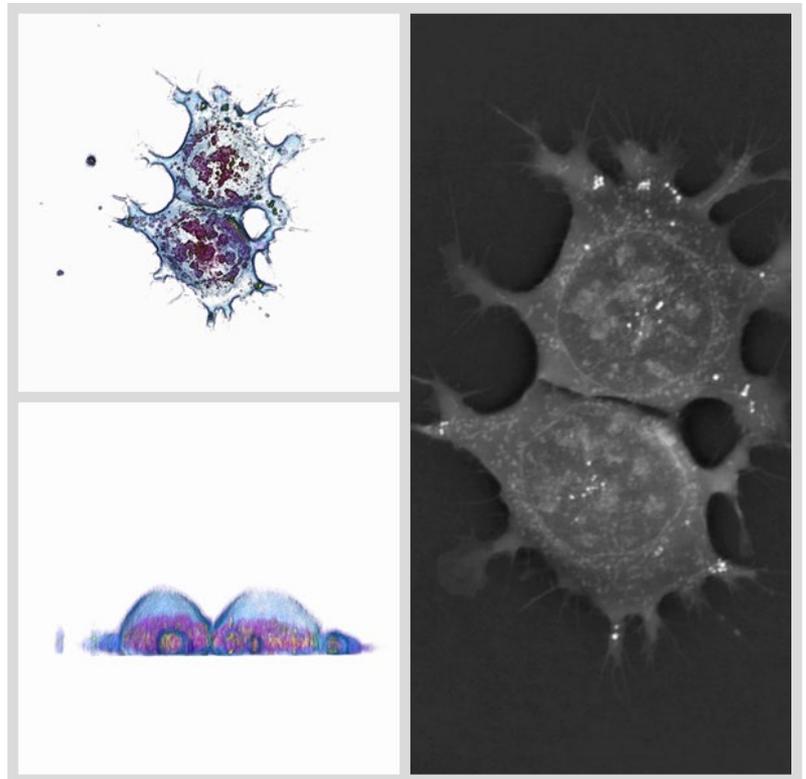




CX-A USER GUIDE



Nanolive SA
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If you encounter an issue or if you have a question, please contact Nanolive Service Support at:



support@nanolive.ch



WHAT IS THIS USER GUIDE?

The purpose of this guide is to provide information about the use of the CX-A and its accessories. For information please refer to the provided documentation and information available on our website www.nanolive.ch.



Scan me or click [here!](#)

WHAT IS THE CX-A?

The CX-A is an automated imaging platform. It allows to measure the refractive index of multiple samples, offering researchers the possibility to acquire super resolution 3D images. Moreover, thanks to the non-phototoxic laser and the stage-top incubation system compatibility, the CX-A is suitable for long-term live cell imaging.

QUICK START

Minimum 2 hours in advance, ideally the day before:

- Switch on the prior motor (switch at the back) and the microscope (switch at the back)
- Switch on the Tokai Hit incubator (orange button on the front), add water in the incubator

Day of experiment:

- Switch on CO₂
- Make sure there is still enough water in the incubator chamber
- Place your sample in the chamber
 - 96 WP: Thermalize the sample minimum 45 min. if sample was at room temperature, minimum 15 minutes if it was at 37°C
 - 35 mm dish: Thermalize the sample minimum 30 min. if sample was at room temperature, minimum 10 minutes if it was at 37°C
- Switch on the computer and open the software EVE
- Prepare your experimental setup in EVE and follow the checklist indications (wait for thermalization, define parameters, file name, ...)
- Click "load well plate"
- Continue to follow the checklist indications, click "calibrate well plate" and carefully set the manual focus
- Click "start acquisition"
- View your data in EVE or STEVE (adjusting brightness and contrast for better visualization)



HOW DO I RUN AN EXPERIMENT?

