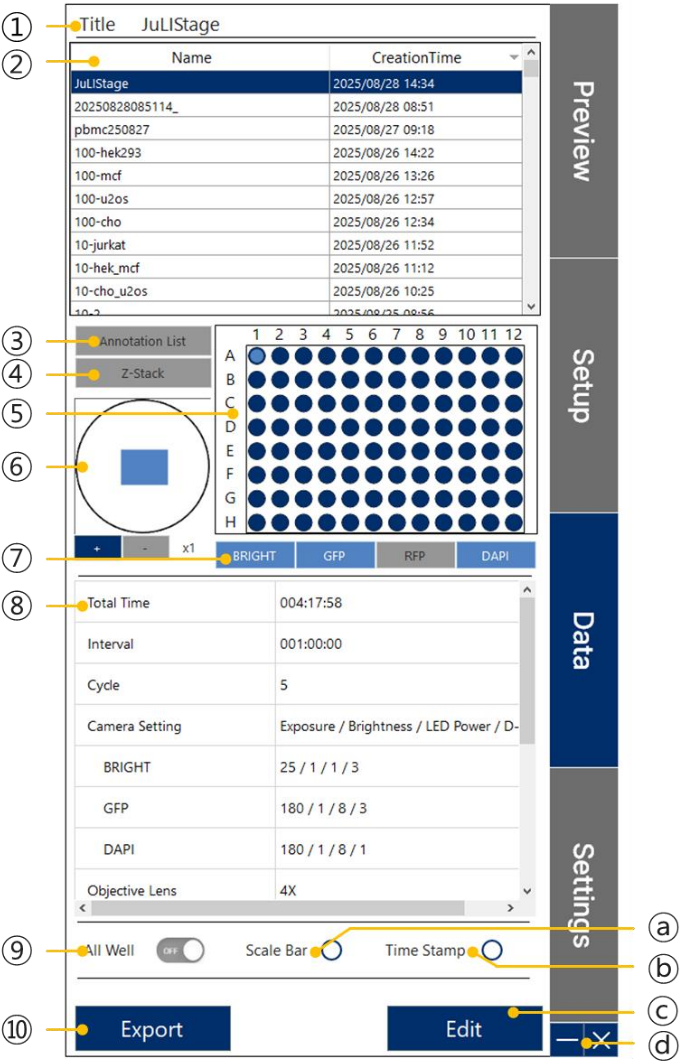
Cycle 00005 | 00001

All Well ● Scale Bar ○ Time Stamp ○

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.



Title JuLIStage

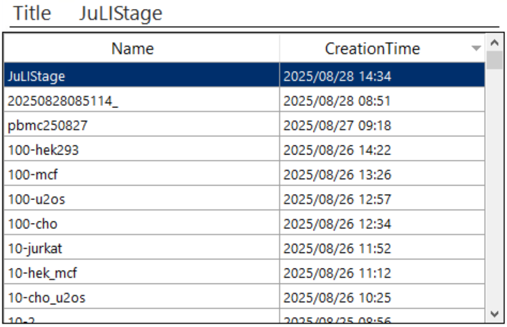
Name	CreationTime
JuLIStage	2025/08/28 14:34
20250828085114_	2025/08/28 08:51
pbmc250827	2025/08/27 09:18
100-hek293	2025/08/26 14:22
100-mcf	2025/08/26 13:26
100-u2os	2025/08/26 12:57
100-cho	2025/08/26 12:34
10-jurkat	2025/08/26 11:52
10-hek_mcf	2025/08/26 11:12
10-cho_u2os	2025/08/26 10:25

Total Time	004:17:58
Interval	001:00:00
Cycle	5
Camera Setting	Exposure / Brightness / LED Power / D-
BRIGHT	25 / 1 / 1 / 3
GFP	180 / 1 / 8 / 3
DAPI	180 / 1 / 8 / 1
Objective Lens	4X

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 button
ⓑ 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.

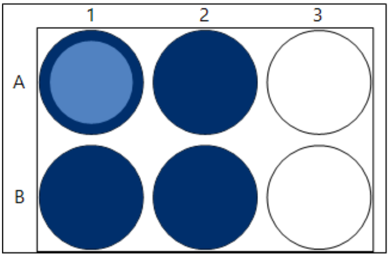


Title JuLIStage

Name	CreationTime
JuLIStage	2025/08/28 14:34
20250828085114_	2025/08/28 08:51
pbmc250827	2025/08/27 09:18
100-hek293	2025/08/26 14:22
100-mcf	2025/08/26 13:26
100-u2os	2025/08/26 12:57
100-cho	2025/08/26 12:34
10-jurkat	2025/08/26 11:52
10-hek_mcf	2025/08/26 11:12
10-cho_u2os	2025/08/26 10:25
10-2	2025/08/25 08:56

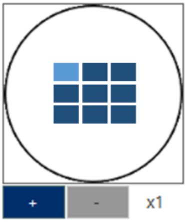
Plate layout

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



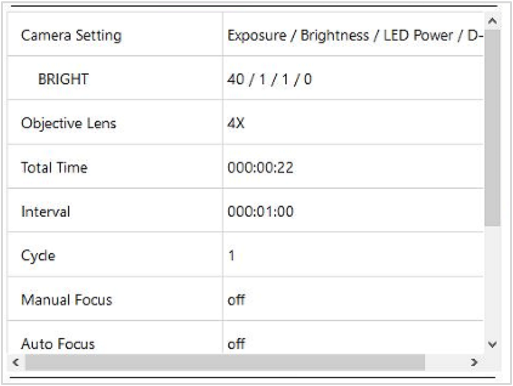
Well navigation and channel selection

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

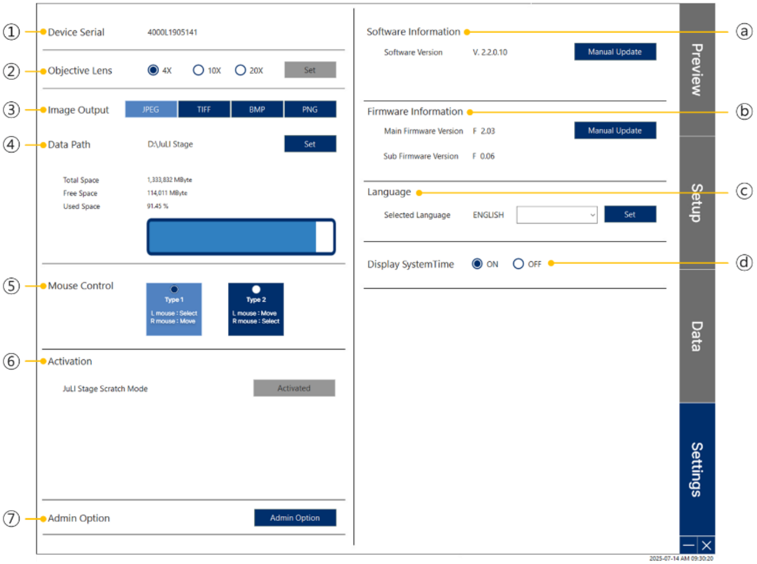
Export

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

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

7.4 Settings menu

The **Settings** menu contains information about the JuLI^{TM} 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.



Device Serial 4000L1905141

Data Path D:\JuLI Stage

Total Space 1,333,832 MByte
Free Space 114,011 MByte
Used Space 91.45 %

JuLI Stage Scratch Mode Activated

Software Version V. 2.2.0.10

Main Firmware Version F 2.03

Sub Firmware Version F 0.06

Selected Language ENGLISH

① Device serial number

② Current objective lens

③ Image output

④ Data path and hard drive info

⑤ Mouse button configuration

⑥ Activation information

⑦ Admin option (service access only)

ⓐ Software information

ⓑ Firmware information

ⓒ Language information

ⓓ System time

ⓔ Minimize & close button

Device Serial

The serial number of the JuLI^{TM} Stage instrument.

Device Serial 4000L1905141

Objective Lens

Provides the information which lens is currently used.

Objective Lens ● 4X ○ 10X ○ 20X Set

☞ Note: The field of view for the 4x, 10x, and 20x objectives is $3.55\ \text{mm}^2$, $0.58\ \text{mm}^2$, and $0.14\ \text{mm}^2$, 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.

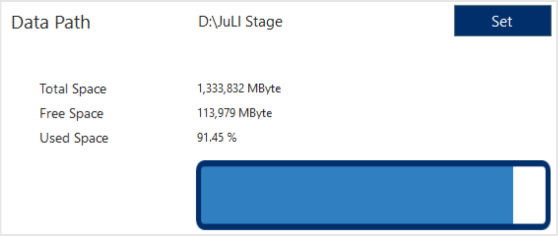
Image Output

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

Image Output: JPEG

Data Path

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



Data Path D:\JuLI Stage

Total Space 1,333,832 MByte

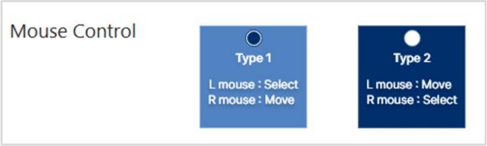
Free Space 113,979 MByte

Used Space 91.45 %

Mouse Control

Switches for mouse buttons: configurations 1 and 2.

☞ Note: Type 1 is the factory setting.



Activation information

JuLI^{TM} Stage Scratch and JuLI^{TM} 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^{TM} Stage Scratcher (Cat No. JSCT100) separately. Please refer to page 64 for Ordering information.

Admin Option (service access only)

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

Software information

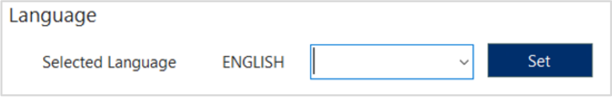
Gives information about the current software version. In order to update the software, click **Manual Update** select the provided .exe-file and follow further instructions on the screen.

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 JuLI™ Stage control software and go to the **Setup** menu.
4. Enter an appropriate Project Title.
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 **Vessel**, 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 Setup** 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 lid of the well plate should be wetted with cell culture media before monitoring.

This step is recommended to prevent condensation from forming inside the lid of the well plate during monitoring.

⚠ IMPORTANT!

Avoid exposing the JuLI^{TM} 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^{TM} Stage.

⚠ IMPORTANT!

When the JuLI^{TM} 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

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

Monitoring	
Poor image (Too dark and too bright)	Re-optimize the brightness and exposure settings. Restart the device.
Dirty image	Wipe the stage carefully. Eliminate any dust on the device and culture dish. Check for and remove any condensation on the lid or the bottom of the vessel. Let the sample warm up before imaging.
Time-lapse images become dark or bright	Warm up the instrument for 2 hours before monitoring (power should be on when warming up the instrument). Cell culture flask should be wetted with culture media before imaging. Eliminate any dust from the culture dish. Remove any condensation from the lid or bottom of the vessel. 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.
Problem saving movie file	Check the available volume of the hard disk.

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 **PC specifications may change without notice*
Operating power	100 - 240 V, 1.5 A, 50/60 Hz
Electronic input	12 VDC, 2.0 A
Operating environment	5 - 40 ℃, 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^{TM} Stage, Stater Pack	JuLI^{TM} Stage basic set (JS1000), Desktop computer (JP0200), 3 Objective lenses (4X, 10X & 20X)
JS1000	JuLI^{TM} 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^2)
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^{TM} 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^{TM} 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

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.

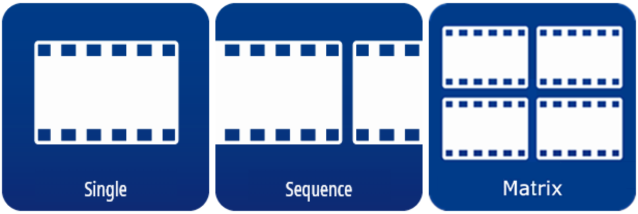
For this, two image tools are available:

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.

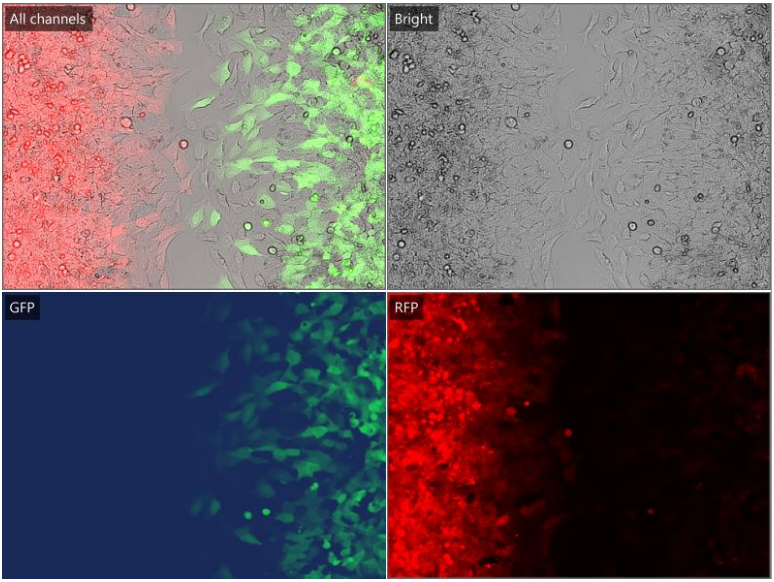
Movie Maker

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, side by side in a customizable grid layout, ranging from 1x1 up to 24x16. For example, a 2x2 grid is shown below.



When working with well plate-based projects, the **All Wells** option lets you quickly add all wells to the matrix in the standard plate layout (e.g., 12x8 for a 96-well plate).

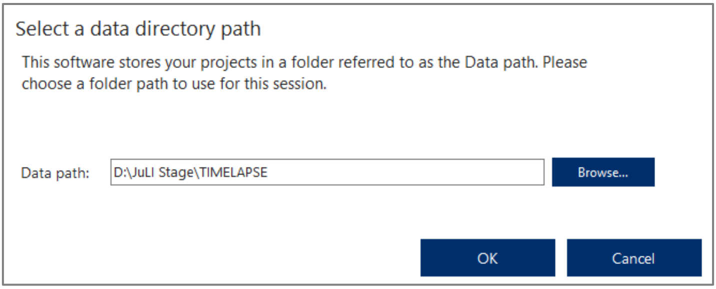
11.2 *Operation*

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

☞ Note: To install ‘**JuLI EDIT**’ on a personal PC, refer to chapter 13 (page 153).

Click **File** and **Data Path**.

Select a data directory path. Click **Browse…** to choose the parent folder (either local or network-based) that contains the measurement projects.
Then click **OK**.



Select a data directory path

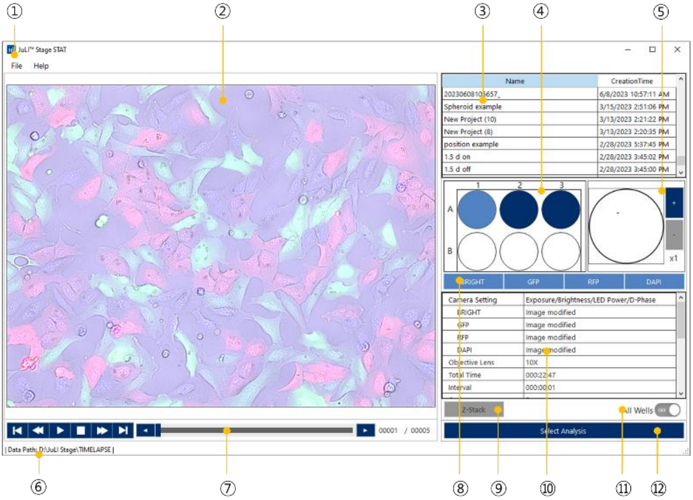
This software stores your projects in a folder referred to as the Data path. Please choose a folder path to use for this session.

Data path: D:\JuLI Stage\TIMELAPSE

☞ 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.



Name	CreationTime
20230608105657_	6/8/2023 10:57:11 AM
Spheroid example	3/15/2023 2:51:06 PM
New Project (10)	3/13/2023 2:21:22 PM
New Project (8)	3/13/2023 2:20:35 PM
position example	2/28/2023 5:37:45 PM
1.5 d on	2/28/2023 3:45:02 PM
1.5 d off	2/28/2023 3:45:00 PM

Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	Image modified
GFP	Image modified
RFP	Image modified
DAPI	Image modified
Objective Lens	10X
Total Time	000:22:47
Interval	000:00:01

| Data Path: D:\JuLI Stage\TIMELAPSE |

① Menu bar

② Image view

③ Project list

④ Vessel display

⑤ Well navigation

⑥ Data path

⑦ Time lapse buttons and slider

⑧ Channel selection

⑨ Z-stack

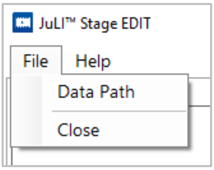
⑩ Project information

⑪ Display all wells

⑫ Edit

① **Menu bar**

- **File** menu

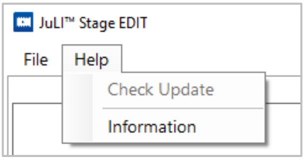


  - **Data Path:** Click to change the current data path

    Always select the parent folder that contains the project or measurements.

  - **Close**: Click **Close** to close the **JuLI™ Stage EDIT** software.

- **Help** menu

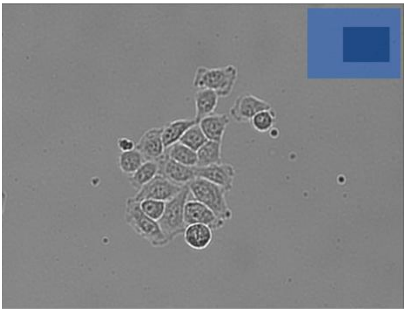


  - **Information:** Software version and copyright information of **JuLI™ Stage EDIT** are displayed.

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.



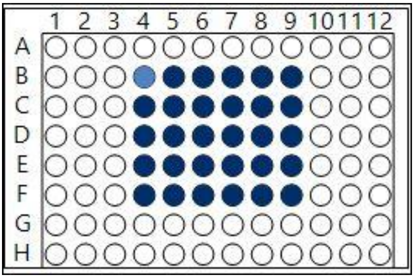
③ Project list

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, and by clicking the “Name” header, the list can be sorted by name.

④ Vessel display

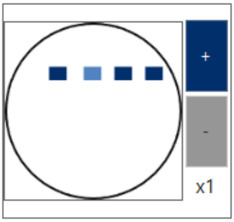
Of the selected measurement project, all wells of the plate type are displayed:

- 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 dark blue squares. By clicking a dark blue square it is selected to be displayed and turns light blue.



⑥ Data path

Display of the data path in use.

| Data Path: D:\JuLIStage\TIMELAPSE |

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

⑦ 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.

⑧ Channel selection and overlay

Display of all 4 channels is 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:

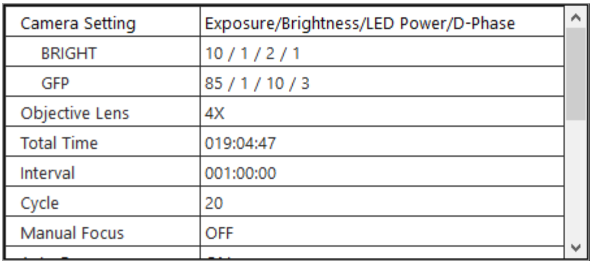
Name	CreationTime
20240130-spheroid	3/19/2025 9:36:50 AM
12well multiposition_z-stack_3cycle	3/17/2025 10:23:28 AM
20250228182626_ AF stitching	3/10/2025 11:19:01 AM
All channel Every cycle	3/10/2025 11:17:28 AM
Bright	2/26/2025 6:05:56 PM
rfp 16-18	2/26/2025 6:00:48 PM
20240130-cell migration	1/23/2025 11:19:34 AM

Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	30 / 1 / 1 / 1
GFP	110 / 4 / 2 / 1
RFP	200 / 13 / 10 / 1
DAPI	160 / 6 / 4 / 1
Objective Lens	4X
Total Time	000:24:53
Interval	000:01:00

☞ 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:

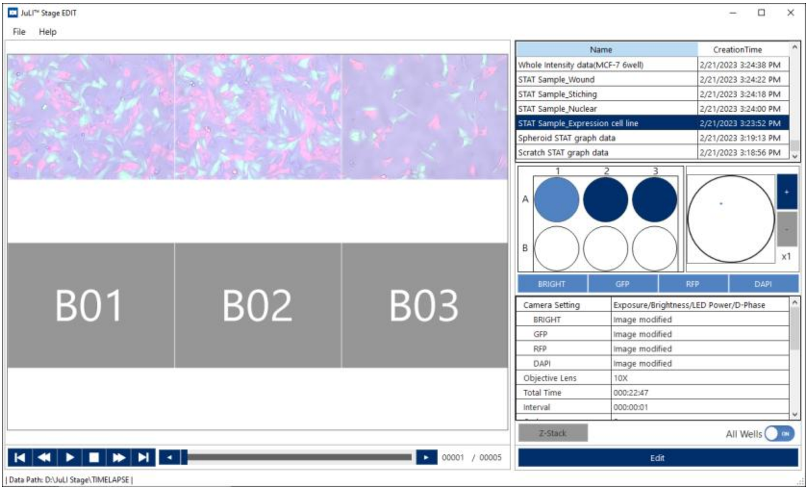


Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	10 / 1 / 2 / 1
GFP	85 / 1 / 10 / 3
Objective Lens	4X
Total Time	019:04:47
Interval	001:00:00
Cycle	20
Manual Focus	OFF

⑪ Display All Wells

Switching the toggle switch of **All Wells** to **ON** will display all wells of the selected plate, allowing both measured and unmeasured wells to be viewed at a glance.

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:



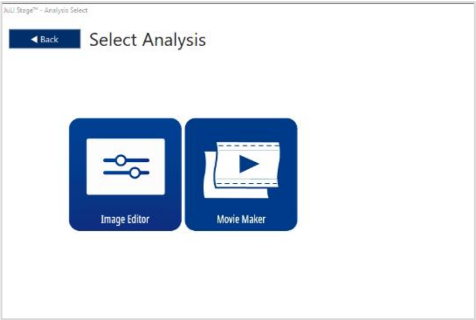
Name	CreationTime
Whole Intensity data(MCF-7 6well)	2/21/2023 3:24:38 PM
STAT Sample_Wound	2/21/2023 3:24:22 PM
STAT Sample_Stiching	2/21/2023 3:24:18 PM
STAT Sample_Nuclear	2/21/2023 3:24:00 PM
STAT Sample_Expression cell line	2/21/2023 3:23:52 PM
Spheroid STAT graph data	2/21/2023 3:19:13 PM
Scratch STAT graph data	2/21/2023 3:18:56 PM

Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	Image modified
GFP	Image modified
RFP	Image modified
DAPI	Image modified
Objective Lens	10X
Total Time	000:22:47
Interval	000:00:01

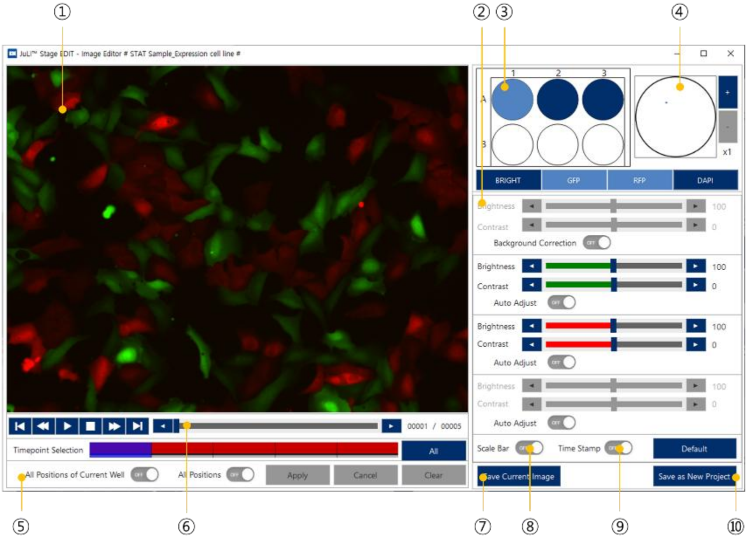
| Data Path: D:\JuLI Stage\TIMELAPSE |

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

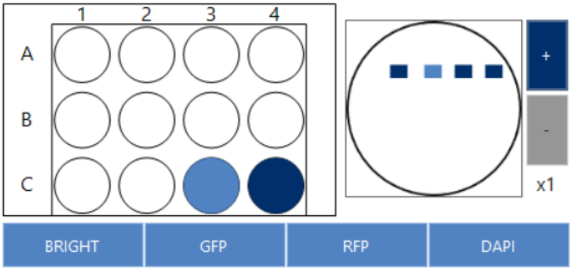


① Image view
② Edit image
③ Vessel display
④ Well navigation
⑤ Edit panel

⑥ Time lapse slider
⑦ Save Current Image
⑧ Scale bar
⑨ Time stamp
⑩ Save as New Project

Vessel display & navigation

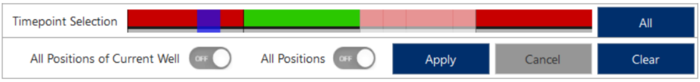
Select well, channel and position inside the well to review and edit image brightness and contrast.



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



This panel allows you to select individual time points or a range of time points for editing.

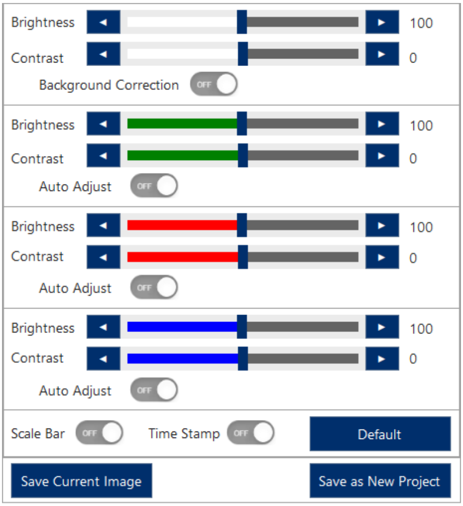
- Blue Bar: Indicates the currently displayed time point, matching the position on the time lapse slider bar
- Red: Time points where edits have not been applied yet
- Grey: Time points currently selected for editing
- Green: Time points where edits have been applied by clicking **Apply**

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 green after pressing **Apply**)
- **Cancel**: Cancel the edits in the selected time points
- **Clear**: Cancel all edits in the selected positions

Edit images

Each selected 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**: Reset all changes to default

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 displayed in image view and also included when using **Save Current Image**, but they will not be saved in **Save as New Project**- images.

Save as 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.

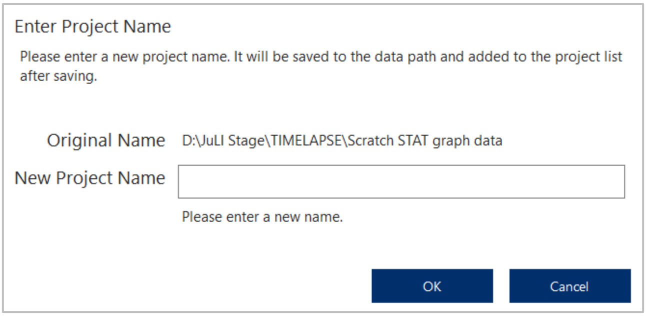
☞ Note: All data of this project will be duplicated and saved, even if only one or a few images were modified.

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

☞ Note: To apply the same changes not only to the current position but also to the entire well or project, one of the two **All Positions** buttons must be used before **Save as New Project**.

Quick Workflow to Export a Modified Data Set

1. Select a well and position
2. Choose whether to apply modifications to all positions in the data set, to all positions within the current well, or to only the current position
3. Select the time points to be edited
4. Adjust channel settings (e.g., brightness and contrast) as needed
5. Click **Apply** to apply changes
6. Click **Save as New Project**, enter a **New Project Name**, and click **OK**. The new project will be saved in the same folder as the original project



Enter Project Name

Please enter a new project name. It will be saved to the data path and added to the project list after saving.

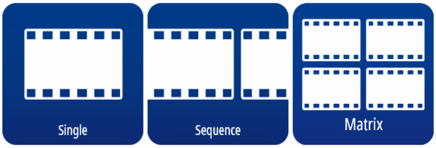
Original Name D:\JuLI Stage\TIMELAPSE\Scratch STAT graph data

New Project Name

Please enter a new name.

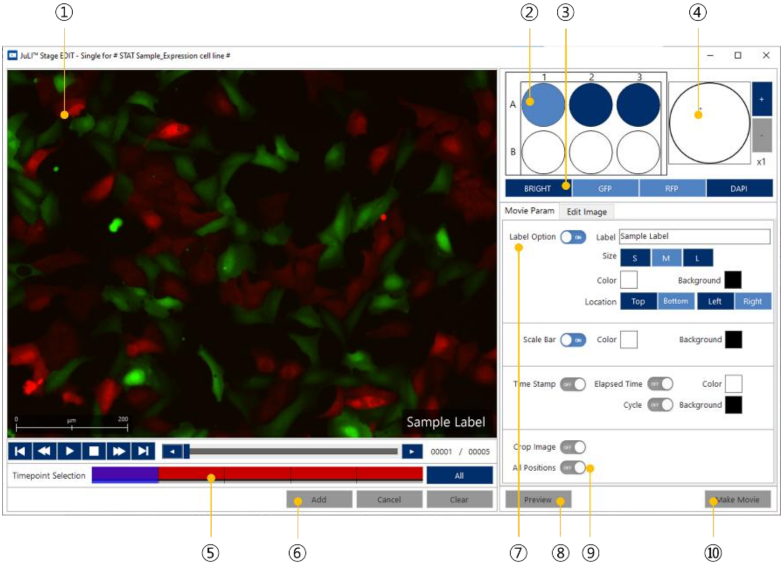
11.2.3 Movie Maker

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

② Vessel display

③ Channel selection

④ Well navigation

⑤ Time point selection

⑥ Edit panel

⑦ Label options

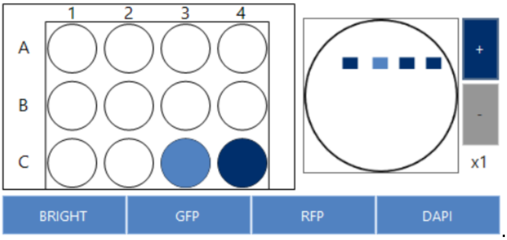
⑧ Preview

⑨ All positions

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.

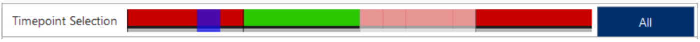
Channels shown in light blue have been acquired and are automatically displayed in the image view as overlays. Clicking a light blue channel will deactivate its overlay and change the button to dark blue. Channels show in grey were not acquired and cannot be displayed.

e.g. display of GFP and RFP channel

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

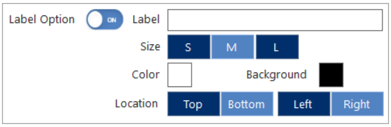


This panel allows you to select individual time points or a range of time points for editing.

- Blue bar: Indicates the currently displayed time point, matching the position on the time lapse slider bar
- Red: Time points where edits have not been applied yet
- Grey: Time points currently selected for editing
- Green: Time points where edits have been applied by clicking **Add**
- **All**: Select all time points
- **Cancel**: Remove settings saved at the selected time points
- **Clear**: Clear all settings across all time points

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

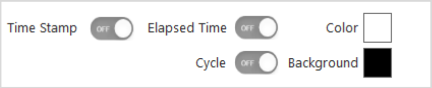
Insert a scale bar into the movie

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

Time Stamp

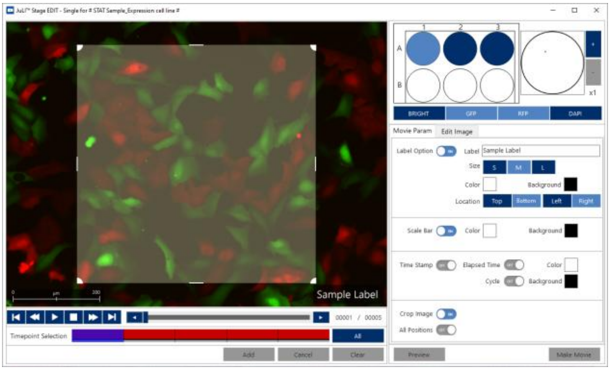
To display a Time Stamp in the movie:

- Elapsed Time: Shows the elapsed time for each captured image
- Cycle: Displays the image acquisition cycle number
- Color: Change color of Time Stamp
- Background: Change the background color of Time Stamp



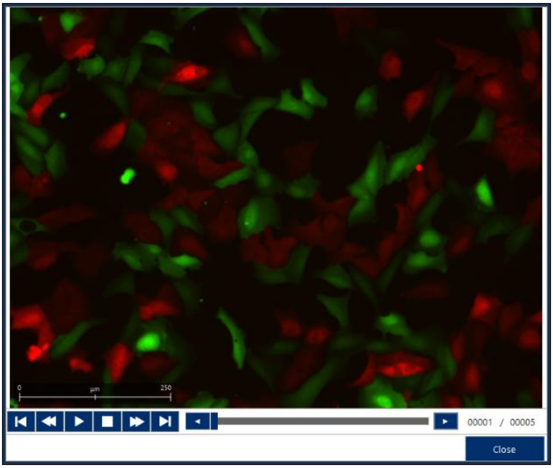
Crop Image

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



Preview

View a preview of the edited movie.



All Positions

Apply all settings to all positions.

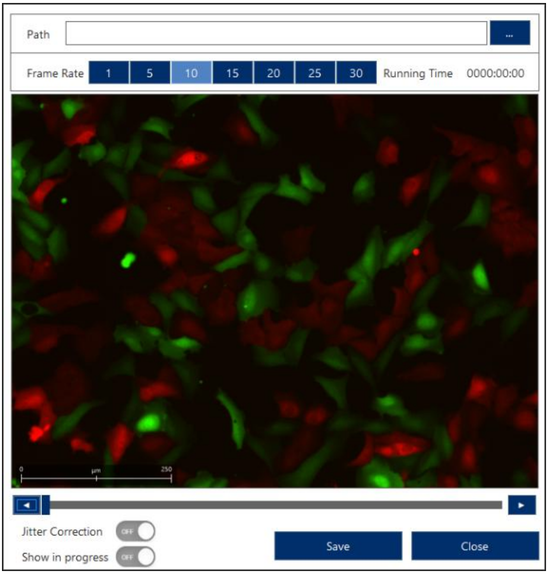
☞ Note: If **All Positions** is **ON**, movies for all positions in all wells will be created with automatically given, individual names. E.g. a measurement containing 6 wells and 5 positions within a well will create 30 movies named for example B02_00005.avi for well B2 and position 5 in well B02.

☞ Note: If the area within a position is cropped using **Crop Image**, and **All Positions** is **ON**, the same cropping area and location will be applied to all positions in all wells 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 : Displays the total duration of the movie
- **Jitter Correction** : Corrects image shifts over time to stabilize object positions across frames
- **Show in Progress** : Preview the movie in real time while it is being generated
- **Save**: Save the movie file
- **Close**: Close the window without saving



Running Time 0000:00:00

Jitter Correction OFF

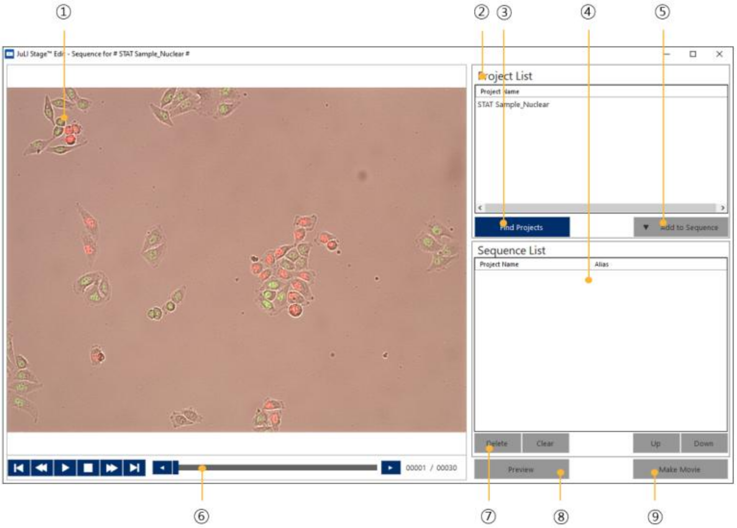
Show in progress OFF

Quick Workflow to create a Single movie

1. Select a well, position, and channels
2. Add labels, a scale bar, or a time stamp
3. Crop the image, if needed
4. Choose the desired time window in the Timepoint selection
5. Click **Add**
6. Decide whether to apply the settings to all positions
7. Click **Make Movie**
8. Set the save path, file name, and choose a frame rate
9. Click **Save** to export the movie

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

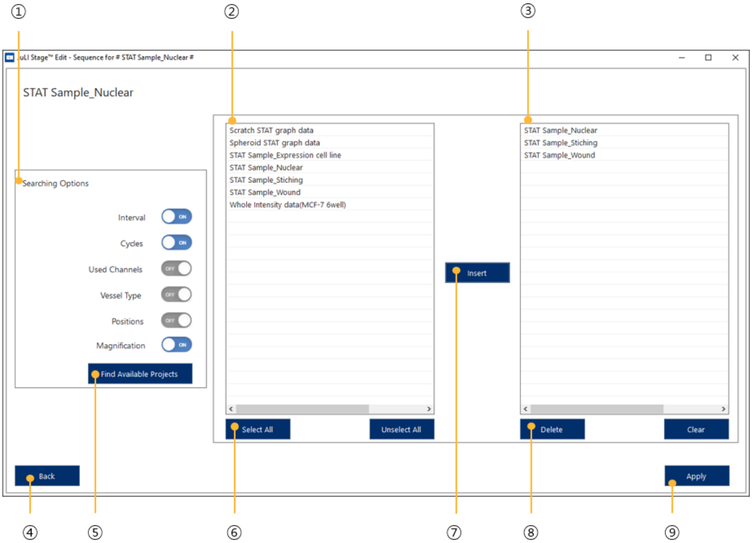
⑦ Edit sequence panel

⑧ Preview

⑨ Make movie

Find Projects

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



Interval ON
Cycles ON
Used Channels OFF
Vessel Type OFF
Positions OFF
Magnification ON

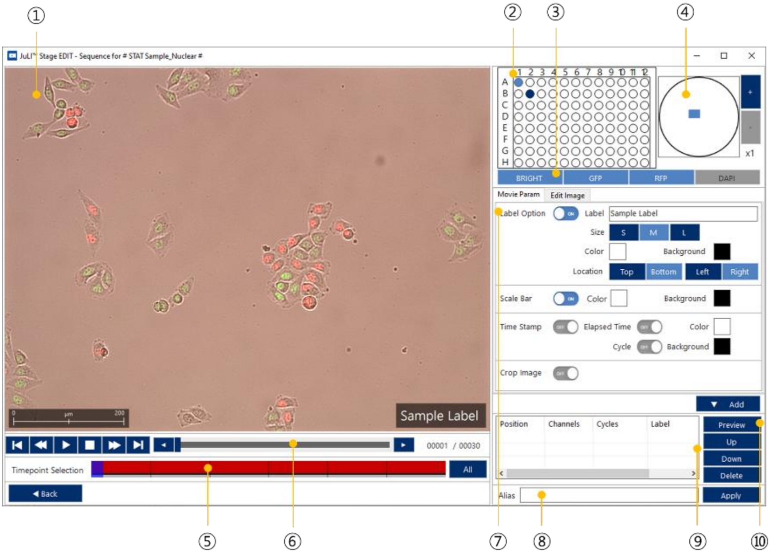
Scratch STAT graph data
Spheroid STAT graph data
STAT Sample_Expression cell line
STAT Sample_Nuclear
STAT Sample_Stiching
STAT Sample_Wound
Whole Intensity data(MCF-7 6well)

STAT Sample_Nuclear
STAT Sample_Stiching
STAT Sample_Wound

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 option
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.