System Start Up

1. Turn on the components: Tokai Hit incubation (orange button on the front), prior motor (switch at the back), microscope (switch at the back) and computer. The rotating arm of the microscope should start to move after it is turned on. The Tokai Hit incubator should be switched on at least 2 hours, ideally 24 hours, prior to starting the experiment to allow enough time for thermalization to be completed.
2. Turn on microscope and stage, at least 2 hours before experiment, ideally 24 hours before.
3. Use the syringe provided to fill the incubation chamber reservoir with ~25mL of distilled (DI) water. It is important that the water level does not rise above the level indicated on the gas-flow tube, located in the back right of the incubation chamber. If this occurs, this will create bubbles when the gas is turned on, which will then cause vibrations that will disrupt the system. Refill the reservoir using the syringe as necessary during experiments (approximately every 12 hours).
4. Open the software EVE. You should see the automated stage move.
5. Confirm that the “placeholder” block (if using 96-well format plates) or the 4-dish holder is in the incubation chamber and cover the chamber with the glass lid located in the lid holder at the front of the microscope. If you are using the 4-dish holder, place empty dishes or lids to block the holes. The system will need 2 hour to thermalize after everything is turned on for the first time.

Taking your first acquisition

1. Carefully place the well plate into the holding insert. Use minimal force around the edges to ensure the plate is inside the insert. If you are using dishes, replace the empty dishes or lids used to block the holes by your samples. For long-term imaging, empty dishes (i.e. those without samples) can be filled with DI water to increase humidity. Pay attention to the orientation; the metal lips on the sidewalls of the insert should face right. The letters on the well plate should face you, and the numbers on the plate should face left. Carefully push the insert/well-plate down into the chamber using the metal pieces on the front and back of the plate. Place the glass lid back on top of the incubation chamber. Each sample must be allowed to thermalize:
 - 96 WP: Thermalize the sample minimum 45 min. if sample was at room temperature, minimum 15 minutes if it was at 37°C
 - 35 mm dish: Thermalize the sample minimum 30 min. if sample was at room temperature, minimum 10 minutes if it was at 37°C
 Selecting “Load well plate” in the software EVE will automatically start a 10 minute timer. If you are using a 96-well format plate, replace the traditional 96-well plate lid for your sample with the reusable DIC lid for imaging.



- Starting from the top left, fill out the general experimental information (media type, well-volume, save destination).

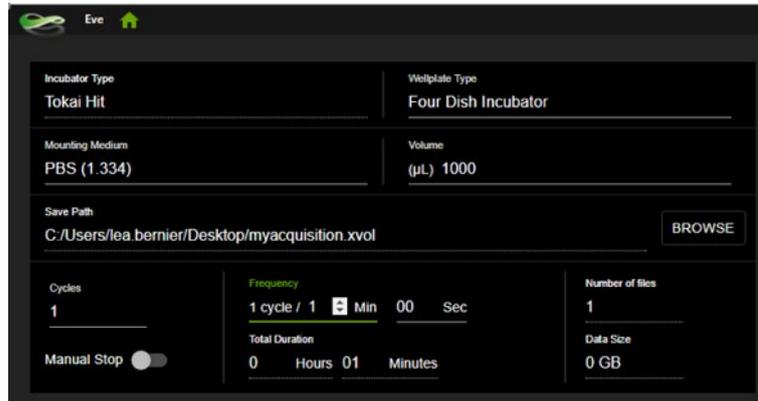


Figure 1: Complete experimental information in EVE

- Customize the protocol based on your experimental procedures. Click and drag each well to establish experimental groups and assign the desired imaging details to each group in the interface on the right side of the screen (add fluorescence channels, grid-scans etc.).

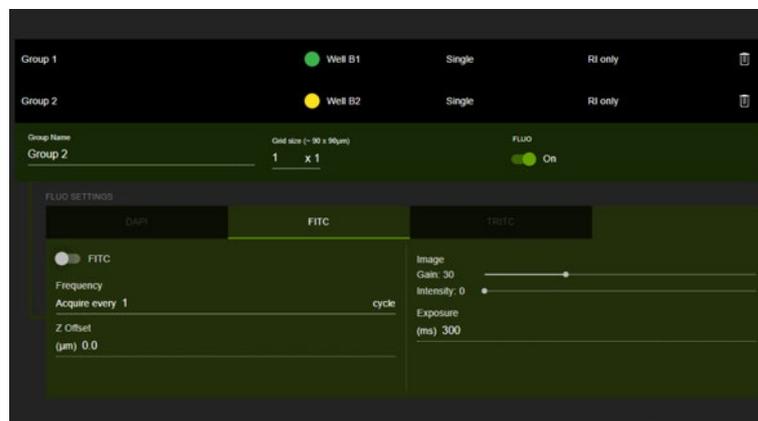


Figure 2: Choose the desired parameters for each group (fluorescence channels, grid-scans etc.)