Sequence List

The sequence list displays the order and alias names of movies (from wells, positions or projects) that will be used to create the final sequence movie.

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

Make Movie

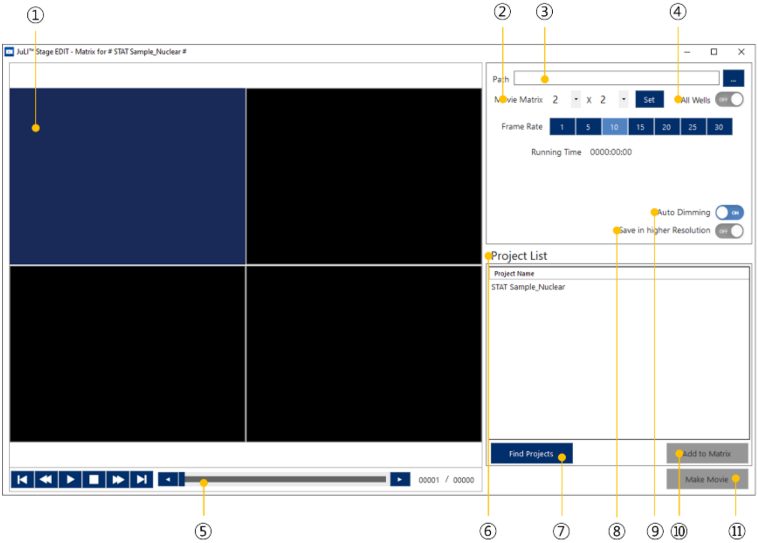
Apply final settings and save the movie sequence.

Quick Workflow to Save a Sequence Movie

1. Select a project in the Project List by clicking on the line (Use **Find Projects** to add another project if needed)
2. Click **Add to Sequence**
3. Select a well, position, and channels
4. Add labels, a scale bar, or a time stamp
5. Crop the image, if needed
6. Choose time window in the Timepoint Selection panel
7. Click **Add**
8. Assign an **Alias**
9. Click **Apply**
10. Repeat steps 1-9 for additional movies in the sequence
11. Arrange the order in the **Sequence List**, then click **Make Movie**
12. Set the save path, file name, and frame rate
13. Click **Save**

11.2.6 Movie Maker – Matrix

The **Movie Maker – Matrix** allows you to combine multiple movies into a tiled layout, making it easier to compare different conditions side by side. Each tile can display either a single time-lapse movie or a combined sequence of multiple time-lapse measurements.



Movie Matrix 2 x 2

Running Time 0000:00:00

Auto Dimming ON

Save in higher Resolution OFF

Project Name: STAT Sample_Nuclear

00001 / 00000

① Matrix view
② Movie Matrix options
③ Data path
④ All Wells
⑤ Time lapse slider
⑥ Project List
⑦ Find Projects
⑧ Save in higher Resolution
⑨ Auto Dimming
⑩ Add to Matrix
⑪ Make Movie

Movie Matrix

Choose the number of tiles of the movie matrix from 1x1 and up to 24x16.

All Wells

When using a vessel with a well plate format, enabling **All Wells** automatically sets the matrix size based on the layout of the plate. Movies are added only to wells that contain actual sample data.

By editing one well and turning **ON All Wells**, the same settings – including position, time window, labels, and image edits (e.g., brightness and contrast) – will be applied across all wells in the project.

For example, if position A01:00003 is selected, point 00003 will be added to the matrix for every well in the plate.

Movie Matrix 2 x 2

All Wells OFF

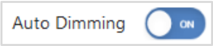
Save in higher Resolution

If activated, the movie will be saved in a resolution of 3872 x 2912 pixels. (If not activated, the original resolution of 1936 x 1456 pixels will be used.)

Save in higher Resolution OFF

Auto Dimming

When enabled, movies that are shorter than others will automatically fade to black after they finish, helping to visually indicate which tiles have completed playback earlier.



Auto Dimming ON

Quick Workflow to save a matrix movie

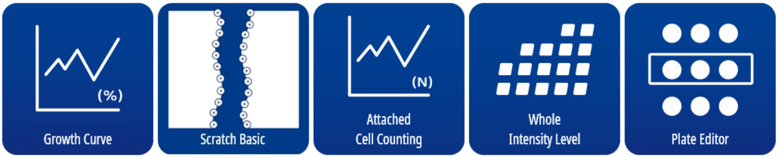
1. Set the save path, file name, the number of tiles for the movie matrix (confirm with **Set**), and select a frame rate
2. Enable **Auto Dimming** and **Save in higher Resolution**, if needed
3. Click a black tile in the matrix to select it
4. In the **Project List**, click the project name (use **Find Projects** to add more projects)
5. Click **Add to Matrix**
6. Select the well, position, and channels

7. Add any desired labels, a scale bar, or a time stamp
8. Select the desired time window
9. Press **Add**
10. (Repeat steps 6-9 for each additional movie you want to include)
11. Press **Apply**
12. To add movies to other tiles, select a new tile and repeat steps 4-11
13. When all tiles are ready, Press **Make Movie** to export

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**.



Using the **Plate Editor,** detailed experimental conditions can be defined and saved as plate maps for the selected project. Each well can be assigned to specific assay conditions (e.g., cell type, cell number, compound concentration), which can then be used to calculate statistics such as mean values of triplicates and their standard deviations.

In addition, the **View Results** section in each evaluation module allows users to create or load a saved experiment layout.

All four evaluation modules generate result tables and ready-to-use graphs that can be copied and pasted. Numerical results are also provided in an exportable .csv format for further analysis in external software such as Microsoft Excel® or other spreadsheet programs.

Evaluation module	Input channel	Output metrics	Example assays
Growth Curve	BRIGHT GFP, RFP, DAPI	**Confluence-based metrics** - Growth Rate [%] - Growth Area [µm²/image], [µm²/well], [mm²/image], [mm²/well] **Confluence ratio** - Growth Rate e.g., [BRIGHT/GFP (%)] **Fluorescence intensity** - Mean fluorescence intensity (in confluence area, unitless)	Cell health and viability assays, Cytotoxicity, Transfection rate, Cell culture quality control

Scratch Basic	BRIGHT GFP, RFP, DAPI	**Wound area confluence** - Wound confluence [%], [µm²/image], [µm²/well] [mm²/image] [mm²/well] **Fluorescence intensity** - Mean fluorescence intensity (in confluence area, unitless)	Scratch wound assay, Migration and invasion - Analyzable at 4x, 10x, and 20x magnifications
Attached Cell Counting	GFP, RFP, DAPI	**Cell count** Counting result : [n/image], [n/mm²], [n/well] (n=number of adherent cells) **Fluorescence intensity** - Mean fluorescence intensity (in detected cells, unitless)	Cell health and viability assays, Cytotoxicity, Transfection rate
Whole Intensity Level	GFP, RFP, DAPI	**Fluorescence intensity** - Mean fluorescence intensity (whole image, unitless)	Calcium-flux

☞ Note: **Analysis is only supported for measurements using multi-well plates** (6-, 12-, 24-, 48-, 96-, or 384-well).

Workaround: To enable analysis for other vessel types such as flasks, dishes, or slides, please configure the measurement using a compatible plate format.

☞ Note: **Attached Cell Counting** and **Whole Intensity Level** are only available if at least one fluorescence channel was acquired during the measurement.

12.2 Operation

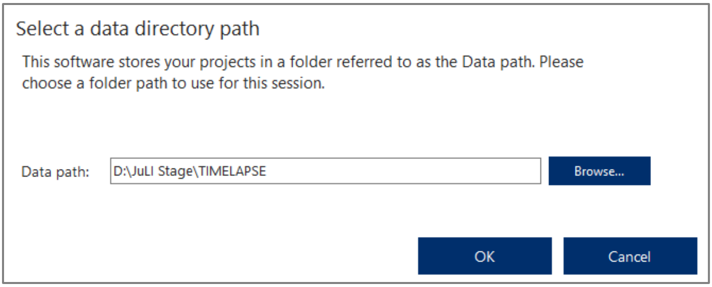
Double click the ‘**JuLI STAT**’ icon on the desktop to run the program.

☞ Note: To install ‘**JuLI STAT**’ on a personal PC, refer to chapter 13 (page 153).

Click **File** and **Data Path**.

Select a data directory path. Click **Browse…** to choose the parent folder (either local or network-based) that contains the measurement projects.

Then click **OK**.



Select a data directory path

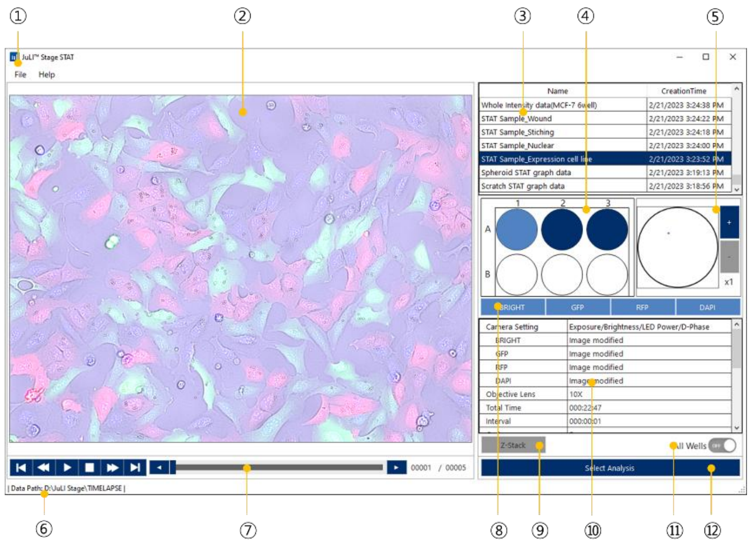
This software stores your projects in a folder referred to as the Data path. Please choose a folder path to use for this session.

Data path: D:\JuLI Stage\TIMELAPSE

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

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.



Name	CreationTime
Whole Intensity data(MCF-7 6well)	2/21/2023 3:24:38 PM
STAT Sample_Wound	2/21/2023 3:24:22 PM
STAT Sample_Stiching	2/21/2023 3:24:18 PM
STAT Sample_Nuclear	2/21/2023 3:24:00 PM
STAT Sample_Expression cell line	2/21/2023 3:23:52 PM
Spheroid STAT graph data	2/21/2023 3:19:13 PM
Scratch STAT graph data	2/21/2023 3:18:56 PM

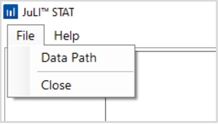
Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	Image modified
GFP	Image modified
RFP	Image modified
DAPI	Image modified
Objective Lens	10X
Total Time	000:22:47
Interval	000:00:01

| Data Path: D:\JuLI Stage\TIMELAPSE |

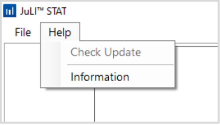
- ① Menu bar
- ② Image view
- ③ Project list
- ④ Vessel display
- ⑤ Well navigation
- ⑥ Data path
- ⑦ Time lapse buttons and slider
- ⑧ Channel selection
- ⑨ Z-stack
- ⑩ Project information
- ⑪ Display all wells
- ⑫ Select analysis

① Menu bar

- **File** menu
  - **Data Path**: Click to change the current data path.
    Always select the parent folder that contains the project or measurement.
  - **Close**: Click **Close** to close the JuLI^{TM} STAT Analysis software.



- **Help** menu
  - **Information**: Software version and copyright information of JuLI^{TM} STAT are displayed.



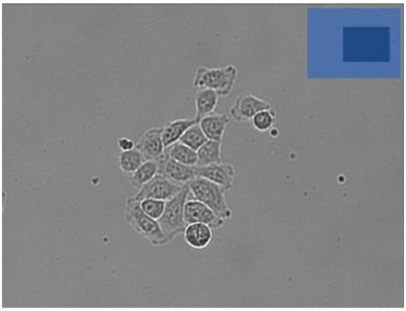
JuLI™ STAT

Software Version: 2.1.0.1

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 window will appear in blue on the top right side. You can drag the small dark blue box in the navigation window 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.



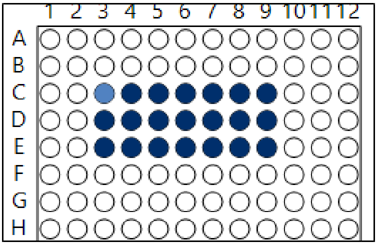
③ Project list

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, and by clicking the “Name” header, the list can be sorted by name.

④ Vessel display

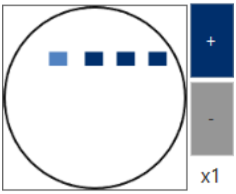
Of the selected measurement project, all wells of the plate type are displayed:

- 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 Data path

Display of the data path in use.

| Data Path: D:\JuLIStage\TIMELAPSE |

Use File > Data Path to change the data path.

⑦ 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.**

⑧ Channel selection and overlay

Display of all 4 channels available on the JuLI^{TM} 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:

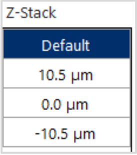
Name	CreationTime
new project (1)	2/28/2023 6:31:52 PM
position example	2/28/2023 5:37:45 PM
1.5 d on	2/28/2023 3:45:02 PM
1.5 d off	2/28/2023 3:45:00 PM
1.0 d on	2/28/2023 3:44:59 PM
1.0 d off	2/28/2023 3:44:58 PM
0.5 d on	2/28/2023 3:44:56 PM

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

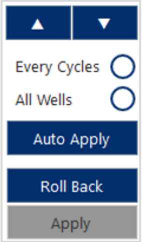
| Data Path: D:\JuLI Stage\TIMELAPSE |

☞ 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.



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

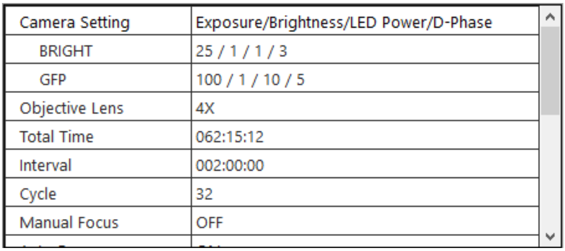


- Checking the circles **Every Cycles** and **All Wells** followed by **Apply** will use only this plane of the selected channel in further analyses.
- Clicking **Auto Apply** will automatically search for the best plane based on the contrast of the image in the selected channel.
- **Roll Back** will 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	25 / 1 / 1 / 3
GFP	100 / 1 / 10 / 5
Objective Lens	4X
Total Time	062:15:12
Interval	002:00:00
Cycle	32
Manual Focus	OFF

⑪ Display All Wells

Switching the toggle switch of **All Wells** to **ON** will display all wells of the selected plate-based project 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:

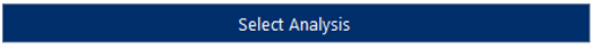
Name	CreationTime
Whole Intensity data(MCF-7 6well)	2/21/2023 3:24:38 PM
STAT Sample_Wound	2/21/2023 3:24:22 PM
STAT Sample_Stiching	2/21/2023 3:24:18 PM
STAT Sample_Nuclear	2/21/2023 3:24:00 PM
STAT Sample_Expression cell line	2/21/2023 3:23:52 PM
Spheroid STAT graph data	2/21/2023 3:19:13 PM
Scratch STAT graph data	2/21/2023 3:18:56 PM

Camera Setting	Exposure/Brightness/LED Power/D-Phase
BRIGHT	Unknown
GFP	Unknown
RFP	Unknown
DAPI	Unknown
Objective Lens	10X
Total Time	000:22:47
Interval	000:00:01

| Data Path: D:\JuLI Stage\TIMELAPSE |

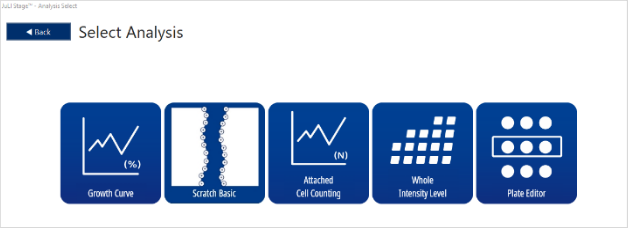
⑫ Select Analysis

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 ◀ Back brings you back to the main viewer.

☞ Note: Analysis is only supported for measurements using multi-well plates (6-, 12-, 24-, 48-, 96-, or 384-well).

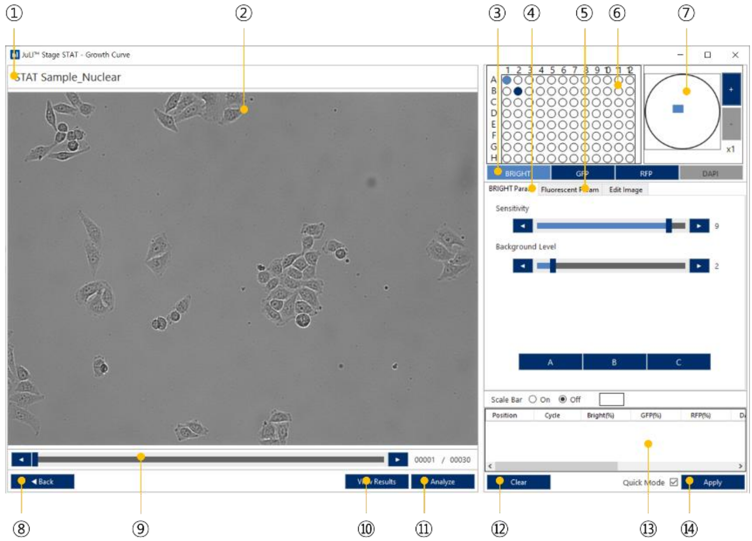
Workaround: To enable analysis for other vessel types such as flasks, dishes, or slides, please configure the measurement using a compatible plate format.

☞ Note: **Attached Cell Counting** and **Whole Intensity Level** are only available if at least one fluorescence channel was acquired during the measurement.

Only the **Growth Curve** is available for projects that 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

② Image view

③ Channel selection

④ Brightfield channel parameter

⑤ Fluorescence channel parameter

⑥ Vessel display

⑦ Well navigation

⑧ Back (to main viewer)

⑨ Time lapse slider

⑩ View results

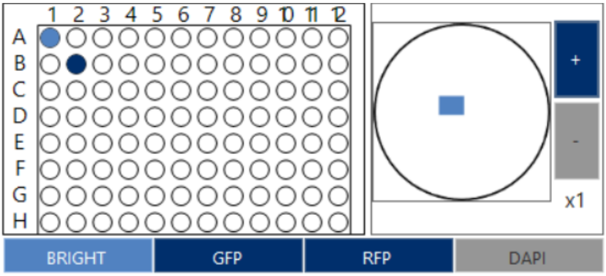
⑪ Analyze

⑫ Clear results list

⑬ Confluence result list

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.

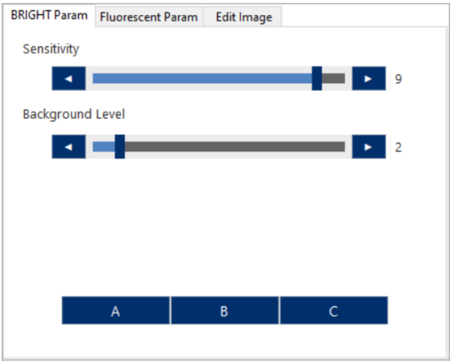
☞ Note: Only one channel can be selected at a time, no other acquired channel can be co-selected to create overlays.

- 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.

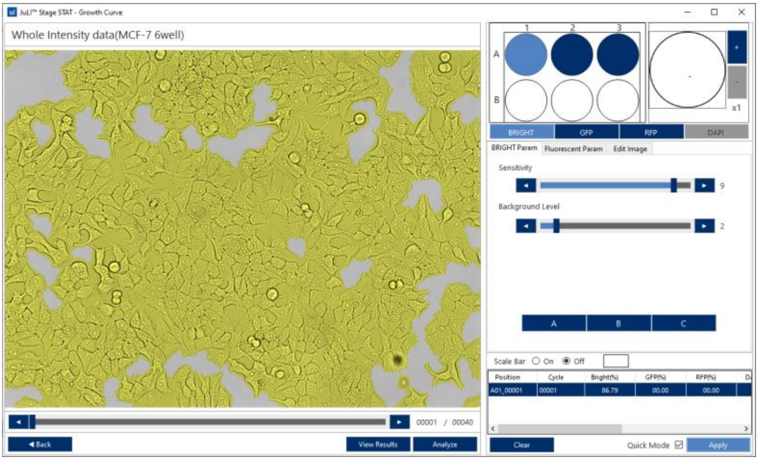
Depending on the brightness and contrast of the image, they lead to different segmentation results.



Sensitivity 9

Background Level 2

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



Whole Intensity data(MCF-7 6well)

Sensitivity 9

Background Level 2

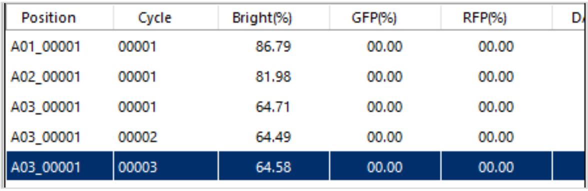
00001 / 00040

Position	Cycle	Bright(%)	GFP(%)	RFP(%)	D
A01_00001	00001	86.79	00.00	00.00	

The yellow-colored area outlines the segmentation result based on the most recent sensitivity and background settings. The table shows the numerical results for the selected well, position, and time point.

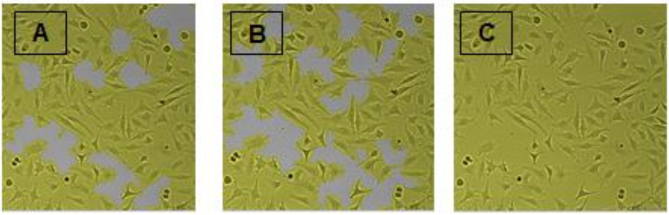
When you select a well, position, and time point, adjust the parameters, and click Apply, the current analysis result is added to the table as a new row.

If the selected image has already been analyzed, clicking Apply with new settings will overwrite the previous values.



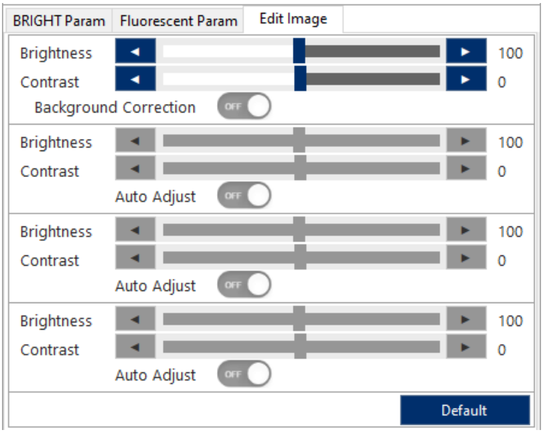
Position	Cycle	Bright(%)	GFP(%)	RFP(%)	D
A01_00001	00001	86.79	00.00	00.00	
A02_00001	00001	81.98	00.00	00.00	
A03_00001	00001	64.71	00.00	00.00	
A03_00001	00002	64.49	00.00	00.00	
A03_00001	00003	64.58	00.00	00.00	

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** are not sufficient, **Edit Image** offers additional options to optimize the brightness and contrast of the original image:



- Adjust the Brightness and Contrast sliders as needed. Click **Apply** to analyze the current image with the selected settings and display the result in the table. To apply these settings to the entire project, click **Analyze**.
- Click **Default** to reset all changes to default values.

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

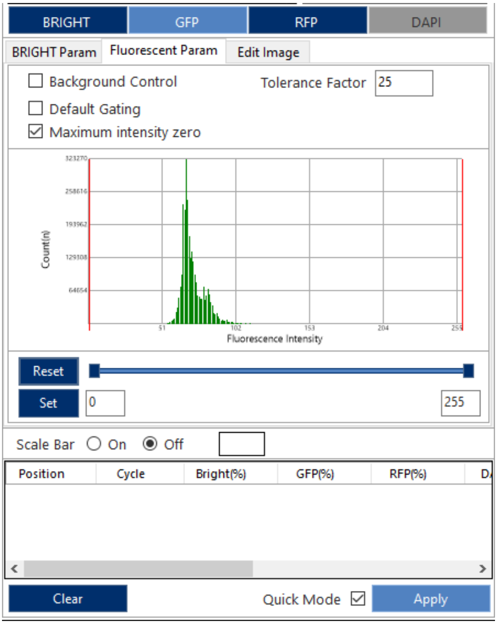
☞ Note: Parameters to optimize confluence segmentation on fluorescent images (**Fluorescent Param**) are deactivated for the brightfield channel.

☞ 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**.



☐ Background Control

Tolerance Factor 25

☐ Default Gating

☑ Maximum intensity zero

0 255

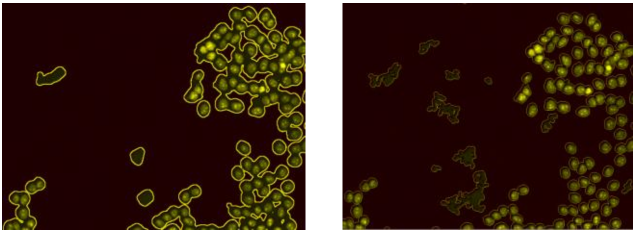
Scale Bar ○ On ● Off

Quick Mode ☑

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.



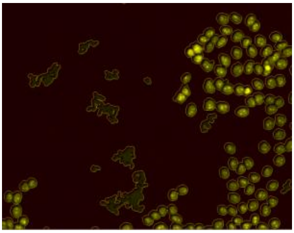
Quick Mode: on

Quick Mode: off

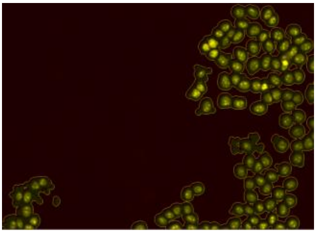
In this example, the Quick mode improved segmentation, but some background is still falsely detected in both cases.

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

Background Control: off



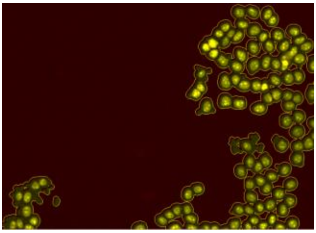
Background Control: on



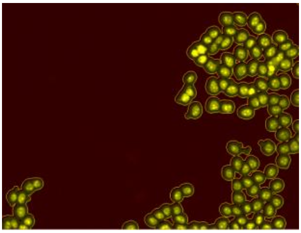
This greatly improved the separation of background and foreground in this example image.

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



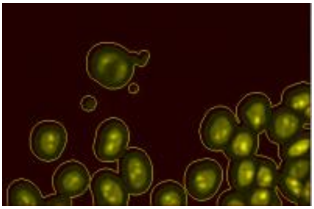
Default Gating: on



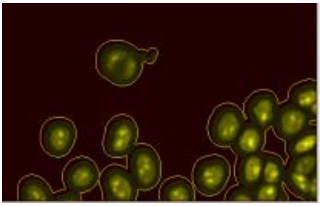
Here, in the lower left corner a difference can be seen.

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

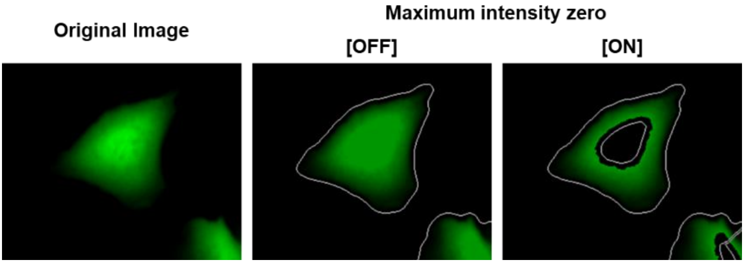
Tolerance Factor: 1



Tolerance Factor: 25



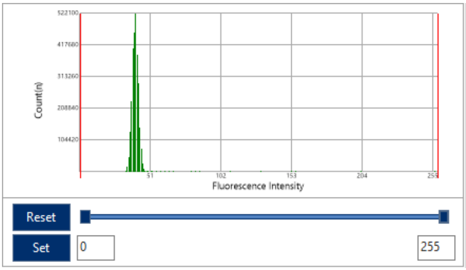
Check the **Maximum intensity zero** box to exclude areas exceeding the maximum intensity (option when **Default Gating** is unchecked):



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 levels on the x-axis and number of pixels on the y-axis.



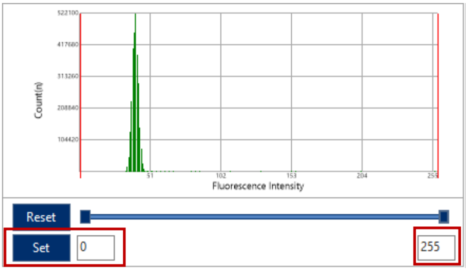
0

255

☞ 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.

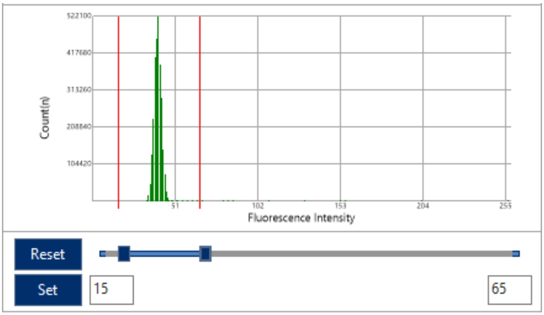
To zoom the graph, enter a minimum and/or maximum intensity value or use the slider, then Click **Set**.



0

255

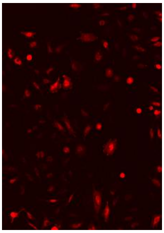
Red lines indicate the new gating window.



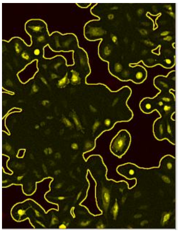
Click **Reset** to return to the initial graph.

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

Original

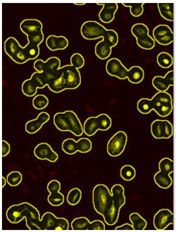


Default gating



54 % confluence

Manual gating: 50 to 255

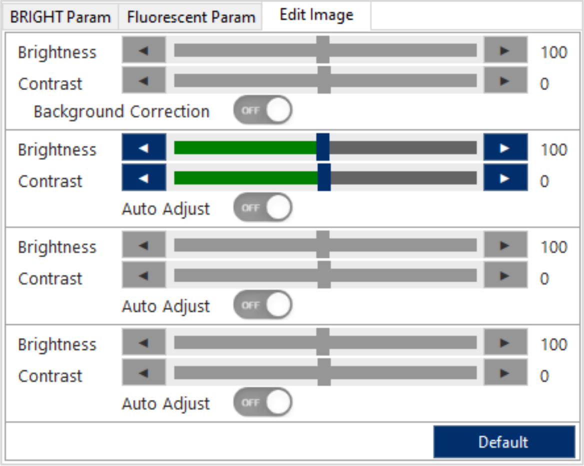


28 % confluence

Edit Image

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

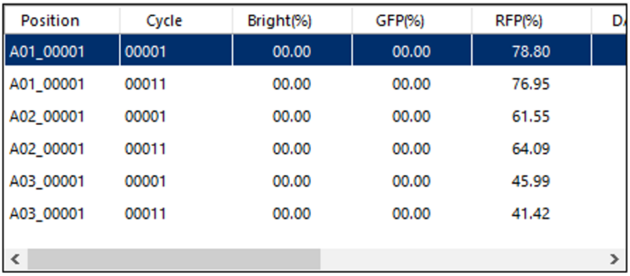
- By moving the **Brightness** and **Contrast** sliders followed by clicking **Apply** the confluence analysis results can be viewed instantly and the best settings can subsequently applied to the entire measurement.
- Clicking **Default** resets any changes.



☞ Note: To more selectively optimize of **Brightness** and **Contrast** settings of only specific wells or time points of a measurement, open the measurement project in **JuLI^{TM} EDIT** and save the original measurement as a new project. For more details, refer to 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 µm²) 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.



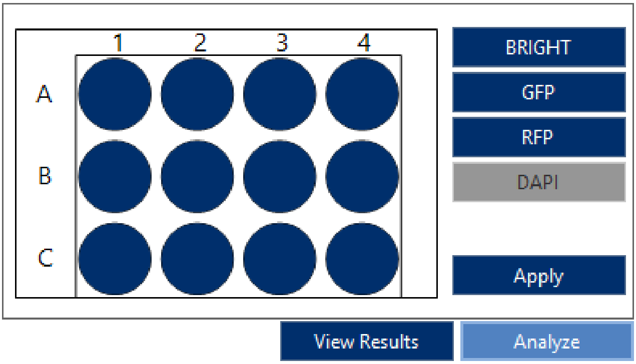
Position	Cycle	Bright(%)	GFP(%)	RFP(%)	D
A01_00001	00001	00.00	00.00	78.80	
A01_00001	00011	00.00	00.00	76.95	
A02_00001	00001	00.00	00.00	61.55	
A02_00001	00011	00.00	00.00	64.09	
A03_00001	00001	00.00	00.00	45.99	
A03_00001	00011	00.00	00.00	41.42	

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

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

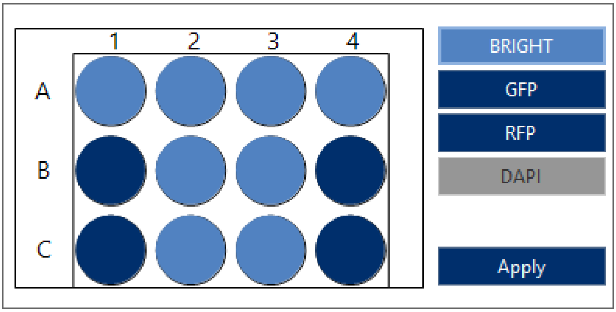
Analyze: Create exportable graphs and result tables

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.

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

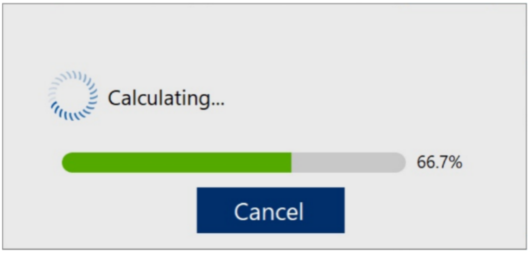


Click **Apply** to start the analysis (and creation of graphs and tables) or click once more on **Analyze** to go one step back.

☞ 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.

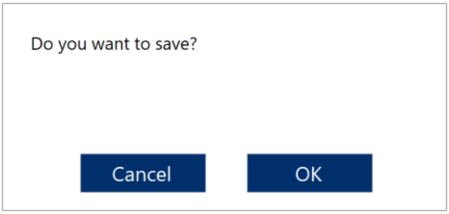
After clicking **Apply**, a window will display the progress of the analysis. Click **Cancel** if you want to stop it.



Calculating...

66.7%

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



Do you want to save?