- Groups should always be defined in horizontal lines (as opposed to vertical lines) to reduce the number of focus points required.

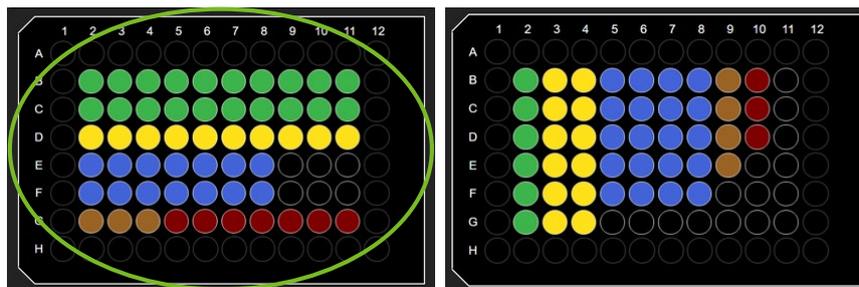


Figure 3: How to define groups in 96-well plate format Nanolive plates



- Set the time frequency at a rate above the minimum imaging time required and adjust the total amount of cycles you wish to run. Take note of the expected data file size and ensure there is enough space in your save destination. At this point, save your setup for later access and reassurance in the event of any system failures or accidents. For future acquisitions, you will be able to load saved setups in EVE directly from the home menu. Please note that you need to change the save location in order to avoid overwriting your files.
- At the top right of the screen, open the checklist and check all steps are complete. Do the same for the "Calibrate Well-Plate" portion of the checklist, and then click the "Calibrate Well-Plate" button at the top right of the screen.

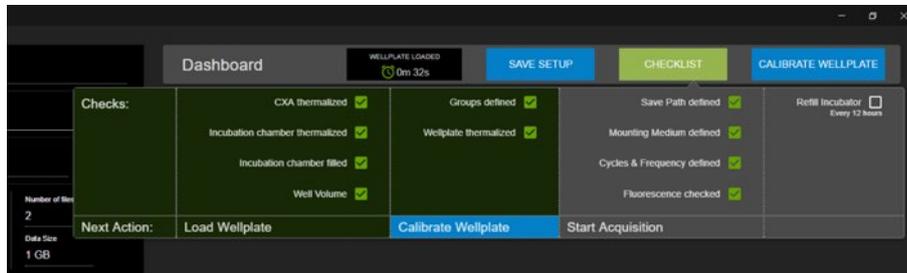


Figure 4: Dashboard in EVE

- Now, you will be asked to manually focus certain wells, which will act as focus points for other wells in that group. Simply move your mouse over the z-value list and scroll using the mouse to adjust the focus. It is recommended to scroll up first (moving the plate away from the objective) and then back down if necessary, to avoid accidentally touching the objective with the bottom of the well-plate. Perform this step for all of the necessary focus points.