View Results

Click **View Results** ⑩ to access various 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.**

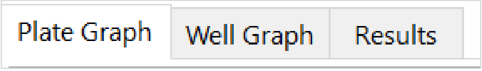
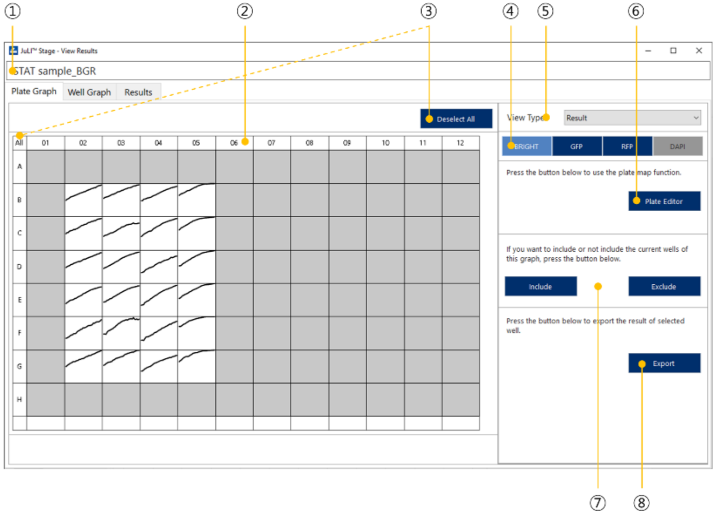


Plate Graph tab

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**.



STAT sample_BGR

View Type: Result

Press the button below to use the plate map function.

If you want to include or not include the current wells of this graph, press the button below.

Press the button below to export the result of selected well.

① Selected project / measurement
② Plate overview
③ Select and deselect All
④ Channel selection
⑤ Readout selection
⑥ Link to plate editor
⑦ Include/exclude wells
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 readout. 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, click on individual wells, or drag a rectangle over multiple wells using the mouse.
- Click **Include** or **Exclude** ⑦ to insert or remove all readouts of all channels belonging to marked wells from the graph and results presentation generated via **Export** ⑧. You can undo the selection by clicking **Deselect All** ③.

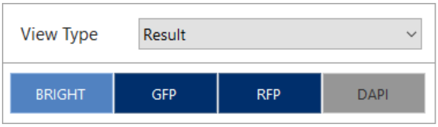
- A direct link to the **Plate Editor** ⑥ allows you to define a detailed experimental layout for the selected measurement project — for example, by assigning names, colors, or compound concentrations to each well. This can be used to adjust curve colors in the graph or to calculate mean values for replicates such as triplicates.

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

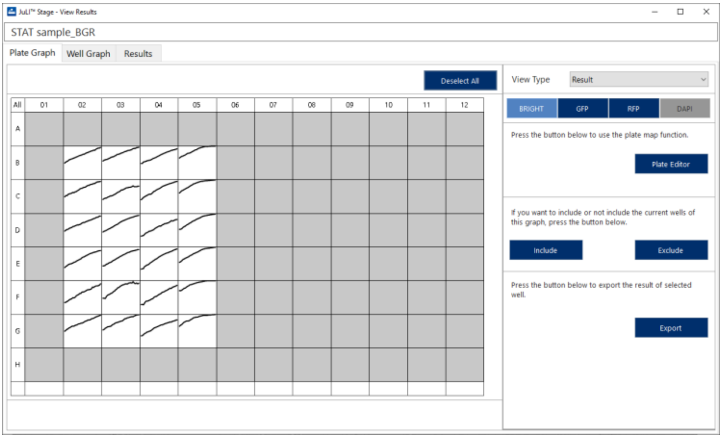
How to create, edit and export graphs and result tables step by step:

In this example, a time series of 24 wells on a 96 well plate were measured using the BRIGHT, GFP and RFP channels, and all three channels were analyzed in the **Growth Curve** module.

The default screen displays the results of the latest analysis, showing the BRIGHT channel by default:



Under **View Type** ⑤ 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:



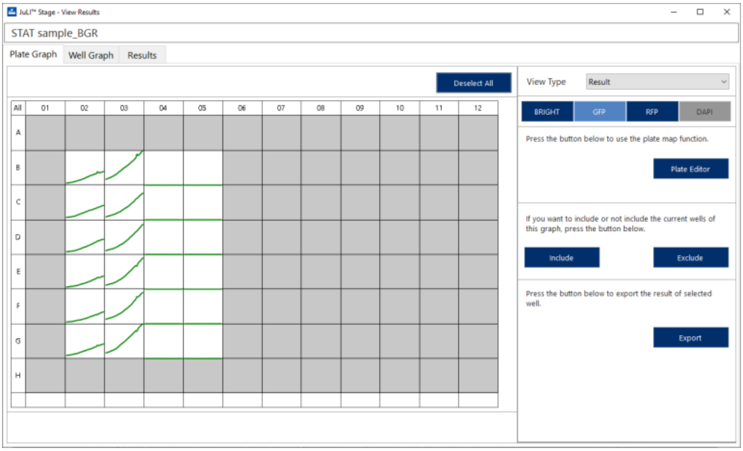
Press the button below to use the plate map function.

If you want to include or not include the current wells of this graph, press the button below.

Press the button below to export the result of selected well.

☞ 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 **GFP**, will display the **View Type Result** of the Growth Rate [%] analyzed on the basis of red fluorescent cells. The default color of the curves will match the color of the selected channel.



STAT sample_BGR

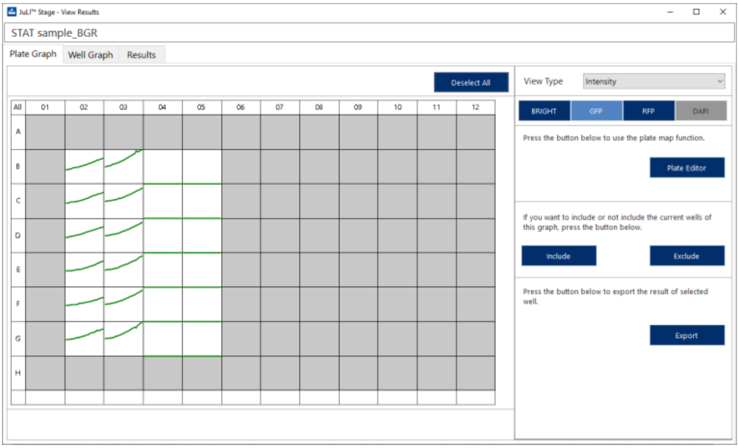
View Type: Result

Press the button below to use the plate map function.

If you want to include or not include the current wells of this graph, press the button below.

Press the button below to export the result of selected well.

☞ 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.



STAT sample_BGR

View Type: Intensity

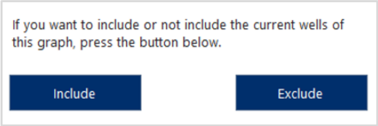
Press the button below to use the plate map function.

If you want to include or not include the current wells of this graph, press the button below.

Press the button below to export the result of selected well.

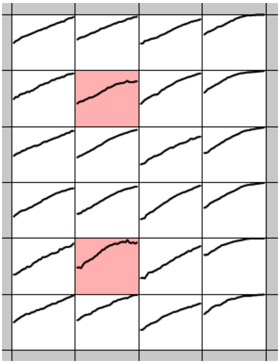
The readout Growth Rate can be changed to (mean) **Intensity** per well by selecting **Intensity** in the drop down menu of **View Type**.

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



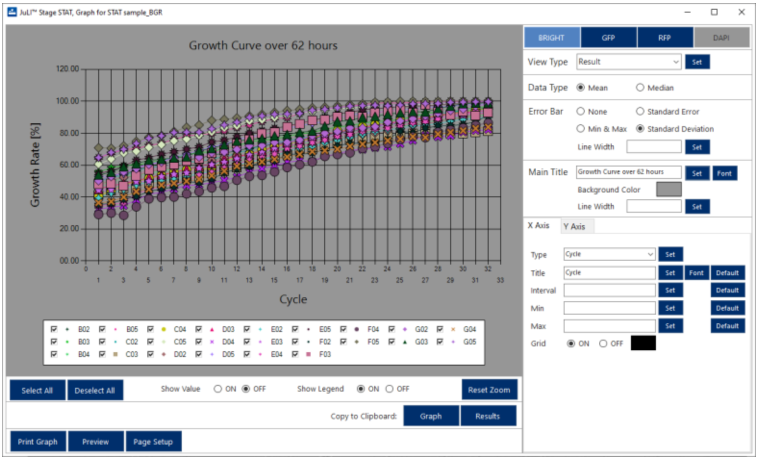
If you want to include or not include the current wells of this graph, press the button below.

Excluded wells are displayed in red. All results from these wells (in all channels) will not be shown in graphs and result tables generated via **Export**:



You can reverse this exclusion by clicking **Include**.

Finally, clicking **Export** will create a graph showing the results of all 24 wells and all time points in individually colored lines:



JuLI™ Stage STAT, Graph for STAT sample_BGR

View Type: Result

Data Type: ● Mean ○ Median

Error Bar: ○ None ○ Standard Error ○ Min & Max ● Standard Deviation

Main Title: Growth Curve over 62 hours

X Axis – Type: Cycle

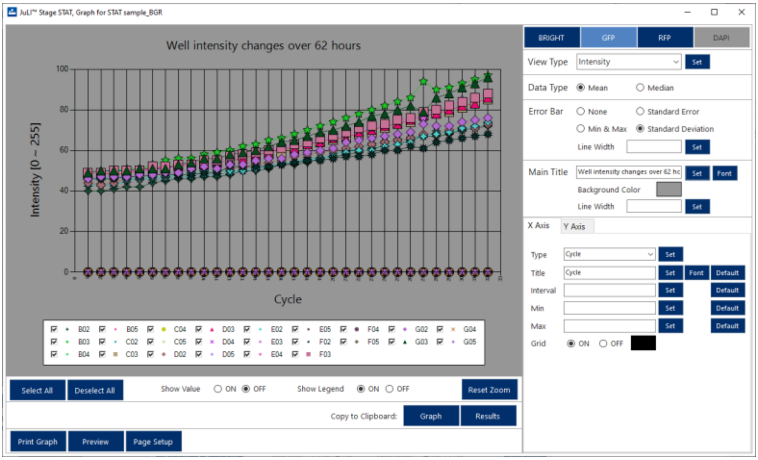
X Axis – Title: Cycle

Grid: ● ON ○ OFF

Show Value: ○ ON ● OFF

Show Legend: ● ON ○ OFF

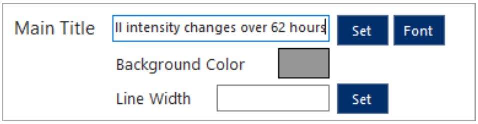
As before, clicking another channel or selecting another readout under **View Type** will display other available readouts. Here for example the mean intensity per well in the **GFP** channel:



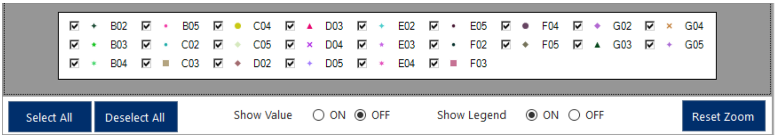
There are various options available to customize the appearance of the graphs, most of which are intuitive and easy to apply.

For example, the **Main Title** - which is set to the project name by default - can be edited to display a different title. The **Background Color** can also be adjusted.

To apply most of these changes, click **Set**.

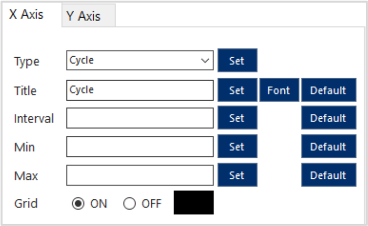

Main Title: ll intensity changes over 62 hours

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**.

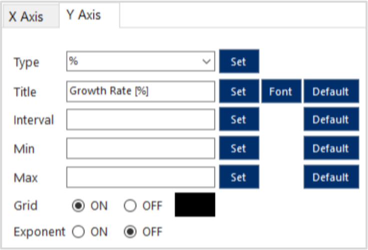

Show Value ○ ON ● OFF

Show Legend ● ON ○ OFF

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



Type: Cycle
Title: Cycle
Interval
Min
Max
Grid ● ON ○ OFF

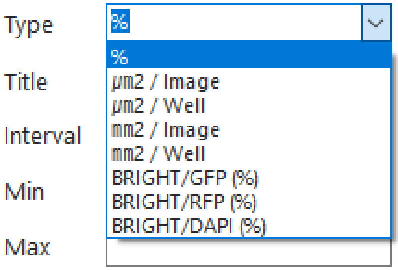


Type: %
Title: Growth Rate [%]
Interval
Min
Max
Grid ● ON ○ OFF
Exponent ○ ON ● OFF

☞ Note: Regardless of the selected **X-Axis Type** (e.g., Total Time or Cycle), the Interval value must be entered in number of cycles.

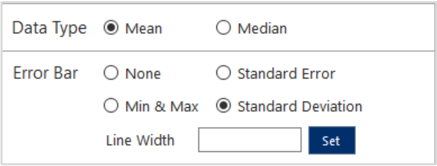
☞ Note: On the **X-Axis** only timelapse describing entries are selectable, not compound dilutions or any other entries defined in a plate map.

Various **Y-axis Type**s are provided for confluence display:



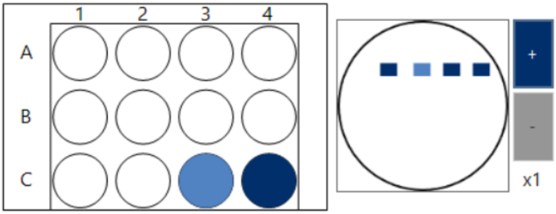
☞ Note: The units µm²/well and mm²/well are theoretical values: based on the detected cell-covered area the confluence in the whole well is extrapolated.

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

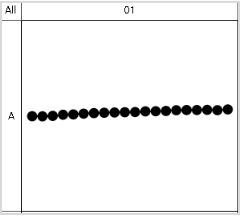


Data Type ● Mean ○ Median
Error Bar ○ None ○ Standard Error
○ Min & Max ● Standard Deviation
Line Width

One such condition occurs when multiple fields are acquired in an experiment, as in this example where four positions within the wells were measured:



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



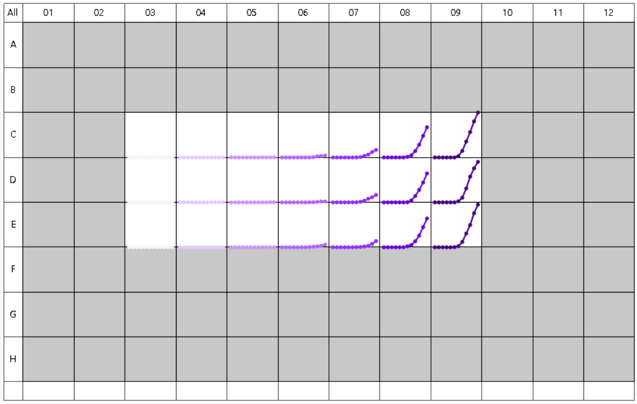
☞ Note: The results for each position in a well will be accessible in the **Well Graph** tab and can be exported as Graph or numerical readouts there. See later in this chapter.

☞ Note: It is not possible to exclude single positions in a well from the analysis or from the statistical calculations (mean, standard deviation, etc.), but they can be excluded from graphs.

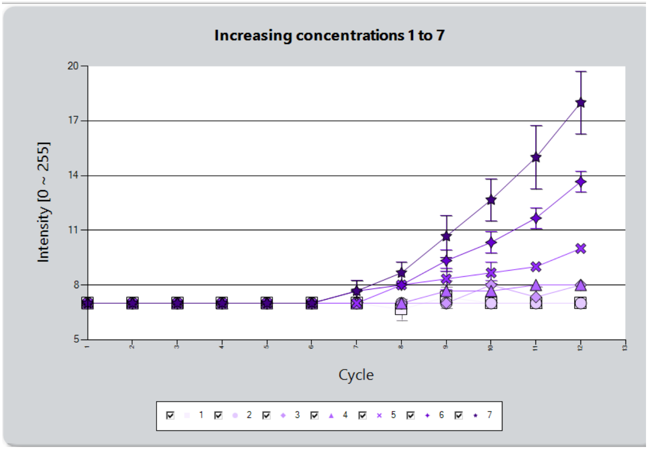
The other case, where **Data Type Mean** or **Median**, as well as all **Error Bar** options are accessible is when a plate map (created in **Plate Editor**) is available for the measurement project. Here, the software needs to know where well repeats are located in order to calculate mean values and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

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

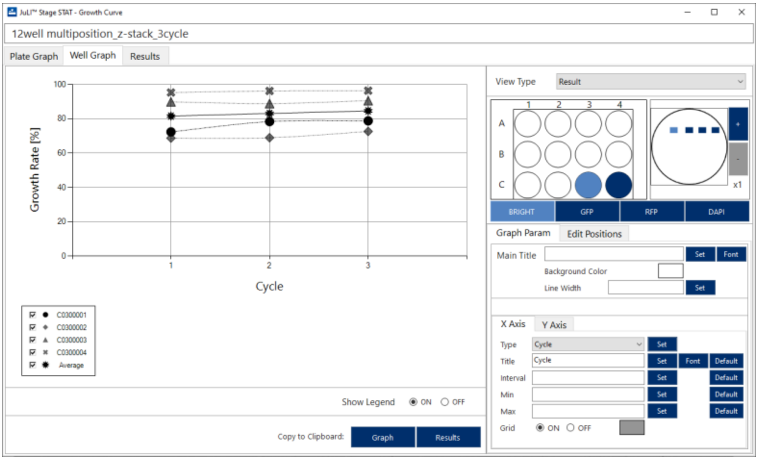
However, copying **Results** to the clipboard, exports the actual results data table (csv-format) (of the selected channel and readout), which can be opened and further processed with external software such as Excel or other spreadsheets. In case of mean values and standard deviations being available, 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.



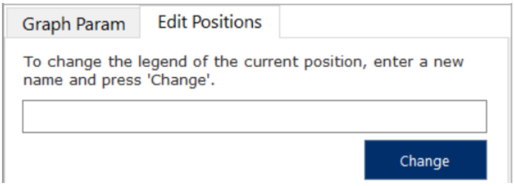
View Type: Result

Type: Cycle

Title: Cycle

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**.

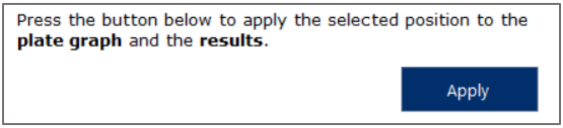
While the configuration options for graphs in **Graph Param** are mostly identical to those already described in detail on page 121, one additional option is available here: **Edit Positions**. In **Edit Positions**, each position in a well can be assigned a new legend name after selection, which is confirmed by clicking **Change**, and this change is reflected only in the **Well Graph**.



To change the legend of the current position, enter a new name and press 'Change'.

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

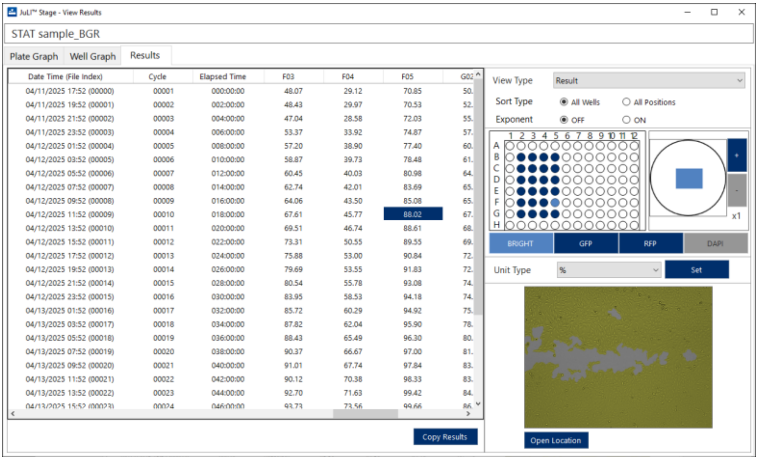
In addition, positions can be excluded from the **Plate Graph** and **Results** by deselecting them in the legend and click **Apply**.



Press the button below to apply the selected position to the **plate graph** and the **results**.

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**.

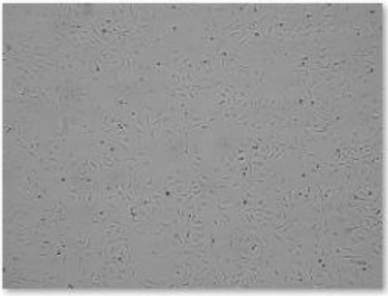


STAT sample_BGR

Date Time (File Index)	Cycle	Elapsed Time	F03	F04	F05	G02
04/11/2025 17:52 (00000)	00001	000:00:00	48.07	29.12	70.85	50.
04/11/2025 19:52 (00001)	00002	002:00:00	48.43	29.97	70.53	52.
04/11/2025 21:52 (00002)	00003	004:00:00	47.04	28.58	72.03	55.
04/11/2025 23:52 (00003)	00004	006:00:00	53.37	33.92	74.87	57.
04/12/2025 01:52 (00004)	00005	008:00:00	57.20	38.90	77.40	60.
04/12/2025 03:52 (00005)	00006	010:00:00	58.87	39.73	78.48	61.
04/12/2025 05:52 (00006)	00007	012:00:00	60.45	40.03	80.98	64.
04/12/2025 07:52 (00007)	00008	014:00:00	62.74	42.01	83.69	65.
04/12/2025 09:52 (00008)	00009	016:00:00	64.06	43.50	85.08	65.
04/12/2025 11:52 (00009)	00010	018:00:00	67.61	45.77	88.02	67.
04/12/2025 13:52 (00010)	00011	020:00:00	69.51	46.74	88.61	68.
04/12/2025 15:52 (00011)	00012	022:00:00	73.31	50.55	89.55	69.
04/12/2025 17:52 (00012)	00013	024:00:00	75.88	53.00	90.84	72.
04/12/2025 19:52 (00013)	00014	026:00:00	79.69	53.55	91.83	72.
04/12/2025 21:52 (00014)	00015	028:00:00	80.54	55.78	93.08	74.
04/12/2025 23:52 (00015)	00016	030:00:00	83.95	58.53	94.18	74.
04/13/2025 01:52 (00016)	00017	032:00:00	85.72	60.29	94.92	75.
04/13/2025 03:52 (00017)	00018	034:00:00	87.82	62.04	95.90	78.
04/13/2025 05:52 (00018)	00019	036:00:00	88.43	65.49	96.30	80.
04/13/2025 07:52 (00019)	00020	038:00:00	90.37	66.67	97.00	81.
04/13/2025 09:52 (00020)	00021	040:00:00	91.01	67.74	97.84	83.
04/13/2025 11:52 (00021)	00022	042:00:00	90.12	70.38	98.33	83.
04/13/2025 13:52 (00022)	00023	044:00:00	92.70	71.63	99.42	84.
04/13/2025 15:52 (00023)	00024	046:00:00	93.73	73.56	99.66	86.

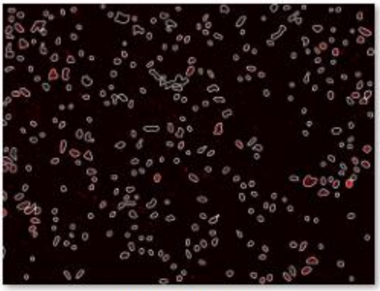
View Type: Result
Sort Type: ● All Wells ○ All Positions
Exponent: ● OFF ○ ON
Unit Type: %

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: Image files are named starting from “00000”, whereas the cycle numbers shown in the analysis results begin at 1. For example, if 12 cycles were acquired, images from cycle 1 are saved as “00000”, and those from cycle 12 as “00011”.

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.

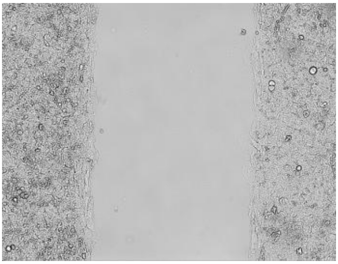
12.2.2.2 Scratch Basic

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

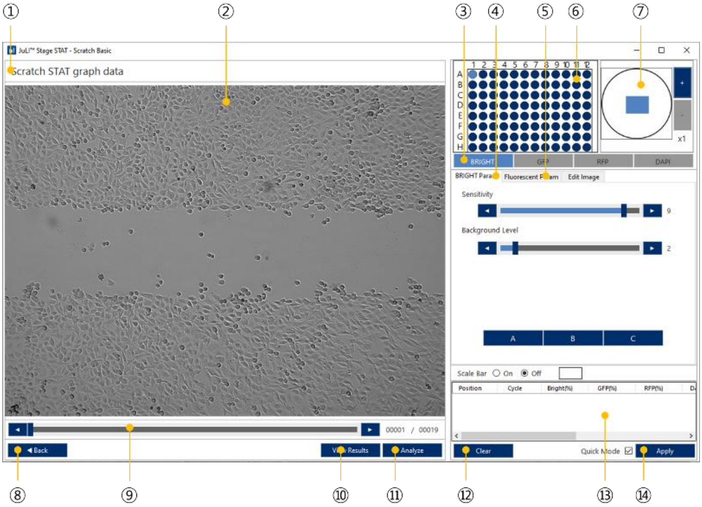
The main differences compared to the optionally available software **JuLI™ Stage Scratch STAT** are that this software provides only one readout – wound confluence over time – instead of 6, such as relative wound density and speed of wound closure. Additionally, it can be applied to all magnifications,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

The wound can be oriented either horizontally or vertically.

10x, horizontal orientation



4x, vertical orientation



① Project name

② Image view

③ Channel selection

④ Bright channel parameter

⑤ Fluorescence channel parameter

⑥ Vessel display

⑦ Well navigation

⑧ Back

⑨ Time lapse slider

⑩ View results

⑪ Analyze

⑫ Clear results list

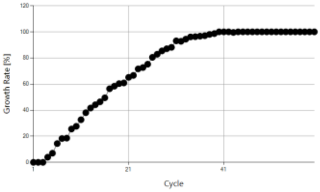
⑬ Confluence result list

⑭ Apply

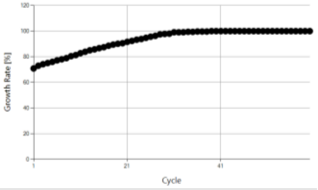
In principal, the interface for optimizing the analysis parameters and to analyze and view the results, is identical to the **Growth Curve** analysis. Therefore please refer to chapter “12.2.2.1 Growth Curve” for further details.

The main difference between **Scratch Basic** and **Growth Curve** lies in the output readout. **Scratch Basic** provides Wound confluence [%], which starts at 0 % in cycle 00001 as it detects the closing wound area over time. In **Growth Curve,** the graph starts at an initial value reflecting the cell confluence in the image in cycle 00001, 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 per wound). However, after running the analysis by clicking **Analyze**, wound-related confluence results are displayed.

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

12.2.2.3 Attached Cell Counting

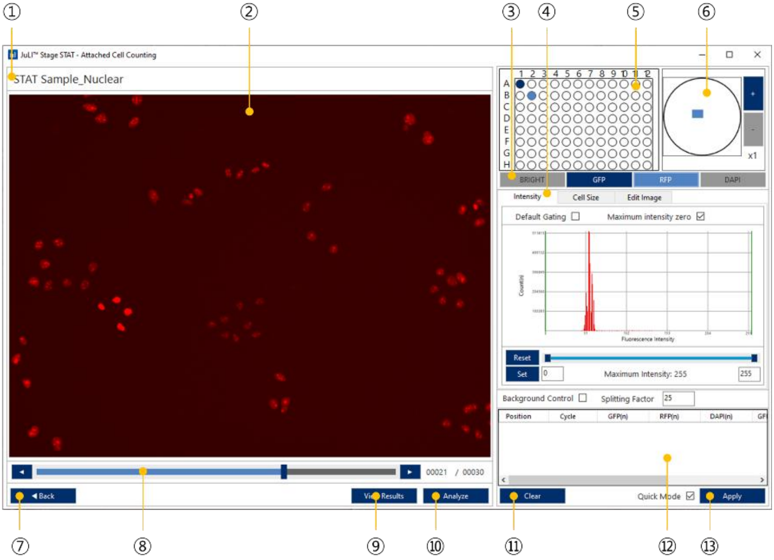
(N)
Attached
Cell Counting

With the **Attached Cell Counting** evaluation module, fluorescently labeled adherent 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 available when a fluorescence channel is acquired; it is automatically disabled (shown in grey) for brightfield-only projects.

☞ 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

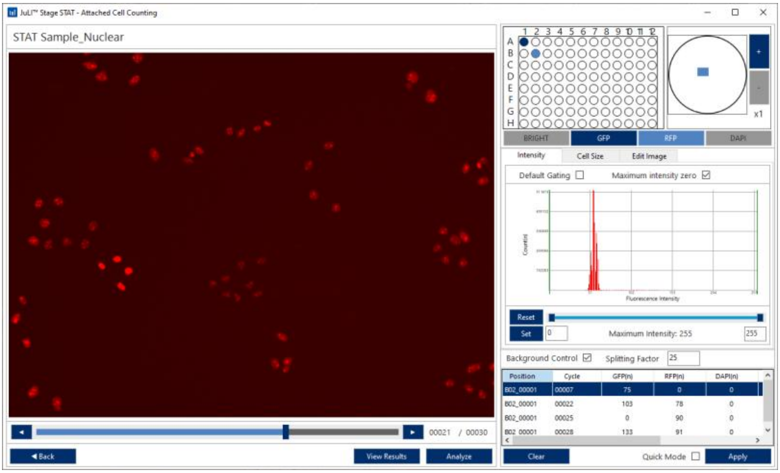
⑪ Clear

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.



Default Gating ☐ Maximum intensity zero ☑

0 Maximum Intensity: 255 255

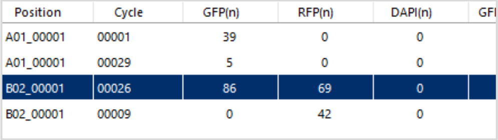
Background Control ☑ Splitting Factor 25

Position	Cycle	GFP(n)	RFP(n)	DAPI(n)
B02_00001	00007	75	0	0
B02_00001	00022	103	78	0
B02_00001	00025	0	90	0
B02_00001	00028	133	91	0

Quick Mode ☐

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):

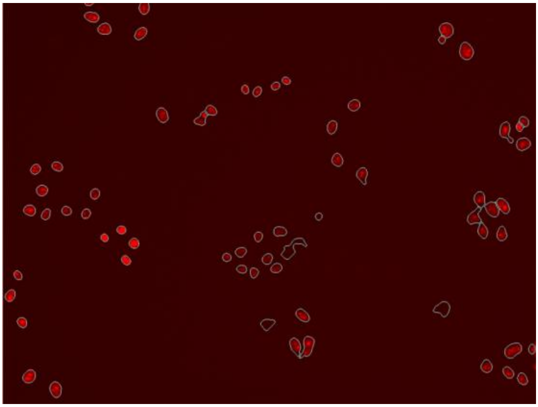


Position	Cycle	GFP(n)	RFP(n)	DAPI(n)	GFI
A01_00001	00001	39	0	0	
A01_00001	00029	5	0	0	
B02_00001	00026	86	69	0	
B02_00001	00009	0	42	0	

Scrolling in the results table to the right will display the result of counted cells per mm². The size of the imaged position in a well = one Field of View (FOV) depends on the objective used in the measurement: for

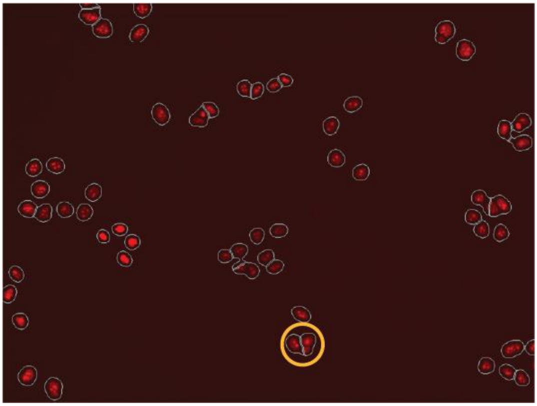
- 4x the size of one FOV is 3.55 mm²
- 10x the size of one FOV is 0.58 mm²
- 20x the size of one FOV is 0.14 mm²

Default settings, **Apply**



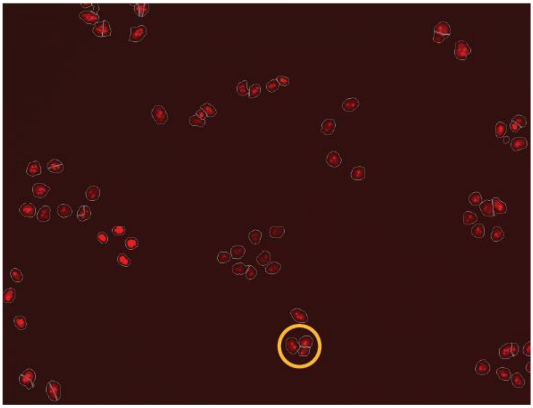
Applying the default settings to this image results in dim cells not being segmented and some falsely selected background signals:

Background Control checked



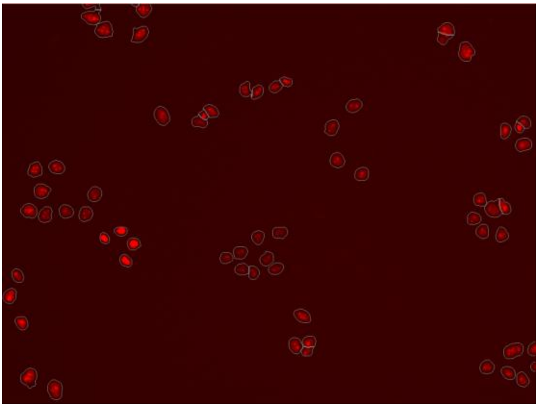
Using the **Background Control** (effecting an automatic background brightness correction) results in the false signal being excluded, but still some falsely fused (see yellow circle for example).

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.)

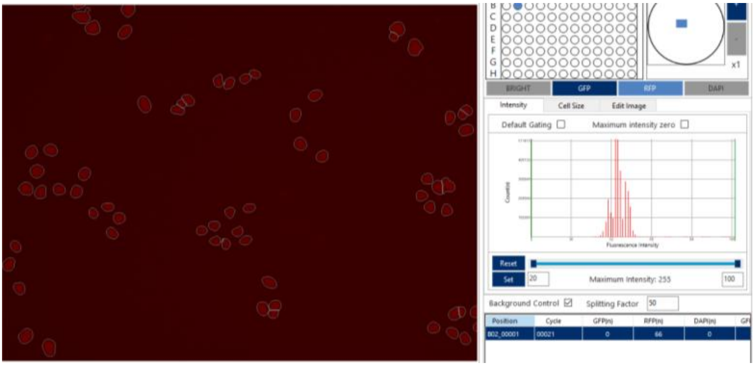
Splitting Factor changed from 25 to 50



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 of 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**



Default Gating ☐ Maximum intensity zero ☐

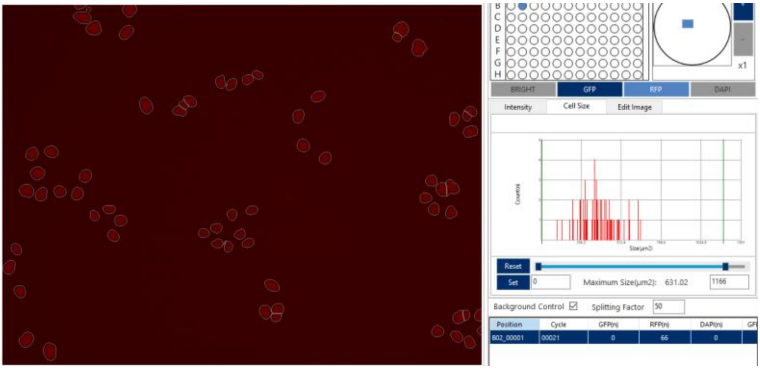
Set 20 Maximum Intensity: 255 100

Background Control ☑ Splitting Factor 50

Position	Cycle	GFP(n)	RFP(n)	DAPI(n)	GF
B02_00001	00021	0	66	0	

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 [µm²], 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).



Set 0 Maximum Size(µm2): 631.02 1166

Background Control ☑ Splitting Factor 50

Position	Cycle	GFP(n)	RFP(n)	DAPI(n)	GF
B02_00001	00021	0	66	0	

☞ 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 “12.2.2.1 Growth Curve” in detail.

☞ Note: If multiple positions were acquired within a well, the value shown as [n/well] is calculated based on the cell density (cells/mm²) derived from the acquired images, which is then extrapolated to the total well area.

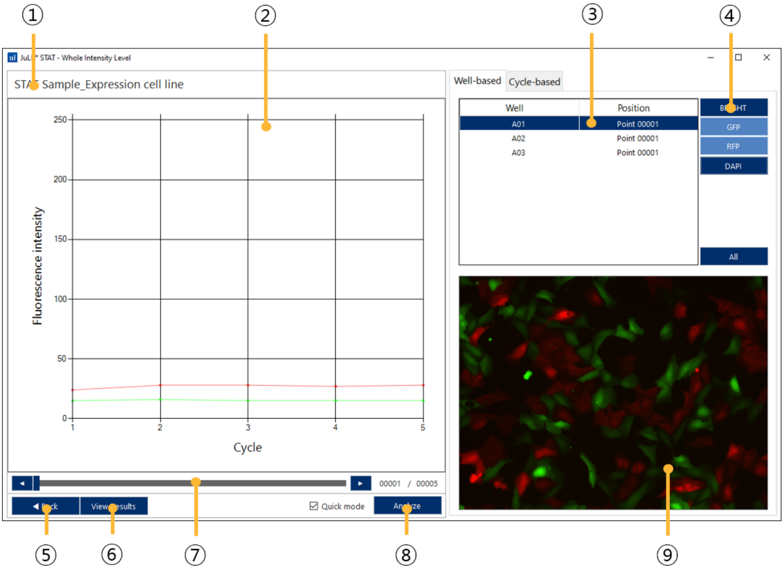
For example, three positions were acquired using a 10× objective (0.58 mm² per field of view) in a 384-well plate. Cell counts for the three images were 180, 126, and 85, resulting in a mean of 130 cells/image. Based on this, the average cell density is 224 cells/mm². Using this density and the total well area, the estimated total number of cells per well ([n/well]) is then calculated.