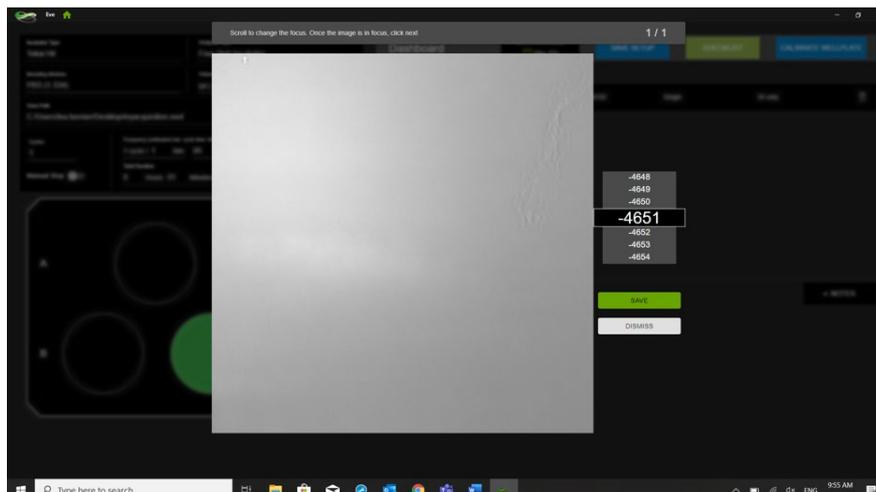


Figure 5: Setting focus at different positions before launching an acquisition



- If you are also acquiring fluorescence, you will need to adjust the parameters for these specific wells. Click on a well that has been designated to acquire fluorescence, and then click the “view” button on the right side of the screen. Towards the bottom of the screen you will see tabs for your fluorescent channels (Dapi, fitc, tritc/cy5). Click on the relevant tab and adjust the fluorescence parameters (exposure, intensity, gain, focus). To visualize this, you can leave each fluorescent channel open while you adjust these parameters, or you can take snapshots and adjust between each snap. The latter method is recommended to reduce exposure time while setting parameters. In this interface, you will also be given a chance to look at surrounding grid-scans for that well (if applicable) and to adjust the brightfield focus to a finer level.

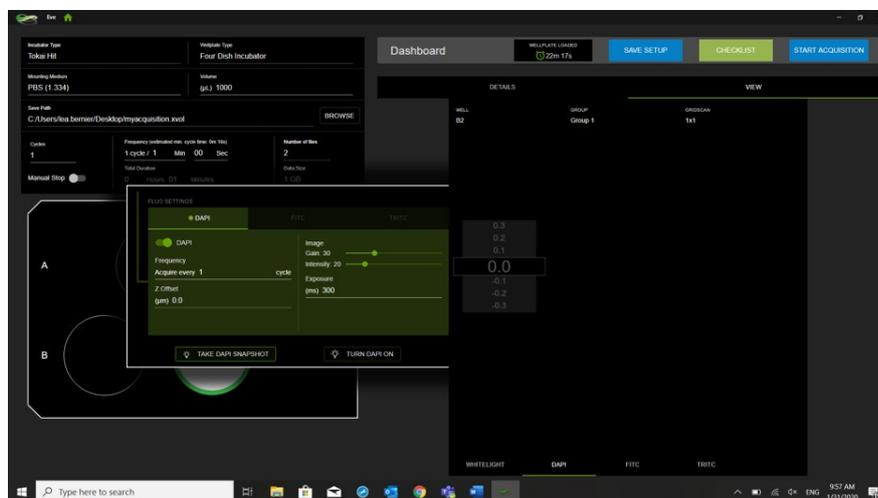


Figure 6: Adjusting fluorescence in EVE

- After you have adjusted your fluorescent channels, look over the last part of the checklist and confirm everything has been completed, and then click “Start Acquisition”. The system will perform a round of calibration for each well (the first well will take the longest). Any calibration fails are marked by a red exclamation point. Once all wells are calibrated, the system will proceed with the imaging protocol. Congratulations, your part is done! You can now leave the rest to the CX-A.

System cooldown

- When finished using the system, perform the opposite of the steps described in the start-up procedure: Turn off incubator > microscope > prior stage > PC.
- Remove the glass chamber lid and place it in the lid holder on the front of the microscope. The lid can be cleaned with 70% ethanol or isopropanol and optical paper.
- Remove the well-plate/insert from the incubation chamber, and then remove the well-plate from the insert. Remove the glass well-plate lid and carefully clean with ethanol and lens paper. Discard well-plate or save for future use.
- Put the “placeholder” block in the incubation chamber and leave set-up ready for next user.



WHAT DO I DO WITH THE ACQUIRED DATA?

Exporting data/data analysis

There are 3 exporting options available: Auto Export, exporting from EVE or exporting from STEVE.