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.

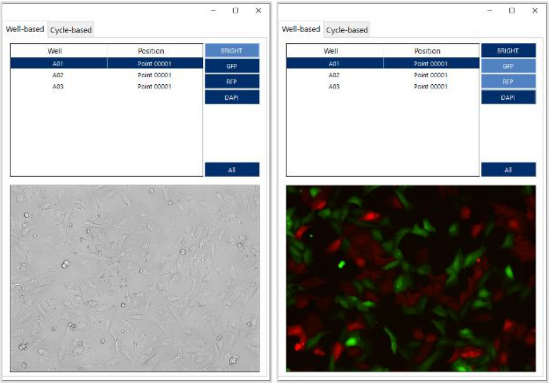
☞ Note: **Whole Intensity Level** cannot be calculated for the BRIGHT channel.



Well	Position
A01	Point 00001
A02	Point 00001
A03	Point 00001

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

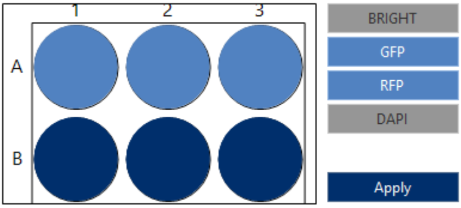
Start by selecting a project/measurement and click **Select Analysis - Whole Intensity Level**: in the initial screen, images of all acquired channels can be viewed separately or as an overlay. To do so, select rows in the Well list ③ in the **Well-based** tab and choose the desired channels in the Channels selection ④.



Well	Position
A01	Point 00001
A02	Point 00001
A03	Point 00001

Well	Position
A01	Point 00001
A02	Point 00001
A03	Point 00001

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

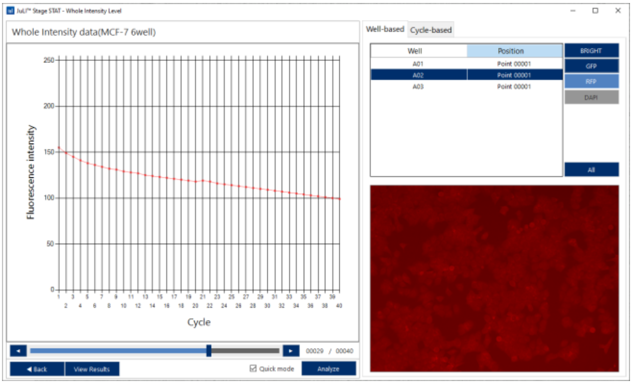


☞ Note: Measurements acquired with more than one position per well can be displayed (named Point 00001, Point 00002, etc.) in the initial screen, but the analysis will only calculate mean values per well.

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

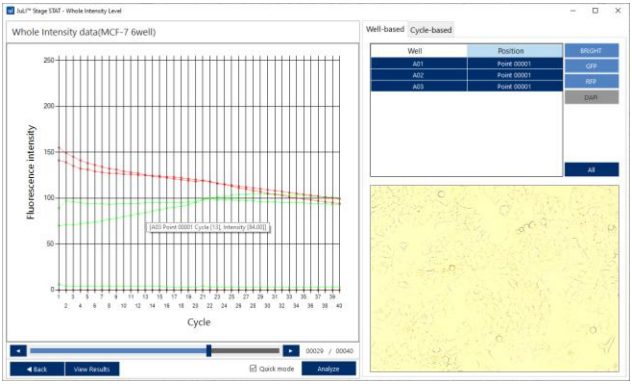
Well-based result display

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.

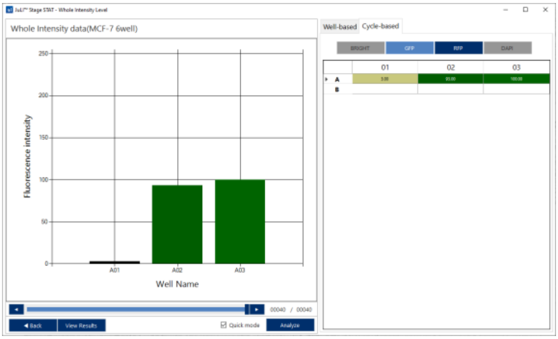
Cycle-based results display

In the **Cycle-based** display, bar charts with heatplot coloring are shown on the left (brighter colors for higher values, darker for lower), while numerical results for each well are listed on the right as a well-based display. Using the time-lapse slider, different cycles can be browsed; however, only one channel is displayed at a time:

GFP channel, Cycle 00001

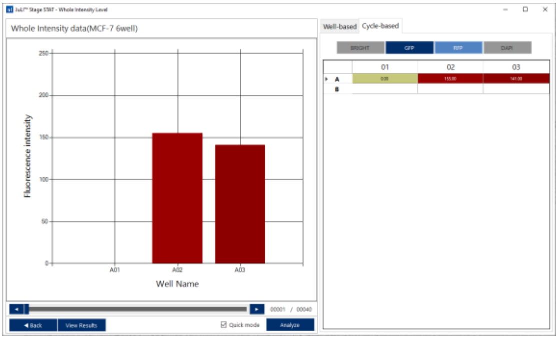
	01	02	03
A	6.00	88.00	70.00
B			

GFP channel, Cycle 00040



	01	02	03
A	3.00	93.00	100.00
B			

RFP channel, Cycle 00001



	01	02	03
A	0.00	155.00	141.00
B			

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

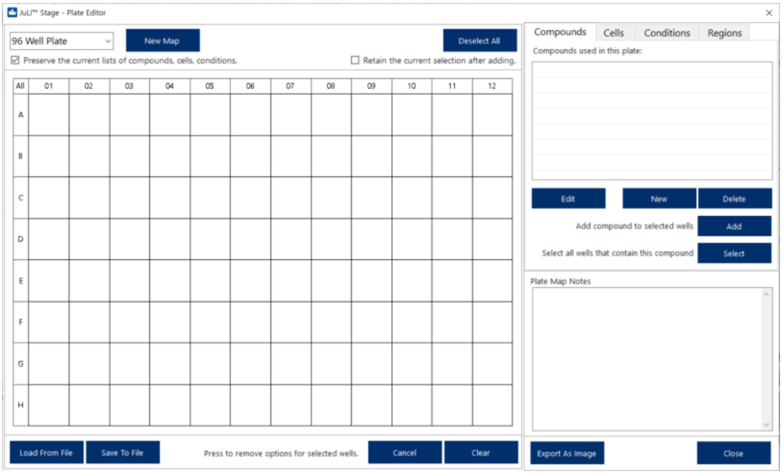
12.2.3 Plate Editor

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.

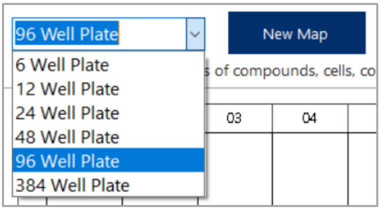
There are two ways to create and save plate maps: either via the **Plate Editor** icon or in the **Plate Graph** tab of the **View Results** section of each analysis module.

Creating a plate map step-by-step:

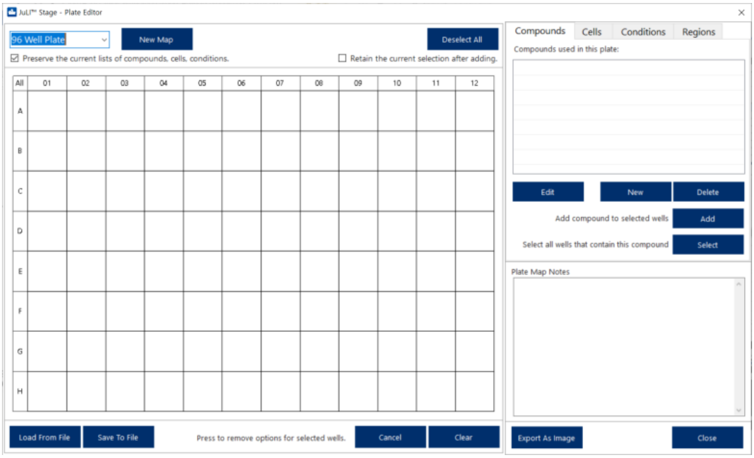
Start by clicking the **Plate Editor** icon to open it:



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**: 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:

- First, any entries made in any of the tabs will appear in the header of result tables and in legends of graphs.
- Second, 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_{50} calculations.

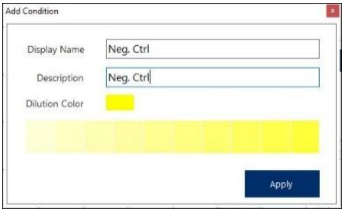
All plate map entries are only aiming at

- 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:

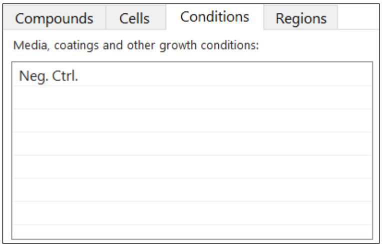


Display Name: Neg. Ctrl

Description: Neg. Ctrl

Dilution Color

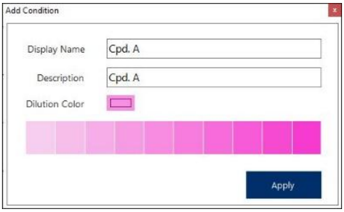
The new entry appears in the list of the **Conditions** tab:



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:

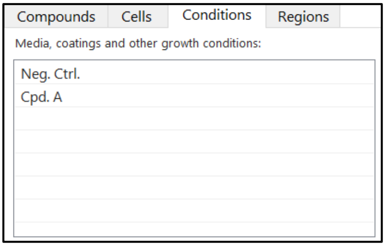


Display Name: Cpd. A

Description: Cpd. A

Dilution Color

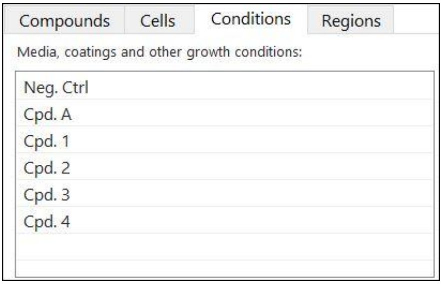
Now, two entries are displayed:



Media, coatings and other growth conditions:

Neg. Ctrl.
Cpd. A

Continue, until 6 conditions are defined using different colors:

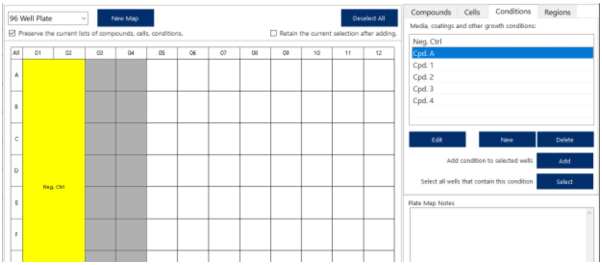


Media, coatings and other growth conditions:

Neg. Ctrl
Cpd. A
Cpd. 1
Cpd. 2
Cpd. 3
Cpd. 4

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**.



96 Well Plate

☑ Preserve the current lists of compounds, cells, conditions.

☐ Retain the current selection after adding.

Media, coatings and other growth conditions:

Neg. Ctrl
Cpd. A
Cpd. 1
Cpd. 2
Cpd. 3
Cpd. 4

Add condition to selected wells

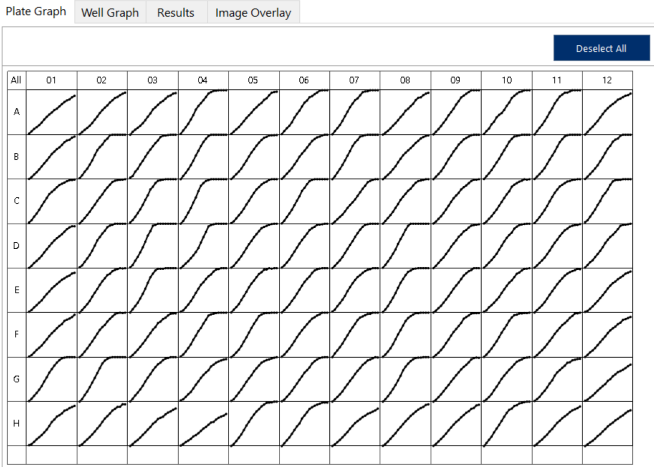
Select all wells that contain this condition

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

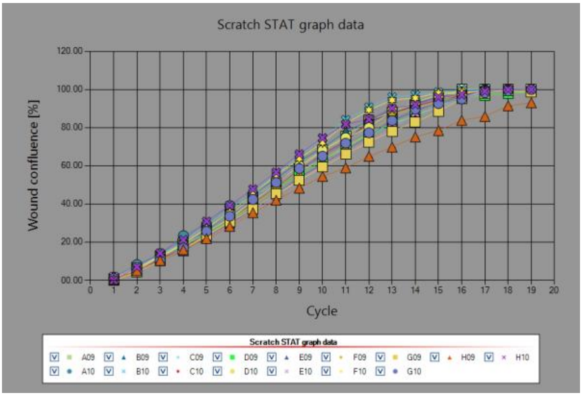
Click **Save To File** to save the plate map to a folder of your choice.

Now, we can compare the results of an existing analysis with and without plate map:

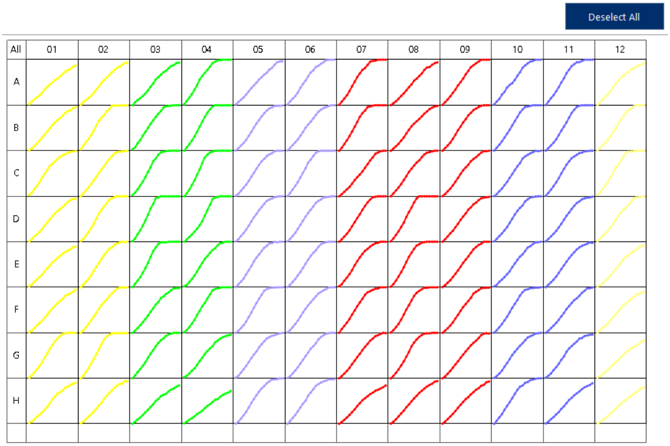
Via **Select Analysis** and **View Results** open a **Plate Graph**. Graphs of the analysis will be displayed in one color:



Click on Export: After excluding all columns except 9 and 10, 16 curves belonging to 16 wells are displayed - each in an individual color:

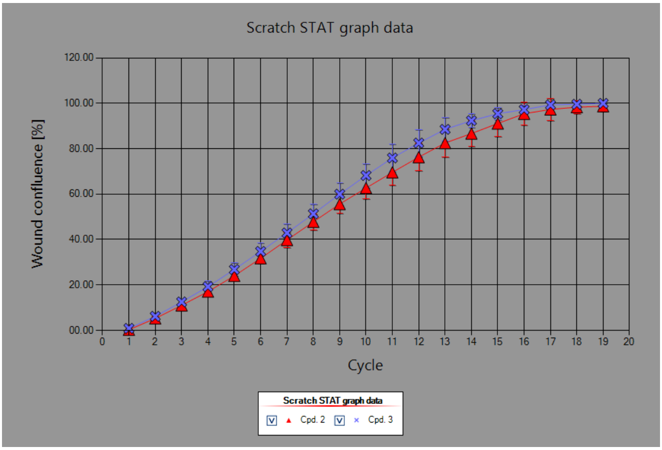


In order to apply the created plate map, close the **Export** window again, and in the **Plate Graph** tab click **Plate Editor – Load From File.** Open the .**StageMap** file and click **Apply:**



The colors of the displayed curves automatically update to match the compound colors set in the plate map.

Click Export again: Now, instead of 16 curves only 2 curves are displayed, showing the mean of 8 repeats from column 9 and 10, and their standard deviation; also, the legend entries display the compound names from the plate map.



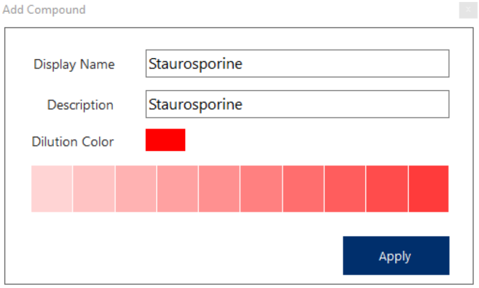
☞ Note: The plate map belonging to a measurement is not attached permanently to a measurement but needs to be loaded again every time the analysis is opened.

Until now, only the **Conditions** tab – one of four in the **Plate Editor** – has been used.

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:

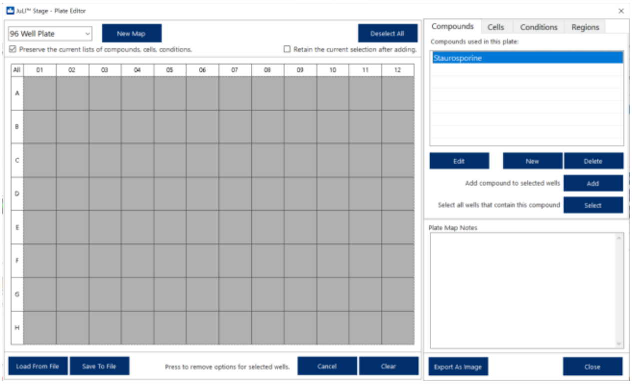
1. Click **New** to enter the compound name followed by **Apply**.



Display Name: Staurosporine

Description: Staurosporine

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:



96 Well Plate

☑ Preserve the current lists of compounds, cells, conditions.

☐ Retain the current selection after adding.

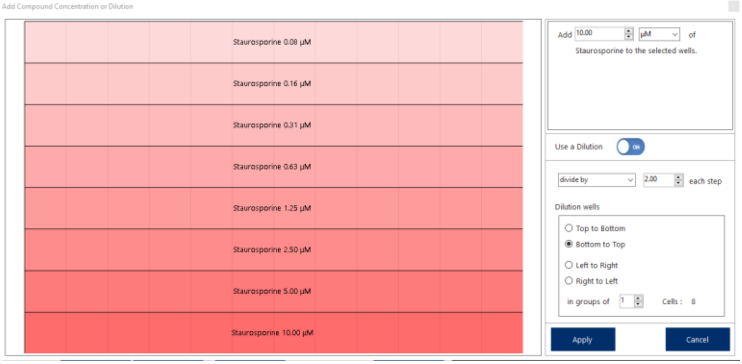
Compounds used in this plate:
Staurosporine

Add compound to selected wells

Select all wells that contain this compound

Press to remove options for selected wells.

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 µM diluted step wise by a factor 2, one row per concentration, was defined:



Add 10.00 µM of Staurosporine to the selected wells.