1. The Auto Export feature allows the automatic exportation of the data as MIP.tiff (float). After an acquisition has been finished, data can be exported to .tiff form, or transferred to STEVE software to perform post-processing and subsequent exporting.
2. To export directly from EVE into a tiff, simply click the export button at the top right. You may export all the wells, or a set of selected wells (click target wells prior to pressing the export button). You will then be able to customize the information you wish to export. You can vary this by the number of frames, the type of data (RI, fluo intensity, overlay, etc), the number of z-slices (for RI), and number of grid-scans (single FoV or grid-scan). Once you have selected the information you wish to export, you will be asked to specify the file name, location, and organization. Click "export", and your tiff files will be saved to the specified location, ready to manipulate in ImageJ or other analysis software. Although a tiff file is the recommended export format, there is also an option to export as other file types.
3. To visualize and perform post-processing in STEVE, click on the icon at the top right of your screen in EVE. Alternatively, open the STEVE shortcut on the desktop and click on the folder icon towards the bottom left side of the screen to load acquired data. In the save location originally designated in EVE, you will find the .vol files for each well belonging to the data set. It is important to note that only .vol files can be opened in STEVE. .xvol files are only compatible with EVE.
4. After opening your file in STEVE, you can do any post-processing that you wish to perform on your data set. The post-processing tab on the top of the screen will allow you to adjust the histogram, background subtraction, and edge softening for both your RI data and fluorescence data. In STEVE, you can also look at the 3D rendering and fluorescence overlay.
5. Once you have processed the data, click the export button on the bottom left side of the screen. You will then be taken through the same exporting process as in EVE, but with the option here to export your post-processed data.

The raw data is never modified

- Post-processing can be saved inside the acquisition file, simply select "Save" when closing the file.
- Select "Raw" or "Processed" in the Modes selection to visualize raw or processed data (raw data is shown by default).
- Post-processing changes the values of refractive indices. This changes the outcome of data analysis. The user is responsible for the outcome and Nanolive cannot be held responsible. It is recommended to always use raw data for analysis.



WHAT IF SOMETHING GOES WRONG?

Troubleshooting

In general, restart the software and/or reboot the computer.

For 96 WP, test one cycle on the first well. If it works, perform your experiment as desired.

- **Calibration errors**

1. Make sure the laser is not obstructed (by the edge of the dish, the edge of the incubation chamber, any opaque object, etc.)
2. Make sure that the volume is identical in each well (75 to 90µl)
3. Try a different well/dish
4. Clean the microscope objective (using > 70% ethanol or isopropanol and the provided swabs)

- **Acquisition errors**

1. Try a different well/dish
2. Clean the microscope objective (using > 70% ethanol or isopropanol and the provided swabs)

- **Cells are not in focus errors**

1. Make sure that the 96 WP or dishes are correctly thermalized before starting the calibration step.
 - 96 WP: Thermalize the sample minimum 45 min. if sample was at room temperature, minimum 15 minutes if it was at 37°C
 - 35 mm dish: Thermalize the sample minimum 30 min. if sample was at room temperature, minimum 10 minutes if it was at 37°C
2. 96 WP: If there is no cell in one focus position, re-create the groups avoiding the well without cells in the center
3. Dish: if there is no cell in the center, open the chamber, rotate the dish, close the chamber, unload/reload and let thermalize 10 min. Restart calibration

- **Connection errors**

1. Verify all cable connections – unplug and re-plug all cables
2. Make sure the CX-A is connected to electrical power and switched on
3. Verify that your USB 3.0 drivers are up to date

- **Processing errors**

1. Make sure your computer meets the EVE hardware requirements. Specifically, the graphics card should be NVIDIA and have at least 8GB of dedicated memory
2. Make sure your graphics card drivers are up to date

GETTING HELP



support@nanolive.ch



WHAT KIND OF SAMPLE CAN I IMAGE?

- Adherent cells in culture (mammalian, bacteria, yeast, fungi, etc)
- Non-adherent cells in culture
- Deparaffinized histological sections in aqueous mounting medium with thickness up to 10 μm
- Cells embedded in hydrogel matrices with thickness up to 30 μm
- Transparent materials with thickness up to 30 μm

You can find more information and examples of imaged samples on our website.



WHICH LABWARE CAN I USE?

Nanolive imaging requires clean optical conditions throughout the entire optical system which comprises the microscope and the sample, as well as full accessibility for the 45-degree angle light beam coming from the rotating arm. This means that the labware quality and geometrical shape are crucial for optical imaging results.

Our 4-dish holder is designed for use with the following 35mm dishes:

- 35mm dishes, high borders**



	μ-Dish 35 mm, high	μ-Dish 35 mm, high	μ-Dish 35 mm, high
	Polymer Coverslip ibiTreat / Uncoated	Glass Bottom	Grid-500 Glass Bottom
Cat.No	81156, 81151	81158	81168
Bottom Thickness	180 μm (+10/- 5 μm)	170 μm (+/- 5 μm)	170 μm (+/- 5 μm)
Bottom Material	#1.5 Polymer coverslip (ibidi)	#1.5H glass coverslip (D 263 M Schott high precision glass)	#1.5H glass coverslip (D 263 M Schott glass)
Lid	Lid lock for minimal evaporation and safe handling	Lid lock for minimal evaporation and safe handling	Lid lock for minimal evaporation and safe handling
Surfaces	ibiTreat (tissue culture treated), Uncoated (hydrophobic), Plastic	Uncoated glass	Uncoated glass
Gas permeable	Yes	No	No
Grid	No	No	Grid-500

- Image WITHOUT the lid (not compatible with DIC lids)

- 35mm dishes, low borders**



	μ-Dish 35 mm, low	μ-Dish 35 mm, low Grid-500
	Polymer Coverslip ibiTreat / Uncoated	Polymer Coverslip ibiTreat / Uncoated
Cat.No	80136	80156
Bottom Thickness	180 μm (+10/- 5 μm)	180 μm (+10/- 5 μm)
Bottom Material	#1.5 Polymer coverslip (ibidi)	#1.5 Polymer coverslip (ibidi)
Lid	Lid lock for minimal evaporation and safe handling	Lid lock for minimal evaporation and safe handling
Surfaces	ibiTreat (tissue culture treated), Uncoated (hydrophobic)	ibiTreat (tissue culture treated), Uncoated (hydrophobic)
Gas permeable	Yes	Yes
Grid	No	Grid-500

- Handle with care - high risk of medium spilling out
- Use with DIC lids for μ-dishes (special lids with glass inserts for use in Differential Interference Contrast (DIC) imaging)
- The DIC lids can be cleaned sterilized with high grade ethanol and then reused



Always make sure your sample (e.g. cells) are present in the centre of the dish.

- Nanolive 96-well format plates

Our 96-well plate format plates are used with our imaging lid and placed on the 96-well plate holder.



Figure 1: 96-well plate format Nanolive plate with imaging lid



HOW SHOULD I PREPARE MY SAMPLE?

General notes

- All optical surfaces should be as clean as possible
- The maximum thickness that can be imaged is 30 μm

Cell confluency

There are no limitations on cell confluency providing the maximum thickness of 30 μm is not exceeded. However, the best image quality can be obtained by imaging single layers or low confluency cell cultures. Additionally, cells should be confluent enough to ensure that there are cells in the field of view at the centre of the dish/well.

Culture medium

- All samples should be embedded in liquid medium during acquisition. Dry samples cannot be imaged. The best image quality can be obtained by imaging with transparent media such as DMEM, PBS, etc.
- Make sure there is enough liquid in the culture dish to cover the entire bottom surface.
- High levels of liquid may affect the acquisition quality (medium level should never exceed half of the dish height).
- For 35 mm dishes, use a maximum of 1.5 ml of optically clear medium, or a maximum of 1 ml with scattering medium (e.g. agar or agarose hydrogels).
- For 96-well plate format plates, use between 75 μl and 95 μl , making sure there is as little variation as possible between wells.
- The refractive index (RI) value of the medium should be close to the RI of the sample to be imaged. For mammalian cells, media should have RI values between 1.33 and 1.44. A list of compatible media is proposed directly in our software EVE. Please note that different media will be required to image biological samples with RI values out of this range (vegetal cells, fungi, etc).

Cleanliness of the sample

The cleanliness of the sample is crucial to ensure good image quality. Wash the cells to remove dead cells and cell debris before imaging. Use lint-free optical paper and ethanol to clean glass bottoms and DIC lids (if applicable).

You can find more detailed information about sample preparation on our website in the "Supporting material" section.