Use a Dilution ON

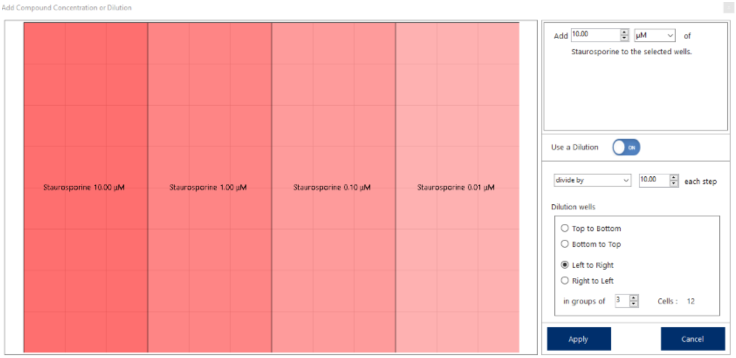
divide by 2.00 each step

Dilution wells

○ Top to Bottom
● Bottom to Top
○ Left to Right
○ Right to Left

in groups of 1 Cells : 8

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.)



Add Compound Concentration or Dilution

Add 10.00 µM of Staurosporine to the selected wells.

Use a Dilution ON

divide by 10.00 each step

Dilution wells

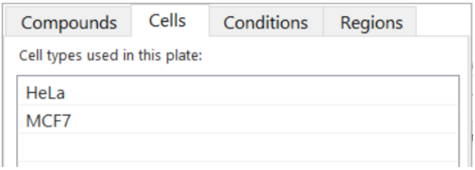
○ Top to Bottom
○ Bottom to Top
● Left to Right
○ Right to Left

in groups of 3 Cells : 12

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.

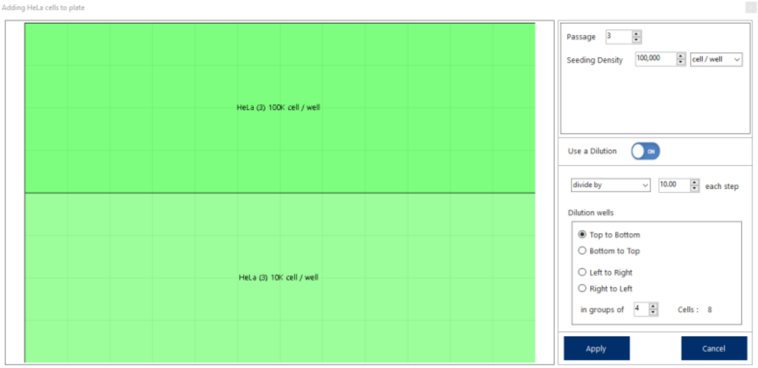
1. Open the **Cells** tab, click **New** to enter e.g. two new cell types, followed by **Apply**.



Cell types used in this plate:

HeLa
MCF7

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.
3. 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:



HeLa (3) 100K cell / well

HeLa (3) 10K cell / well

Passage 3

Seeding Density 100,000 cell / well

Use a Dilution ON

divide by 10.00 each step

Dilution wells

● Top to Bottom

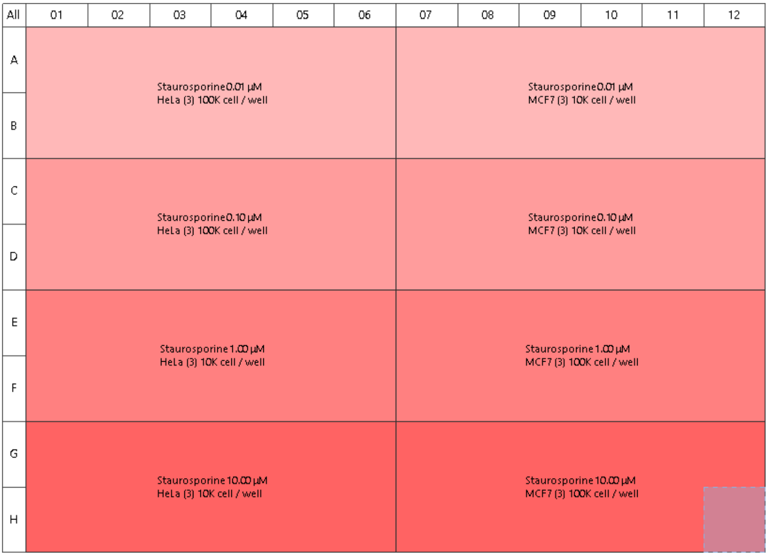
○ Bottom to Top

○ Left to Right

○ Right to Left

in groups of 4 Cells : 8

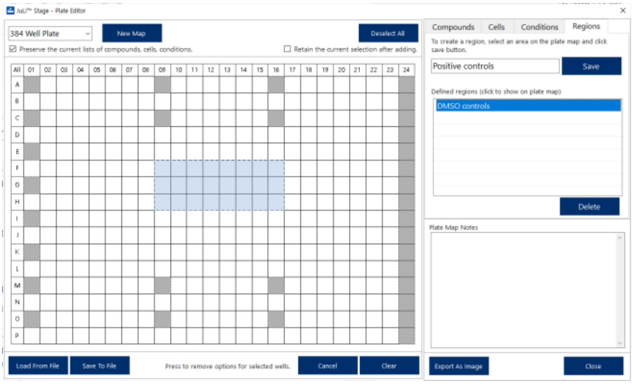
4. This can be repeated for all 3 tabs and the final layout will contain all information at a glance: compound name, concentration, cell type, seeding density, and condition.



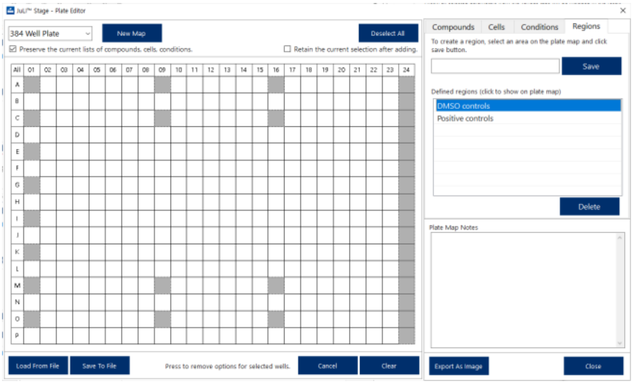
All	01	02	03	04	05	06	07	08	09	10	11	12
A	Staurosporine0.01 µM HeLa (3) 100K cell / well						Staurosporine0.01 µM MCF7 (3) 10K cell / well					
B												
C	Staurosporine0.10 µM HeLa (3) 100K cell / well						Staurosporine0.10 µM MCF7 (3) 10K cell / well					
D												
E	Staurosporine 1.00 µM 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												

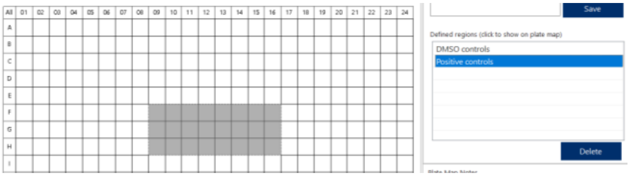
The tab **Regions** can be used to mark specific wells to bundle them into regions, which will ease the selection of wells in the other tabs.

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:



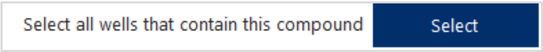
Then click on one region in the regions list and wells belonging to the regions will be marked accordingly.

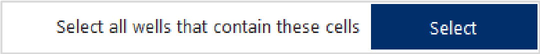




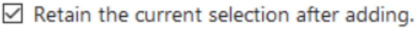
When the tab is changed to e.g. **Compounds**, the wells belonging to a region can be edited more easily.

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.





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 **Retain the current selection after adding** was checked: then the formerly marked wells will be available after a further tab change:


☑ Retain the current selection after adding.

Selecting the **Preserve the current lists of compounds, cells, conditions** checkbox ensures that the entered list is retained when changing the plate type.


☑ Preserve the current lists of compounds, cells, conditions.

Button **Export As Image**:

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

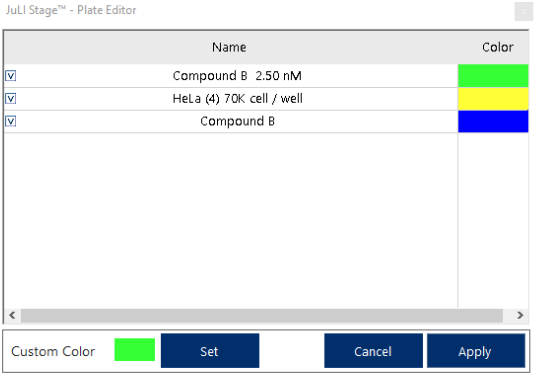
Buttons **Cancel** and **Clear**:

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

Buttons **Edit** and **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.



	Name	Color
☑	Compound B 2.50 nM	🟩
☑	HeLa (4) 70K cell / well	🟨
☑	Compound B	🟦

13 Software Installation

13.1 Codec installation

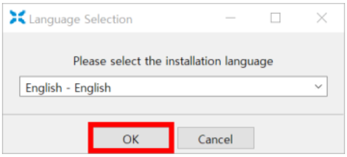
☞ Note: The codec file must be installed on **drive C** and needs to be installed only once for all JuLI™ Stage Apps.

☞ Note: If you use the JuLI™ Stage EDIT or STAT software on the JuLI™ Stage Desktop PC, codec installation is not required.

☞ Note: If you want to install the JuLI™ Stage EDIT or STAT software on your **personal PC**, copy the **'codec setup.exe'** and the **'JuLI™ Stage EDIT Setup.exe'** or **'JuLI™ Stage STAT Setup.exe'** file to your personal PC. Then please follow the steps below.

1. Double click the **'codec setup.exe'** file to initiate the installation.

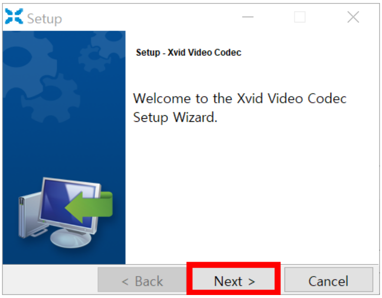
2. Select language and click **'OK'** button.



Please select the installation language

English - English

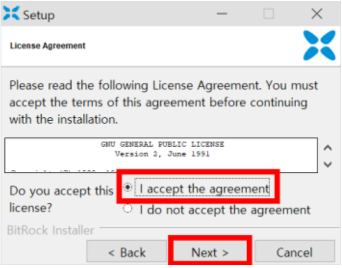
3. Click **'Next'** button.



Setup - Xvid Video Codec

Welcome to the Xvid Video Codec Setup Wizard.

4. Accept the agreement and click **'Next'** button.



License Agreement

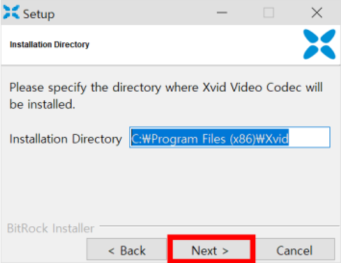
Please read the following License Agreement. You must accept the terms of this agreement before continuing with the installation.

GNU GENERAL PUBLIC LICENSE
Version 2, June 1991

Do you accept this license?

● I accept the agreement
○ I do not accept the agreement

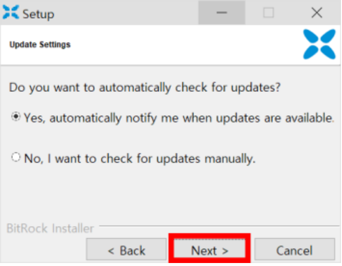
5. Enter/confirm the installation directory and click **'Next'** button twice.



Installation Directory

Please specify the directory where Xvid Video Codec will be installed.

Installation Directory C:₩Program Files (x86)₩Xvid

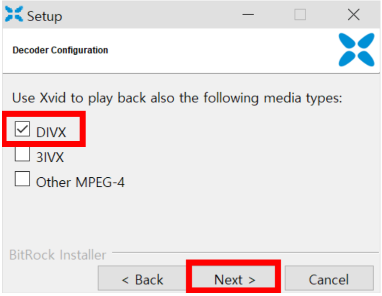


Update Settings

Do you want to automatically check for updates?

● Yes, automatically notify me when updates are available.
○ No, I want to check for updates manually.

6. Select **'DIVX' type** and click **'Next'** button

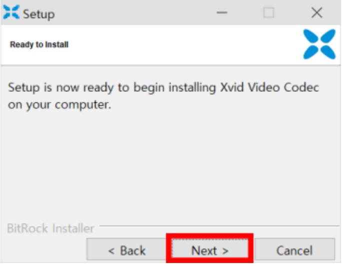


Decoder Configuration

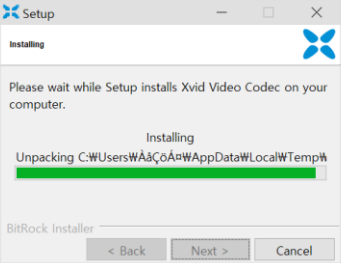
Use Xvid to play back also the following media types:

☑ DIVX
☐ 3IVX
☐ Other MPEG-4

7. Click **‘Next’** button again to start the installation



Setup is now ready to begin installing Xvid Video Codec on your computer.

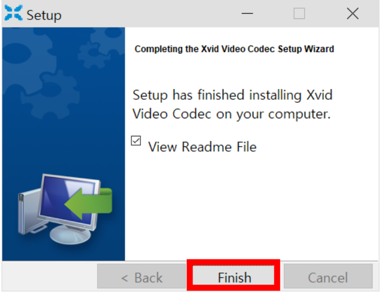


Please wait while Setup installs Xvid Video Codec on your computer.

Installing

Unpacking C:₩Users₩ÀåÇöÁ¤₩AppData₩Local₩Temp₩

8. Click **‘Finish’** button to complete the installation.



Completing the Xvid Video Codec Setup Wizard

Setup has finished installing Xvid Video Codec on your computer.

☑ View Readme File

13.2 JuLI™ Stage EDIT / STAT Software Installation

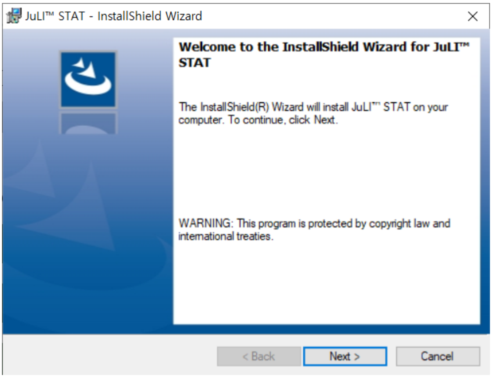
☞ Note: The **JuLI™ Stage EDIT or STAT** software must be installed on drive C.

☞ Note: If you want to install the JuLI™ Stage EDIT or STAT software on your personal PC, please refer to below recommend specification,

Description	Specification
Windows	Windows 10
CPU	Multi Core Processor (Intel i5 or over)
RAM	8 GB
Hard disk space	1 GB

☞ Note: If you want to install the JuLI™ Stage EDIT or STAT software on your **personal PC**, copy the **'codec setup.exe'** and the **'JuLI™ Stage EDIT Setup.exe'** or **'JuLI™ Stage STAT Setup.exe'** file to your personal PC. Then, please follow the steps below.

1. Double click **JuLI™ Stage EDIT Setup.exe** or **JuLI™ Stage STAT Setup.exe** to initiate the installation routine.
2. Click **Next**.



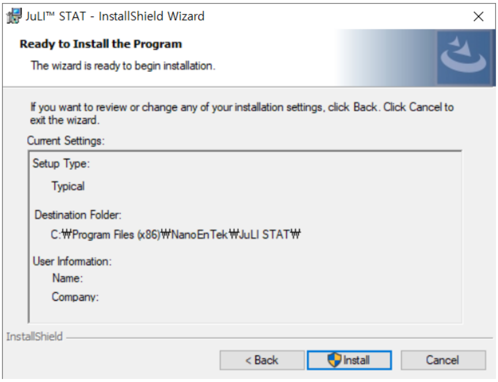
JuLI™ STAT - InstallShield Wizard

Welcome to the InstallShield Wizard for JuLI™ STAT

The InstallShield(R) Wizard will install JuLI™ STAT on your computer. To continue, click Next.

WARNING: This program is protected by copyright law and international treaties.

3. Click **Install**.



Ready to Install the Program

The wizard is ready to begin installation.

If you want to review or change any of your installation settings, click Back. Click Cancel to exit the wizard.

Current Settings:

Setup Type:

Typical

Destination Folder:

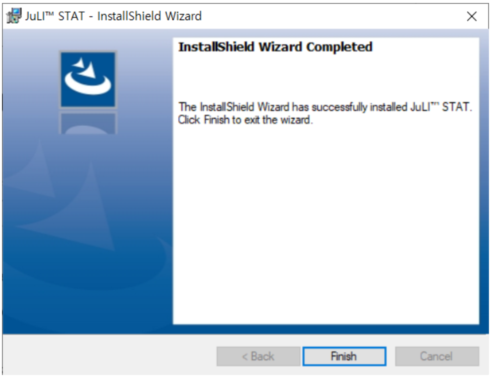
C:₩Program Files (x86)₩NanoEnTek₩JuLI STAT₩

User Information:

Name:

Company:

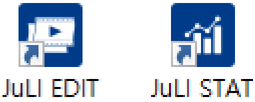
4. Click **Finish** to complete the installation.



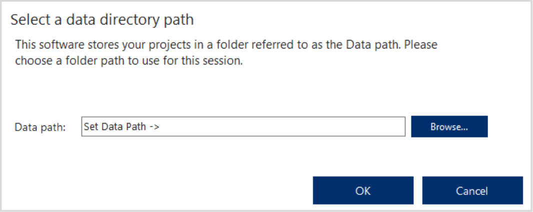
InstallShield Wizard Completed

The InstallShield Wizard has successfully installed JuLI™ STAT. Click Finish to exit the wizard.

5. Double click the **JuLI™ Stage EDIT or STAT** icon on the desktop to run the program.



Select a data directory path. Click **Browse…** to select the parent folder containing measurement projects. Then click **OK**.



Select a data directory path

This software stores your projects in a folder referred to as the Data path. Please choose a folder path to use for this session.

Data path: Set Data Path ->

☞ ***Note: Select a parent folder which contains project folders. Do not select a single project folder.***

JuLI™ Stage

Real-time live cell imaging system

NESMU-JST-001E (V.2.6)

NanoEntek

NanoEntek, Inc.
851-14, Seohae-ro, Paltan-myeon, Hwaseong-si
Gyeonggi-do, 18531, Korea
Tel: +82-2-6220-7940
Fax: +82-2-6220-7999

NanoEntek America, Inc.
220 Bear Hill Road, Suite 102, Waltham, MA 02451, USA
Tel: +1-781-472-2558
Fax: +1-781-790-5649

E-mail
sales@nanoentek.com

Website
www.nanoentek.com